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EDITORIAL

Is there a need for universal double reflex testing of HBsAg-positive individuals for hepatitis D infection?

Zaigham Abbas, Minaam Abbas

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Abstract

Hepatitis D virus (HDV) can infect HBsAg-positive individuals, causing rapid fibrosis progression, early decompensation, increased hepatocellular carcinoma risk, and higher mortality than hepatitis B virus (HBV) mono-infection. Most countries lack high-quality HDV prevalence data, and the collection techniques employed often bias published data. In recent meta-analyses, HDV prevalence in HBsAg-positive patients reaches 5%-15% and is even significantly higher in endemic areas. Since HBV vaccination programs were implemented, HDV prevalence has decreased among younger populations. However, owing to immigrant influx, it has increased in some Western countries. The current practice of HDV screening in HBsAg-positive individuals is stepwise, based on physician's discretion, and limited to at-risk populations and may require numerous visits. Double reflex testing, which includes anti-HDV testing in all HBsAg-positive individuals and then HDV RNA testing for anti-HDV-positive ones, is uncommon. Reflex testing can identify more HDV infection cases and link identified patients to further care and follow-up. Moreover, laboratory-based double reflex screening is less biased than physician-led testing. Therefore, health-care providers should learn about reflex testing, and federal and provincial hepatitis control programs should implement laboratory-based double reflex testing to obtain reliable HDV prevalence estimates. The test's cost-effectiveness depends on the number of HBV-positive patients screened to identify one HDV-positive patient. Such testing may be viable in areas with low HBsAg but high HDV prevalence. However, its economic impact on areas with low HDV prevalence needs further study.

Key Words: Anti-hepatitis D virus antibody; HBsAg; Hepatitis D virus RNA; Hepatitis B; Hepatitis D; Reflex testing

Core Tip: Most countries lack high-quality hepatitis D virus (HDV) prevalence data, and published data are often biased by the collection techniques employed. Currently, HDV diagnosis practice is stepwise. It relies on physician's discretion and requires numerous visits. Generally, only HBsAg-positive patients highly at risk for HDV are screened. Double reflex testing involves anti-HDV testing of all HBsAg-positive individuals, followed by HDV RNA testing for those who test positive for anti-HDV. This test approach is gaining attention because of the severe implications of HDV coinfection, and emerging as an effective strategy for identifying undiagnosed cases.

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INTRODUCTION

Hepatitis D significantly increases the risk of rapid fibrosis progression, early decompensation, hepatocellular carcinoma, and higher mortality than HBV mono-infection[1]. Most countries have no quality prevalence data, and published data are often biased. Meta-analyses indicate that 5%-15% of HBsAg-positive patients have been exposed to hepatitis D virus (HDV), accounting for 12-70 million individuals[2-4]. However, these meta-analyses focused on regions or pockets with a high infection probability. Recently, the adjusted HDV prevalence was lower in most countries and territories than previously reported[5]. In addition, many countries have no nationwide reports of HDV prevalence. The study (crude) prevalence of hepatitis D is not the same as the country prevalence because only those suspected to harbor HBV are tested and reported. Therefore, the reported HDV pockets do not represent country prevalence.

Since hepatitis B vaccination programs were implemented, the epidemiological landscape of hepatitis D has changed. In 2021, the global HDV prevalence was approximately at 262240000 and only a fraction of these infections were newly diagnosed[6]. However, the true prevalence of HDV remains uncertain because of the lack of awareness, limited access to reliable diagnostic tests for HDV antibody and HDV RNA, and high screening cost, resulting in the diagnosis of only 20%-50% of the true population infected with the HDV[1]. Most of the diagnosed population comprises immigrants and refugees in the West and residents of several Asian nations[7]. Although the HDV prevalence is generally decreased in the younger population resulting from robust HBV vaccination programs in some countries, it has increased in Western countries because of the influx of immigrants, as mentioned above.

Early detecting hepatitis D is important because it has implications for public health. With early treatment, disease progression and complications may be prevented. In one study, delaying HDV screening for more than 5 years was independently associated with worsened liver-related outcomes[8]. In addition, knowledge of HDV coinfection influences treatment decisions because certain newly developed antiviral medications are effective against both HBV and HDV, including bulevirtide[9]. Identifying and managing HDV-positive individuals help reduce the risk of disease transmission, particularly in high-risk settings such as healthcare facilities and households. Screening programs also contribute to public health education by increasing awareness of the risks associated with HBV and HDV coinfection.

Double reflex testing involves anti-HDV testing of all HBsAg-positive individuals, followed by HDV RNA testing for those who test positive for anti-HDV. Owing to the severe implications of coinfection, this test has gained attention, emerging as an effective diagnostic strategy. However, the current practice of diagnosing HDV is stepwise, relying on the physician's discretion and requiring several visits. HDV cases must be identified for timely care management. Reflex testing simplifies the process for both healthcare providers and patients and reduces the bias inherent to physician-led testing.

The universal screening of HBsAg-positive patients helps identify more individuals with HDV infection. If implemented in national hepatitis control programs, it will be more cost-effective in areas with a reported lower prevalence of hepatitis B but a higher prevalence of hepatitis D. The cost-effectiveness depends on the number of HBV-positive patients screened to obtain one patient with hepatitis D. However, considering that the number of newly diagnosed HBV cases has decreased globally, the strategy of reflex testing may be easily implemented by the healthcare systems. Cost-effectiveness studies may be needed in areas with high HDV *vs*. low HDV prevalence.

Several pieces of evidence support double reflex testing. A study conducted at the University of Naples Hospital Federico II in Italy highlighted the impact of implementing reflex testing for HDV in HBsAg-positive individuals. Before reflex testing was introduced, only 16.4% of HBsAg-positive participants were tested for anti-HDV, but after implementation, the percentage increased to 100%. Although the anti-HDV positivity prevalence decreased (from 16.6% to 10.7%), the absolute number of identified anti-HDV-positive patients rose from 14 to 52, with a higher prevalence in immigrant populations, leading to the possibility of more targeted interventions[10]. Therefore, reflex testing substantially improves the detection of HDV infection.

In Spain, one study assessed the impact of HDV reflex testing over 8 years by comparing the previous scenario with the current one (7.6% testing rate) using a proposed universal reflex testing strategy. Results revealed that implementing

reflex testing increased anti-HDV detection; thus, more patients received treatment and achieved undetectable HDV-RNA levels. Liver complications and associated costs were also significantly reduced; thus, reflex testing could decrease the clinical and economic burden of chronic hepatitis D by 35%-38% by 2030[11]. Such modeling could be used to support the drive for double reflex testing with long-term savings, contributing to the increased upfront cost.

Guidelines for HDV screening vary internationally. The European Association for the Study of Liver recommends testing all HBsAg-positive individuals[12]. The American Association for the Study of Liver Diseases focuses on patients at high risk of HDV infection or with active liver disease despite low HBV-DNA levels[13]. However, a United States study showed that a risk-based screening approach would miss 18% of HDV-positive patients because of unreported or negative risk factors[14]. Therefore, reflex anti-HDV testing followed by HDV-RNA testing is now increasingly advocated in the United States. Despite Factors such as awareness, reliable test availability, and cost-effectiveness in different epidemiological settings can influence the adoption of such a program[1]. However, the Chronic Liver Disease Foundation has recently recommended universal HDV screening for all HBsAg-positive patients[15].

CONCLUSION

Double reflex testing is highly recommended in hepatitis D-endemic regions. Modeling should be initially employed to project the cumulative savings attained from this program, which can, in turn, justify the economic impact of increased testing. Furthermore, low-HBV-prevalence and high-HDV-prevalence countries should consider double reflex testing as the preferred strategy[6]. Reflex testing for HDV in HBsAg-positive individuals significantly improves HDV infection detection and management and eases the burden on physicians. Therefore, healthcare providers need to be educated on this program, and government-based hepatitis control programs should implement it.

FOOTNOTES

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REVIEW

Non-alcoholic fatty liver disease and sleep disorders

Lu-Fang Bu, Chong-Yu Xiong, Jie-Yi Zhong, Yan Xiong, Dong-Ming Li, Fen-Fang Hong, Shu-Long Yang

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Abstract

Studies have shown that non-alcoholic fatty liver disease (NAFLD) may be associated with sleep disorders. In order to explore the explicit relationship between the two, we systematically reviewed the effects of sleep disorders, especially obstructive sleep apnea (OSA), on the incidence of NAFLD, and analyzed the possible mechanisms after adjusting for confounding factors. NAFLD is independently associated with sleep disorders. Different sleep disorders may be the cause of the onset and aggravation of NAFLD. An excessive or insufficient sleep duration, poor sleep quality, insomnia, sleep-wake disorders, and OSA may increase the incidence of NAFLD. Despite that some research suggests a unidirectional causal link between the two, specifically, the onset of NAFLD is identified as a result of changes in sleep characteristics, and the reverse relationship does not hold true. Nevertheless, there is still a lack of specific research elucidating the reasons behind the higher risk of developing sleep disorders in individuals with NAFLD. Further research is needed to establish a clear relationship between NAFLD and sleep disorders. This will lay the groundwork for earlier identification of potential patients, which is crucial for earlier monitoring, diagnosis, effective prevention, and treatment of NAFLD.

Key Words: Non-alcoholic fatty liver disease; Sleep duration; Sleep quality; Sleep disorders; Obstructive sleep apnea

Core Tip: Non-alcoholic fatty liver disease (NAFLD) is independently associated with sleep disorders. Different sleep disorders may be the cause of the onset and aggravation of NAFLD. An excessive or insufficient sleep duration, poor sleep quality, insomnia, sleep-wake-disorders, particularly obstructive sleep apnea, may increase the incidence of NAFLD and contribute to its development and worsening. Further research is needed to establish a clear relationship between NAFLD and sleep disorders, which can help identify potential patients earlier and facilitate effective prevention and treatment measures.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases, with an estimated global prevalence of 25%[1]. Its epidemiological and demographic characteristics vary around the world, and are positively correlated with obesity prevalence[2]. Due to unhealthy lifestyle behaviors among the population in China, the prevalence of NAFLD has risen sharply from 23.8% in 2001 to 32.9% in 2018, gradually replacing hepatitis B as the main cause of chronic liver disease[3]. NAFLD is a systemic disease characterized by steatosis and abnormal accumulation of fat in hepatic parenchymal cells, metabolically stressed liver damage closely related to insulin resistance (IR), as well as certain genetic factors, possessing a complex multifactorial pathogenesis and heterogeneous clinical manifestations[4,5]. Non-alcoholic steatohepatitis (NASH), a subtype of NAFLD, is a potential progressive liver disease that may lead to cirrhosis, hepatocellular carcinoma, and even death[6]. Various extrahepatic manifestations such as chronic kidney disease, cardiovascular disease and obstructive sleep apnea (OSA), is also associated with NAFLD, imposing a substantial burden and economic impact on patients and society[7]. In the past decades, studies have found that sleep disorders might facilitate the development of NAFLD accompanied by obesity, inflammation, IR, as well as glucose or lipid metabolic disorders[8]. The underlying mechanism may be related to the increased secretion of stress hormones (such as cortisol and catecholamines) by activating the hypothalamic-pituitary-adrenal axis, thereby increasing the risk of the metabolic syndrome[9]. Nowadays, there is an increasing interest in understanding whether different sleep patterns can serve as causative factors for NAFLD. Current research on sleep stage changes in NAFLD patients shows inconsistent findings. Some studies indicate a possible decrease in the percentage of rapid-eye-movement sleep in NAFLD patients [10]. Additionally, other studies suggest changes in non-rapid eye movement sleep structure, such as a potential decrease in the proportion of slow wave sleep. Further large-scale research is needed to gain a better understanding of these sleep characteristics in NAFLD patients[11]. In this review, the association between different sleep traits and NAFLD is investigated, the recent advances concerning the correlations between NAFLD and sleep disorders are summed up, the complicated and interrelated relationship between OSA and NAFLD are elucidated, and their identical and different mechanisms and clinical features are discussed. Furthermore, the effect of continuous positive airway pressure (CPAP) treatment on OSA is also summarized, aiming to provide current and future therapeutic implications for NAFLD.

PATHOGENESIS OF NAFLD

The pathogenesis of NAFLD is complex and multi-factorial. Previous studies have confirmed its positive correlations with metabolic diseases such as obesity, IR, metabolic syndrome, and type 2 diabetes. The pathogenesis of NAFLD has frequently been probed and two hypotheses were successively proposed, namely the early proposed "two-hit" model and the current "multiple-hit theory". The "two-hit" model believes that IR and abnormal hepatic lipid accumulation is the first hit, while the oxidative stress and inflammation is the second hit[12]; however, because other alternative factors including glucose and lipid metabolism disorders, intestinal flora disorder and epigenetic regulation were confirmed to be involved in NAFLD development, the "multiple-hit theory" has been widely accepted nowadays[13]. In addition, a dysregulated circadian rhythm due to sleep mode changes have been implicated in the pathogenesis of NAFLD[14,15]. As one of the most reliable markers of the circadian rhythm, melatonin (MT) is also involved in the pathogenesis of NAFLD. It is known that MT promotes sleep, circadian rhythms, and neuroendocrine processes. Current evidence suggests that MT protects against liver damage by inhibiting oxidation, inflammation, hepatic stellate cell proliferation, and hepatocyte apoptosis, thus inhibiting the progression of NAFLD[16]. Ren *et al*[17] observed that MT could ameliorate high-fat diet/chronic intermittent hypoxia-induced hepatocellular damage by activating sirtuin 1-mediated autophagy signaling.

CORRELATIONS BETWEEN SLEEP AND NAFLD

In this review, we see sleep duration, daytime napping, daytime sleepiness, sleep quality and sleep habits as sleep-related traits (Table 1). A randomized controlled trial indicates a causal relationship between sleep characteristics and NAFLD. The onset of NAFLD is the result of changes in sleep patterns, whereas alterations in sleep characteristics are not the cause of NAFLD. The causal relationship between the two is unidirectional[18]. Recent studies concerning the relationship between sleep duration and NAFLD suggest that short sleep duration and long daytime naps are risk factors for NAFLD[19-21]. A cohort study has shown that in young adults, short sleep duration is independently associated with an increased risk of incident NAFLD, regardless of the presence of intermediate/high fibrosis scores[22]. Furthermore, a cross-sectional study found a decreasing trend in the proportion of NAFLD in pace with increased sleep duration in men, whereas in women, the proportion of NAFLD displayed a U-shaped distribution, with the lowest in the group (6-7 h of sleep) and the highest in the group (≤ 6 h or ≥ 8 h of sleep)[23]. Similarly, a meta-analysis of the relationship between sleep duration (or quality) and NAFLD incidence showed that both short sleep duration (≤ 6 h) and long sleep duration (≥ 8 h) may increase the risk of NAFLD, and the incidence of NAFLD increases as the sleep duration decreased[24,25]. Accordingly, a case-control study on NAFLD demonstrated that optimal sleep duration (7-9 h/d) is negatively associated with IR and liver stiffness in patients with NAFLD[26]. Taken together, too short or too long sleep duration may both increase the risk of NAFLD in both men and women.

In addition, there were differences in the association between sleep duration and NAFLD in different populations: (1) Taking gender into account, a community-based longitudinal cohort study concluded that short sleep duration reduced the risk of NAFLD in men but had no risk in women[27]. Liu *et al*[28] found that sleep duration is an independent influencing factor for male NAFLD. The risk of NAFLD decreases with an increase in sleep duration in males, but there is also no significant correlation observed in females. A cross-sectional survey involving 4828 participants suggested that sleep quality was associated with NAFLD, and there were also gender differences[29]; and (2) Taking age into account, excessive nighttime sleep duration was associated with a moderately increased risk of NAFLD in a retrospective study targeted at middle-aged or elderly men in China[30]. In addition, in another cohort study of middle-aged or elderly people in South Korea, a positive correlation was also found between excessive sleep duration and elevated NAFLD scores[31].

SLEEP DISORDERS AFFECT NAFLD

A population-based study showed that NAFLD is independently associated with sleep disorders after the adjustment of age, gender, and ethnicity[32]. Sleep disorders are present in NAFLD regardless of underlying cirrhosis[33]. The prevalence of sleep disorders was significantly higher in individuals with NAFLD compared to controls; while the prevalence of NAFLD was higher in individuals with sleep disorders compared to good sleepers[34]. Common sleep disorders associated with NAFLD include insomnia, daytime sleepiness, sleep-wake disorders and sleep-disordered breathing such as OSA (Table 2).

Insomnia and daytime sleepiness

A meta-analysis of seven studies showed that people with insomnia or excessive daytime sleepiness have an increased risk of NAFLD[35]. Moreover, patients with NAFLD may have more severe daytime sleepiness and shorter sleep duration[36]. A mendelian randomization demonstrated that trouble getting up in the morning and insomnia were associated with an increased risk of NAFLD[37]. Similarly, a case-control study found that nearly 30% of patients with biopsy-proven NAFLD confirmed insomnia, and the prevalence of NAFLD in insomnia patients was significantly higher than that in non-insomnia patients[38]. Furthermore, daytime sleepiness is significantly linked to the biochemical and histologic surrogates of NAFLD severity. It is not only positively correlated with liver enzymes and IR, independent of cirrhosis, but also positively correlated with the degree of fibrosis[39].

Sleep-wake disorders

Sleep-wake disorder, also known as non-24-h sleep-wake rhythm disorder, is a circadian rhythm sleep-wake disorder characterized by an inability to entrain to the 24-h environment. Sleep-wake disorders may increase the risk of NAFLD in patients suffered from obesity, IR, inflammation, and disorders in glucose or lipid metabolism, resulting in weight gain by increasing the food-sensitive dopaminergic activity[40] and the circulating concentration of growth hormone-releasing peptide[41]. It is well-known that IR plays a central role in the progression of hepatic steatosis and fibrosis. Therefore, IR may be a major intersection between sleep-wake disorders and NAFLD[42]. In addition, sleep-wake disorders can also facilitate glycometabolism, promote lipid mobilization in adipose tissue by increasing cortisol hormone concentrations and weakening the tissue response to insulin, and accelerate the transport of free fatty acids to the liver[43]. Increased sympathetic nervous system and adrenal cortical activity may lead to the adverse metabolic effects of sleep-wake disorders. In a comparative study, the sleep of healthy volunteers was experimentally fragmented at all stages using auditory and mechanical stimuli. After two nights of sleep fragmentation, the results indicated that insulin sensitivity and glucose effectiveness, *i.e.*, the ability of glucose to mobilize itself was independent of the insulin response, were both decreased. In addition, morning cortisol levels were elevated, and the sympathetic nervous system was excited[44]. Sleep-wake disorders are also associated with elevated pro-inflammatory factors such as interleukin (IL)-1β, which are involved in the development of liver inflammation promoting NAFLD[45].

Table 1 Correlations between non-alcoholic fatty liver disease and sleep disorders

Items		Correlations
Sleep	Sleep duration	Short sleep duration and long daytime naps are risk factors for NAFLD[19-22]
		Moderate sleep duration reduces the risk of NAFLD[23-26]
		Excessive sleep duration increases the risk of NAFLD[23,25,30,31]
	Sleep quality	Poor sleep quality was significantly associated with an increased risk of NAFLD[19,25]
Sleep disorders	Insomnia and daytime sleepiness	Increases the risk of NAFLD in participants with insomnia or daytime sleepiness[35,38,39]
	Sleep-wake disturbance	Raises the risk of NAFLD through obesity, IR, disorder of glucose-lipid metabolism and inflammation[40,42-45]

NAFLD: Non-alcoholic fatty liver disease; IR: Insulin resistance.

Table 2 Selected studies investigating associations between sleep disorders and non-alcoholic fatty liver disease

Confounders	OR/HR (95%CI)	OR/HR (95%CI) adjustments for BMI	Ref.
Age, Alcohol, Smoking, Physical activity, Blood pressure, BMI, Marriage, Education level, Presence of job, Loud snoring, and Sleep apnea	M 1.28 (1.13-1.44) W 1.71 (1.38-2.13)	M 1.03 (0.90-1.19) W 1.59 (1.23-2.05)	Kim *et al*[19], 2013
Age, BMI, METs, and IR	1.31 (1.10-1.56)	1.29 (1.04-1.60)	Peng *et al*[20], 2017
Age, BMI, Alcohol, Smoking, ALT, HDL-C, TG, Diabetes, Blood pressure, Physical activity	M 1.39 (1.13-1.72) W 1.46 (1.05-2.04)	M 2.57 (1.88-3.52) W 9.38 (5.84-15.1)	Okamura *et al*[21], 2019
Age, BMI, Smoking and Physical activity	M 0.98 (0.62-1.54) W 1.44 (1.06-1.96)	M 1.18 (0.67-2.08) W 1.38 (0.95-2.01)	Imaizumi *et al*[23], 2015
Age, Sex, BMI, Smoking, Adiponectin, and TNF-α	1.66 (Not available)	1.62 (Not available)	Katsagoni *et al*[26], 2017
Age, Sex, BMI, HDL, Smoking, and Physical activity	2.230 (1.304-3.813)	1.462 (1.029-2.077)	Kim *et al*[31], 2019
Age, Smoking, BMI and Physical activity	1.13 (0.58–2.19)	0.93 (0.41–2.10)	Takahashi *et al*[29], 2020
BMI, Salt intake, Physical activity, and MetS	2.83 (2.63-3.05)	1.64 (1.35-2.00)	Wang *et al*[50], 2020
BMI and Abdominal obesity	2.42 (2.36-2.48)	1.21 (1.17-1.26)	Chung *et al*[51], 2021
BMI, Abdominal obesity, METs, and IR	4.89 (3.08–5.98)	1.78 (1.11–6.82)	Nobili *et al*[79], 2014
Sex, Age, BMI, IR and METs	4.20 (1.88-9.37)	3.85 (1.35-10.94)	Fu *et al*[55], 2022
BMI and Abdominal obesity	1.45 (1.03-1.98)	1.22 (1.02-1.45)	Krolow *et al*[62], 2021

ALT: Alanine aminotransferase; BMI: Body mass index; OR: Odds ratio; HR: Hazard ratio; CI: Confidence interval; TG: Triglycerides; HDL-C: High density lipoprotein-cholesterol; METs: Metabolic syndrome; IR: Insulin resistance; TNF-α: Tumor necrosis factor-α; W: Women; M: Men.

Sleep-disordered breathing

OSA is the most common sleep breathing disorder. A general population-based polysomnography study showed that the incidence of mild OSA was estimated to be 59% in men but 33% in women, while the incidence of moderate to severe OSA was estimated to be 30% in men but 13% in women[46]. It is characterized by episodes of apnea, hypopnea and sleep fragmentation (SF) due to restricted airflow in the collapsed upper airway during sleep[47]. It has been shown that SF-induced intermittent hypoxia (IH) and sleep deprivation are associated with IR and metabolic dysfunction, as well as adipose tissue dysfunction which are thought to play key roles in the metabolic abnormalities of OSA[48,49]. Snoring is the direct consequence of airway collapse in OSA patients, which is independently and positively associated with a higher incidence of NAFLD[50].

There is growing evidence that OSA is involved in the development of NAFLD with IH acting as the most important connecting factor[51,52]. The IH of OSA may also be involved in the progression of NAFLD by affecting the level of liver enzymes. It increased hepatic production of lysyl oxidase, an enzyme that cross-links collagen, and may serve as a biomarker of liver fibrosis in patients with severe obesity and NAFLD[53]. In animal models, IH can directly induce

hepatic steatosis by repeating brief hypoxia and reoxygenation simulating OSA[54]. Fu *et al*[55] found that IH caused by OSA may aggravate NAFLD and lead to a higher risk of NASH in patients with obesity.

OSA affects NAFLD

There are many studies on the aspects of OSA affecting NAFLD. Severe OSA is more likely to be associated with significant liver disease, one possible reason being its independent correlation with increased liver stiffness[56]. A systematic review and meta-analysis demonstrated that OSA is associated with an increased risk of NAFLD, NASH and fibrosis[57]. Jin *et al*[58] found significant correlations between OSA and NAFLD in terms of hepatic steatosis, lobular inflammation and fibrosis, suggesting that OSA may be involved in the progression of NAFLD through elevated liver enzyme levels and hepatic histological changes. In the presence of obesity, patients with OSA may potentially contribute to liver injury in NAFLD through IR and systemic inflammation[59]. Another case-control study showed that in the absence of considering obesity and metabolic syndrome, patients with OSA have a significantly high incidence of NAFLD and exhibit notable hepatic fibrosis[60]. After excluding the confounding factor of obesity, the severity of OSA emerges as an independent risk factor for both NAFLD and liver fibrosis[61]. Krolow *et al*[62] found that patients with moderate to severe OSA had an increased risk of hepatic fibrosis after adjusting for obesity level. Kim *et al*[63] demonstrated that the severity of OSA increased with the prevalence of NAFLD regardless of the gender. Also, compared to non-obese OSA patients, obese patients with OSA were more prone to developing NAFLD. In addition, regarding hepatic steatosis, there was no association between liver fibrosis and the severity of OSA. A retrospective analysis suggested that age and obesity predicted high liver fibrosis risk as assessed by noninvasive scoring systems, but not OSA severity[64]. In a cross-sectional study of human subjects, the risk of hepatic steatosis increased along with the severity of OSA and sleep-related hypoxemia after the adjustment of confounding factors including centripetal obesity[65].

Recent studies have been devoted to determining the influence of IH and OSA-related parameters on NAFLD severity. A multivariate analysis showed that the apnea-hypopnea index (AHI), oxygen desaturation index (ODI), lowest desaturation values, and percentage of sleep duration with mean nocturnal oxygen saturation (SpO2) were independent predictors of NAFLD after adjustment for body mass index (BMI), weight, and IR (it was found that the most correlated parameter for the severity of NAFLD was the duration of IH during sleep)[66]. Furthermore, decreasing SpO2 during sleep was also associated independently with a higher risk of liver cytolysis[65]. Benotti *et al*[67] found that OSA severity (as measured by the AHI) and hypoxia parameters were positively correlated with NAFLD severity in subjects without metabolic syndrome. Cakmak *et al*[68] reported that AHI and ODI values were significantly higher in the moderate and severe NAFLD groups compared to counterparts in the non-NAFLD group, SpO2 and lowest O2 saturation (LaSO2) were significantly lower in the mild and severe NAFLD groups. These results revealed that the parameters AHI, ODI, LaSO2, and SpO2 levels play pivotal roles in the association between NAFLD and OSA. The severity of OSA was also associated with a decrease in high-density lipoprotein-cholesterol and an increase in BMI, triglycerides (TG), homeostasis model assessment IR index, transaminases, and FIB-4 index (a noninvasive score for liver fibrosis)[69]. Human subjects with OSA had significantly higher levels of alanine transaminase (ALT) and aspartate transaminase (AST) than those without OSA[70]. A single-center, cross-sectional study indicated that OSA may be an independent risk factor for dyslipidemia, and that OSA and obesity have a synergistic effect on ALT elevation[71]. A cross-sectional study showed that the risk of developing NAFLD increases in older patients with OSA, and high TG is an important factor leading to the development of liver injury[72]. Given that the pathological mechanism of OSA promotes the development of NAFLD, there are three aspects included, as shown in Figure 1.

Metabolism disorders in glucose and lipid: OSA is independently associated with metabolic dysfunction, including dyslipidemia and IR. Yokoe *et al*[73] found that IH impaired glucose homeostasis and stimulated pancreatic β-cell replication only during periods of hypoxic exposure, but the presence of hyperglycemia may increase the hypoxic susceptibility of β-cells. The mechanism of systemic glucoregulation by glucose-sensing neurons in the ventromedial hypothalamic nucleus is also involved in the process of IH inducing the occurrence of IR by up-regulating the sympathetic nervous system, increasing circulating free fatty acids (FFAs) and hepatic glycogenolysis[74]. In addition, IH induces the occurrence of hyperlipidemia by inhibiting the clearance of TG-rich lipoproteins. Drager *et al*[75] observed that, in male C57BL/6J mice on a high-cholesterol diet under exposure to IH air for 4 weeks, the clearance of lipoprotein lipase, a key enzyme for lipoprotein clearance, was inhibited; resulting in a significant increase in total cholesterol and TG levels. IH-induced hyperlipidemia is also associated with the up-regulation of sterol regulatory element binding protein-1 and the over-expression of stearoyl coenzyme A desaturase 1[76,77]. In conclusion, the mechanism by which OSA promotes the development of NAFLD may be IH-reduced utilization of FFAs by limiting β-oxidation in mitochondria, and excessive FFAs are diverted to the synthesis of TG and cholesterol to trigger hyperlipidemia, which ultimately leads to the development of NAFLD.

Inflammation: The roles of IH in the progression of NAFLD are related to inflammation[78]. IH in OSA patients affects liver histology and activation of inflammatory cells in NAFLD regardless of obesity or IR[79]. In NAFLD animal models, IH has been shown to modulate inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and IL-6 to produce pro-inflammatory effects[80,81]. Savransky *et al*[82] found that the levels of IL-1β, IL-6 and TNF-α were elevated in mice following exposure to IH, lobular inflammation and fibrosis were documented in the liver. Similarly, comparable results were observed in humans. Schaefer *et al*[83] used *in vitro* models of NASH to study the impacts of IH on the liver, they found that IH contributed to a significant induction of IL-6 expression in both hepatocytes and macrophages. Furthermore, *in vitro* and *in vivo* models of NAFLD have shown that IH promotes the production of inflammatory signals by activating inflammatory bodies or caspase-1 in fat-laden hepatocytes, as well as promoting crosstalk between hepatocytes and Kupffer cells by releasing extracellular vesicles to induce hepatocellular damage. This is followed by

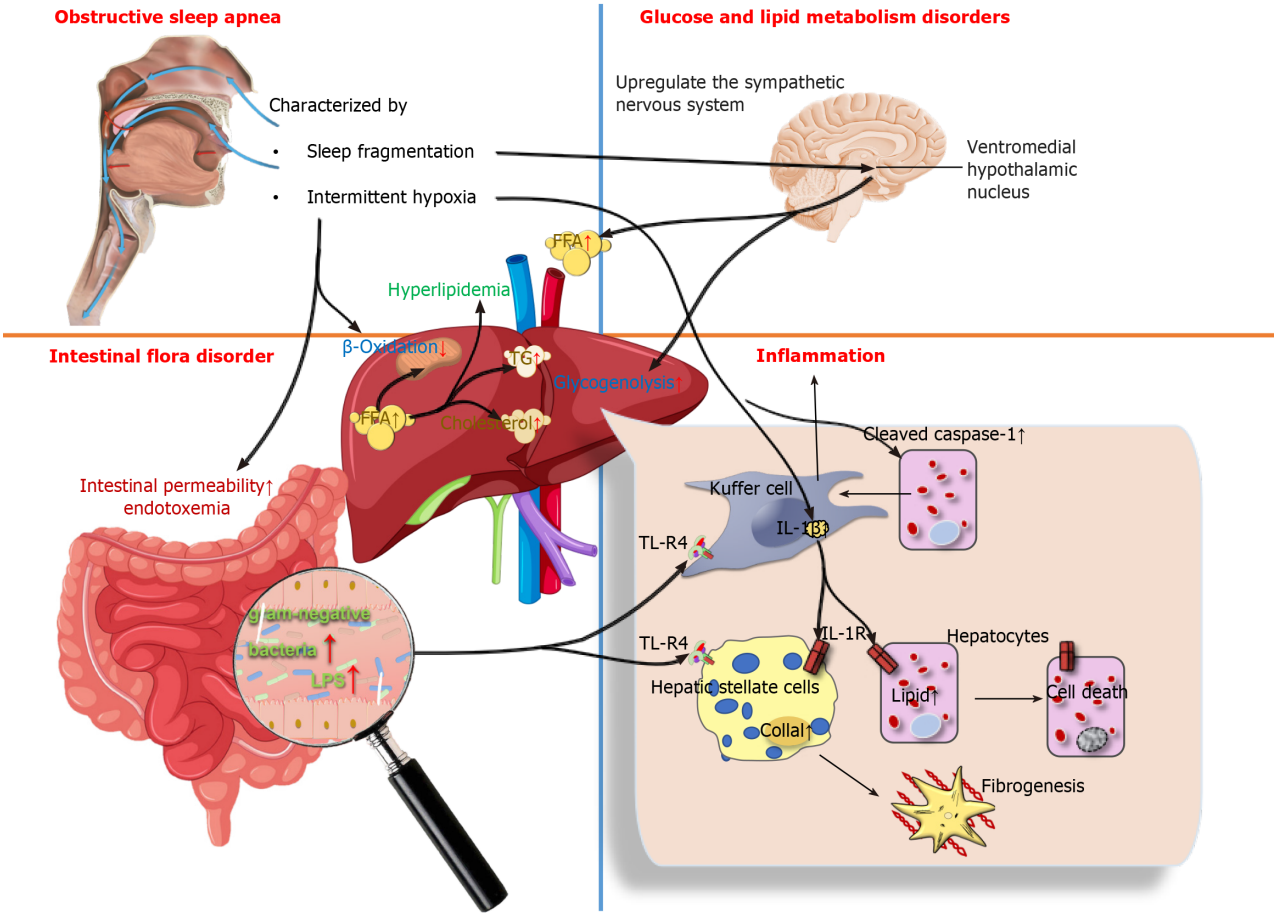

Figure 1 The pathological mechanism of obstructive sleep apnea promotes the development of non-alcoholic fatty liver disease. Obstructive sleep apnea causes glucose and lipid metabolism disorders, intestinal flora disorder and hepatic inflammation through the sympathetic nervous system, endotoxemia and hepatic toll-like receptor-4. TL-R4: Toll-like receptor-4; IL-1R: Interleukin-1 receptor; IL-1β: Interleukin-1 β.

increased cell mortality through a variety of mechanisms, including apoptosis and pyroptosis[84]. Notably, Taylor *et al* [85] discovered that human adipocytes are highly sensitive to IH, which enhances inflammatory gene expression in adipose tissue and the release of inflammatory cytokines involved in the development of NAFLD.

Intestinal flora disorder: There is a wide range of microorganisms in the human intestine, in which various microorganisms interact with each other to form a dynamic ecosystem called the gut microbial ecology. It has been shown that IH in OSA may affect the ecology of the gut microbiota and mediate a variety of cardiovascular diseases that coexist with OSA[86]. OSA is a risk factor for intestinal injury. Regardless of the metabolic status, intestinal permeability may be a possible factor leading to the susceptibility of OSA patients to NAFLD[87]. For example, Nobili *et al*[88] found that a novel correlation exists between OSA and NAFLD, namely that IH may disrupt the intestinal-liver axis in pediatric NAFLD by increasing the number of gram-negative bacteria in the intestine and intestinal permeability, with increased endotoxemia coupled with toll-like receptor-4 (TLR-4) up-regulation in hepatocytes, Kupffer cells and hepatic stellate cells[88,89]. In addition, one of the characteristic manifestations of OSA-SF, induces metabolic alterations in the organism that may be mediated in part by concurrent changes in gut microbiota, which was confirmed using SF-derived microbiota routinized in germ-free mice[90]. Chronic SF-induced reversible gut microbiota changes led to systemic and visceral white adipose tissue inflammation in addition to altered insulin sensitivity in mice, most likely *via* enhanced colonic epithelium barrier disruption.

CPAP treatment on OSA and NAFLD

Currently, CPAP is the globally accepted gold standard for the treatment of OSA. It can keep the airway open and reduce daytime sleepiness, improving cognition and sleep quality in OSA patients[91]. There have been many studies performed to explore the effects of CPAP therapy on OSA patients suffering from NAFLD, but the results obtained were varied. Some observational data suggested that CPAP treatment improves hepatic biochemistry of NAFLD in OSA patients; and that CPAP treatment is statistically significantly associated with improvement of hepatic injury in OSA patients, but a sufficiently long duration of treatment (greater than or equal to 3 months) may be required to achieve a positive effect. Chen *et al*[92] enrolled 160 patients with OSA and measured serum transaminases before and after CPAP treatment. After 3 months of treatment, both ALT and AST levels decreased significantly. A recent meta-analysis also showed that, compared to controls, ALT and AST levels were significantly lower in OSA patients after CPAP treatment, and was more

effective in OSA patients treated with CPAP for more than 3 months[93]. Hirono *et al*[94] found a significant reduction in AST and ALT levels and significant improvement in liver injury after 6 months of CPAP treatment in 50 patients with OSA suffering from NAFLD. In addition, the effect of CPAP treatment on NAFLD in OSA patients was also related to OSA patients' adherence. Patients with good adherence to CPAP showed significantly decreased levels in AST and ALT than those with poor adherence[95]. Sundaram *et al*[96] also found that treatment of OSA with CPAP may reverse liver injury parameters and reduce oxidative stress, indicating that CPAP could be a new therapy for preventing NAFLD progression in obese children with OSA.

However, some randomized controlled trials did not show a benefit of CPAP treatment on liver injury in OSA patients. For instance, Jullian-Desayes *et al*[97] found that six to twelve weeks of effective CPAP did not show any impact on reducing steatosis, NASH or liver fibrosis even after adjustment for gender, BMI, baseline AHI and severity of liver injury. Also, in the randomized controlled trial by Kohler *et al*[98], 94 patients with moderate to severe OSA were randomized to therapeutic or subtherapeutic CPAP treatment. Plasma ALT and AST levels were measured before and after treatment. The results showed that 4 wk of active CPAP treatment did not show any beneficial effect on transaminase levels compared to subtherapeutic CPAP in patients with OSA. Ng *et al*[99] showed that 6 months of CPAP treatment did not lead to improvement in hepatic steatosis and liver fibrosis, despite a significant correlation between hepatic steatosis and markers of OSA severity. Labarca *et al*[100] performed a systematic evaluation and meta-analysis of 5 randomized controlled trials involving patients with OSA and NASH who were treated with CPAP, but did not find obvious changes in hepatic steatosis, liver fibrosis and transaminase levels (ALT and AST) in OSA patients. Differences regarding the effect of CPAP treatment in OSA patients on NAFLD may be related to the duration of CPAP treatment, compliance of OSA patients and the severity of NAFLD progression.

NAFLD AFFECTS SLEEP DISORDERS

The effects of NAFLD on sleep can be observed from some observational studies, although there are no animal experiments to explain the specific mechanism by which NAFLD affects sleep. NAFLD patients have altered sleep status, namely in NAFLD, sleep duration was shortened, sleep onset was delayed and sleep quality poorer[39,101]. Moreover, NAFLD may increase the risk of developing OSA. A study showed that OSA is common in adults who have biopsy-proven NAFLD[102]. Similarly, in a 6-month prospective study, Romdhane *et al*[103] found that the incidence of OSA was relatively higher in patients with NAFLD in comparison with controls. In a nationwide population-based study, Chung *et al*[51] found that NAFLD was significantly associated with an increased risk of OSA after adjusting for multiple metabolic variables. Specifically, in younger, male or obese patients with NAFLD, there is a higher risk of OSA than that in older, female or non-obese patients.

The mechanism by which NAFLD affects OSA may be related to MT metabolism disorder. It is known that sleep is closely related to the metabolism of MT, which is metabolized by the liver. Liver metabolic dysfunction in NAFLD patients increases escalates as disease progresses. Currently, it has been found that key factors in NAFLD-induced sleep disorders include hepatic encephalopathy and circadian rhythm imbalance due to altered MT metabolism. Moreover, in the advanced stages of NAFLD, cirrhosis has an effect on circadian sleep regulation by a delay in the 24-h MT rhythm, which is likely to be related to reduced sensitivity to light signals[104]. The core feature of NAFLD is a discoordination between central and peripheral circadian rhythms[105]. This phenomenon has also been observed in db/db (hereditary obesity) mice[106], and the main circadian rhythm defect lies in the peripheral liver oscillator rather than the behavioral rhythm or master clock, but the mechanism by which peripheral circadian rhythm disorder affects the central circadian rhythm remains to be explored.

CONCLUSION

This paper provides some significant insights into the correlations between sleep disorders and the occurrence or development of NAFLD. Excessive or short sleep duration and poor sleep quality may increase the risk of NAFLD. Similarly, insomnia, daytime sleepiness, sleep-wake disorders and OSA have been associated with the development of NAFLD. NAFLD is also a risk factor for OSA; thus, it is necessary to screen and monitor the occurrence and development of NAFLD in OSA patients. Moreover, CPAP treatment can stabilize and slow down the progression of NAFLD under certain circumstances. Sleep factors can be added to the list of changeable lifestyle behaviors to reduce the risk of NAFLD. This includes maintaining proper sleep duration, improving sleep quality, and addressing sleep disorders.

FOOTNOTES

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REVIEW

Amebic liver abscess: An update

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Abstract

Amebic liver abscess (ALA) is still a common problem in the tropical world, where it affects over three-quarters of patients with liver abscess. It is caused by an anaerobic protozoan *Entamoeba hystolytica*, which primarily colonises the cecum. It is a non-suppurative infection of the liver consisting primarily of dead hepatocytes and cellular debris. People of the male gender, during their reproductive years, are most prone to ALA, and this appears to be due to a poorly mounted immune response linked to serum testosterone levels. ALA is more common in the right lobe of the liver, is strongly associated with alcohol consumption, and can heal without the need for drainage. While majority of ALA patients have an uncomplicated course, a number of complications have been described, including rupture into abdomino-thoracic structures, biliary fistula, vascular thrombosis, bilio-vascular compression, and secondary bacterial infection. Based on clinico-radiological findings, a classification system for ALA has emerged recently, which can assist clinicians in making treatment decisions. Recent research has revealed the role of venous thrombosis-related ischemia in the severity of ALA. Recent years have seen the development and refinement of newer molecular diagnostic techniques that can greatly aid in overcoming the diagnostic challenge in endemic area where serology-based tests have limited accuracy. Metronidazole has been the drug of choice for ALA patients for many years. However, concerns over the resistance and adverse effects necessitate the creation of new, safe, and potent antiamebic medications. Although the indication of the drainage of uncomplicated ALA has become more clear, high-quality randomised trials are still necessary for robust conclusions. Percutaneous drainage appears to be a viable option for patients with ruptured ALA and diffuse peritonitis, for whom surgery represents a significant risk of mortality. With

regard to all of the aforementioned issues, this article intends to present an updated review of ALA.

Key Words: Amebic liver abscess; Amebiasis; Ruptured liver abscess; Percutaneous drainage; Metronidazole

Core Tip: Amebic liver abscess (ALA) is the most prevalent type of liver abscess in the tropical world. It has many peculiar characteristics, such as non-suppurative lesion, strong male predisposition, association with alcohol consumption, predilection for the right liver lobe, and potential for healing without drainage. Differentiating it from a pyogenic liver abscess can be challenging in clinical practice. The role of a serological test is limited in the endemic regions where microbiological evidence often requires molecular tests. Metronidazole continues to be the preferred agent for ALA. However, there are some growing concerns regarding the resistance against this drug. Drainage is often not required for the treatment of uncomplicated ALA. In the case of complicated ALA, a recent paradigm shift has led to the preference for percutaneous treatment over surgery, which carries a high mortality risk.

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INTRODUCTION

Even 150 years after Friedrick Lösch made the initial discovery of *Entamoeba histolytica* (*EH*), this ancient protozoan still continues to pose a threat to public health in developing nations. There has not been a recent global assessment on the epidemiology of amebiasis, nevertheless, previous estimates suggest that amebiasis affects approximately 40 million people worldwide and kills up to 0.1 million people annually[1-3]. It is the second leading cause of parasite disease-related deaths. The Indian subcontinent, Africa, Mexico, Central and South America, and Africa are among the regions with high rates of amebiasis[3-5]. In developed countries, immigrants from endemic areas account for the majority of cases[6].

The major source of infection is water or food contaminated with quadrinucleate cysts of EH. The trophozoites released in the small intestine lumen colonize the cecum. Due to some unclear mechanisms, the parasite turns into a pathogen in around 10% of infected subjects, invades the intestine, and enters the liver through portal venous circulation. The virulent amebic trophozoites in the liver cause inflammation and necrosis leading to the formation of abscess[7]. An amoebic liver abscess (ALA) is the most common extraintestinal manifestation of invasive amebiasis and the commonest form of liver abscess in tropical areas[8]. It is not clear why ALA develops in a small proportion of patients with intestinal amebiasis. The complex interactions between the genetic polymorphism in EH influencing the virulence, the host immune system, and the surrounding environment, particularly gut flora, appear to play an important role in imparting such susceptibility[1,9-11]. While EH is thought to be the only cause of ALA, a recent study from South America has found DNA sequences of *E. dispar* from the liver abscess aspirate, raising doubts about its causal involvement[12]. Recent years have seen the emergence of many new insights into the clinic-epidemiological characteristics, diagnostic techniques, and changing management paradigms for patients with ALA. The purpose of this article is to provide an updated review of ALA, taking into account all the relevant issues. The data source for this review article included PubMed, Google Scholar, the Cochrane Library, and the cross-references from the searched publications. The relevant articles published between January 1980 and October 2023 were searched using appropriate keywords.

CLINICO-EPIDEMIOLOGICAL CHARACTERISTICS

In tropical areas, ALA is the most prevalent type of liver abscess. In the largest series of liver abscess patients reported from India, 81% of 1630 patients had ALA diagnosed by serological testing[8]. In another study that employed nested multiplex polymerase chain reaction (PCR) to identify specific DNA sequences of EH, 87% of patients with liver abscesses were found to have ALA[13]. On the other hand, ALA constitutes < 10% of all liver abscess patients in non-endemic regions[14].

Peculiar characteristics

Although an ALA is clinically indistinguishable from pyogenic liver abscess (PLA), certain peculiar characteristics make ALA stand out from other causes of liver abscess.

Lack of typical characteristics of an abscess: EH causes hepatic apoptosis and trogocytosis rather than a suppurative

infection of the liver. Thus, the aspirate of ALA does not have the typical characteristics of pus. It is a thick brown acellular debris that is odourless and almost always sterile[15,16]. Unlike PLA, which contains leukocytes and appears hot on nuclear scanning, ALA lacks leukocytes and appears as a cold lesion on technetium-99m liver scan[17]. The absence of neutrophils in ALA is mainly due to lysis of the protozoan. The majority of ALA heal without the need for a drainage procedure. Therefore, it seems inaccurate to refer to ALA as an "abscess."

Strong gender and age predilection: Despite a similar distribution of asymptomatic EH infection between the genders, the incidence of invasive amebiasis is much higher in males[18]. Males are 4-9 times more likely to have ALA than females[1,8,19]. This phenomenon has been observed even in travellers from non-endemic countries who acquire the disease[7]. Notably, such a marked gender difference does not exist among children. Males become more susceptible to ALA following puberty, with a peak incidence occurring around 40 years of age[20]. In a large study including over 2000 ALA patients, adult males between ages 30 and 50 years had the highest incidence of ALA[21]. This pronounced predilection for gender and age is thought to be due to the effect of testosterone and alcohol consumption[1,2,22,23]. Early cytokines production by natural killer T (NKT) cells, particularly Interferon (IFN) γ, plays a major role in EH invasion [24]. In response to hepatic samebiasis, gender-specific differences in cytokine production have been found in an animal study that revealed a lack of early IFN-γ response in male mice[23]. Furthermore, it was discovered that testosterone renders male mice more vulnerable to ALA by preventing NKT cells from secreting IFNγ[10]. In a study, immunoglobulin-G levels against EH were significantly higher in female asymptomatic carriers of EH than in corresponding male subjects. This could be one of the additional factors contributing to the lower incidence of ALA in the female population [25].

Predominant right lobar involvement: The EH trophozoites invade the cecum and travel to the liver *via* portal circulation. Because the right lobe of the liver receives portal blood mostly through the superior mesenteric vein, which drains the cecum, ALA more frequently affects this part of the liver. Such lobar predilection is not observed in the case of PLA where bacterial invasion can occur through venous, arterial, or biliary systems.

Strong association with alcohol consumption: Alcohol consumption increases the risk of developing ALA. A history of significant alcohol consumption is present in up to 85% of patients with ALA[1,26,27]. A number of studies from the Indian subcontinent have found a strong correlation between the incidence of ALA and the intake of local alcoholic beverages such as toddy[1,20,26-28]. Moreover, alcoholics have been found to have larger ALA, more complications, and a longer recovery period[27]. The precise mechanism of how alcohol confers the susceptibility to ALA is yet to be defined. Numerous indirect mechanisms, including changes in the microbiota of the gut, elevated gut permeability, elevated expression of alcohol dehydrogenase-2 on EH, decreased immunity, and elevated hepatic iron concentration, have been hypothesised[27,29]. The possibility of direct oral transmission has been ruled out by studies that failed to demonstrate EH cysts in alcoholic beverages[1,30].

Clinical presentation

Uncomplicated cases of ALA usually present with a short history of fever and abdominal pain in the right upper quadrant. Most patients experience symptoms two to four weeks after the exposure; however, in travellers, lag times have been recorded to range from 23 to 563 d[31,32]. At the time of diagnosis, less than one-third of the patients have diarrhea, only about 10% of patients have jaundice, and 5%-14% of cases have pulmonary symptoms like chest pain and shortness of breath. Recently, three distinct clinical presentations of ALA have been described according to the severity and duration of symptoms[22,32]. These include: (1) Acute aggressive ALA - characterized by acute onset of severe symptoms, systemic toxicity, markedly deranged laboratory parameters, and high risk of rupture requiring a drainage procedure; (2) subacute mild ALA - characterized by mild-to-moderate symptoms with onset within 2-4 wk, usually responding to the medical therapy; and (3) chronic indolent ALA - characterized by late presentation (> 4 wk) with mild persistent symptoms including pain in abdomen. Such patients have a well-formed wall with only a negligible risk of rupture; yet, drainage may be necessary to reduce pain. Patients with complicated ALA have variable presentations depending upon the nature and severity of complications. Most complications of ALA are related to its rupture into the adjacent structures, vascular thrombosis or compression, and secondary bacterial infection[22].

COMPLICATIONS OF ALA

Various complications resulting from ALA has been described in the literature (Figure 1). These complications can be broadly divided into two groups: those associated with ALA rupture and those unrelated to it.

Complications due to rupture of ALA

The incidence of ALA rupture varies from 6% to 40%[22]. In terms of the sites of rupture, the intraperitoneal site currently predominates over pleuropulmonary rupture (10%-24% *vs* 4.2%-7%)[33,34]. In a study by Priyadarshi *et al*[19], intraperitoneal rupture accounted for 83% of all 117 patients with ruptured ALA. A larger proportion (80%) of thoracic complications of ALA include sterile reactive pleural effusion rather than amebic empyema secondary to rupture[22,34]. Cardiac tamponade resulting from the rupture of the left lobe of ALA into the pericardium is one of the most serious complications of ALA[35,36]. Rupture of biliary ducts into the abscess cavity can result in biliary fistula, which is more common in large and centrally located ALA[22]. Although rupture of ALA into the hollow viscus leading to result in hepatic

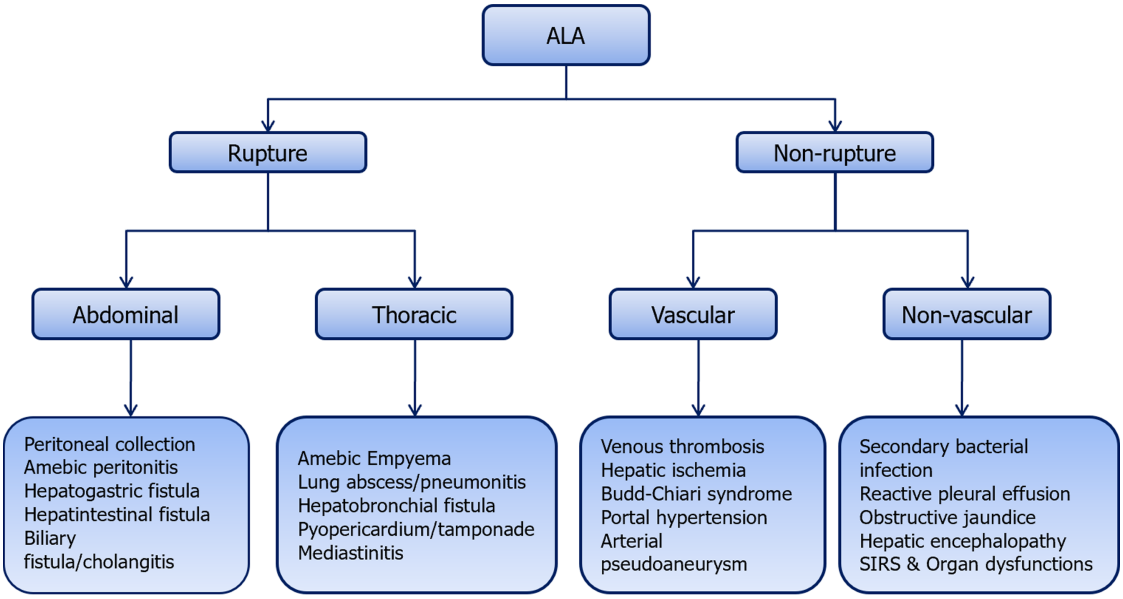

Figure 1 Complications of amebic liver abscess. The complications can be broadly divided into two groups: Those related to amebic liver abscess rupture and those that are not. The common complications included rupture into the adjacent structures, vascular thrombosis, and secondary bacterial infection. ALA: Amebic liver abscess; SIRS: Systemic inflammatory response syndrome.

fistulas (hepatobronchial, hepatogastric, hepatoenteric and hepatocolonic fistula) is rare, such complications are often innocuous because of the spontaneous drainage of the abscess through the fistula[37-39].

Despite the fact that the incidence of rupture has not decreased much over the years, mortality rates have improved dramatically. In 1994, Meng *et al*[40] reported a 22% incidence of rupture in 503 consecutive ALA patients with a mortality rate of 17%. In contrast, the incidence of rupture and mortality rate was 16% and 1.1%, respectively, in a large series of 1321 ALA patients published by Jindal *et al*[8] in 2021. Similarly, the mortality rate for 117 patients with ruptured ALA was merely 0.85% in a study published by Priyadarshi *et al*[19] in 2019.

Non-rupture complications

Thrombosis of major vessels, such as the portal vein, hepatic vein, and inferior vena cava, has been reported in patients with ALA[41,42]. In an autopsy series, Krishnan *et al*[42] have reported IVC thrombosis in 8% of the 95 ALA patients, and all but one also had concomitant hepatic vein thrombosis. Nevertheless, new information obtained with a sensitive multidetector CT scan suggests that thrombosis of smaller branches of the portal and hepatic veins is quite prevalent in ALA patients. When the segmental and subsegmental branches of the hepatic and portal veins were combined, a recent study found that 69% of ALA patients had venous thrombosis. In addition, 53% patients, who had severe clinical course or ruptured ALA, showed a zone of perilesional ischemia[43]. An intracavitary hepatic artery pseudoaneurysm has also been reported in patients with ALA[44]. Moreover, ALA can present as reversible portal hypertension due to the compression of the portal vein and as Budd-Chiari syndrome due to the obstruction of the hepatic veins[45,46]. A large ALA located in the caudate lobe can cause compression of the vena cava, resulting in pedal edema. Obstructive jaundice may occur from an ALA close to the porta hepatis[47]. Jaundice in ALA can also result from a biliovascular fistula created due to the simultaneous injury to the bile ducts and hepatic veins. Interestingly, jaundice in such patients improves after biliary diversion with nasobiliary drainage[48]. Even though ALA is usually considered to be bacteriologically sterile, up to 20% of patients may develop a secondary bacterial infection, which could complicate the disease course[22]. In a recent study, the aerobic bacterial culture positivity rate of ALA aspirate was only about 5%, however, 37% of aspirate revealed molecular evidence of various anaerobes of gut microbiota, such as Fusobacterium, Peptococcus, and Prevotella[13]. These anaerobes are likely to be translocated from the gut and could be crucial in granting virulence to EH. The other rare complications of ALA include hepatic encephalopathy and acute respiratory distress syndrome[49,50].

Factors associated with rupture

The risk of ruptures is high for ALAs in the subcapsular location, left lobe, or caudate lobe[51,52]. Due to the lesser bulk and larger area beneath the left hemidiaphragm, the left lobe ALA is at a higher risk of rupture[51]. Thin, immature, incomplete, and ragged ALA walls are also important risk factors for its rupture[22,32]. Jha *et al*[33] reported that older age, chronic alcoholism, hyperbilirubinemia, hypoalbuminemia, leucocytosis, and hyponatremia were the important predictors of ALA complications, which largely (88%) included rupture. Other factors, such as strain virulence and host immunity may contribute to the risk of rupture, however, there is currently little evidence to support this assumption. A recent study found an increased incidence (53%) of intraperitoneal rupture of ALA in COVID-19-recovered patients. This was presumed to be due to alteration in the immune state of these patients[53]. ALA with concomitant ileocolonic ulceration is associated with a high risk of rupture, which might be due to an infection with a more virulent strain of EH [54].

DIAGNOSTIC ISSUES AND ADVANCES

For liver abscess *per se*, ultrasonography is the preferable initial test. It is a rapid, widely available, inexpensive and sensitive test for detection of an abscess. Computed tomography (CT) scan is usually required for complicated ALA patients (Figure 2). Recently, Priyadarshi *et al*[32] have described three distinct morphological patterns of ALA on CT scans with clinical correlation. Type I ALA, the clinically aggressive type, had absent or incomplete wall and ragged edges with irregular enhancement, Type II ALA had a complete rim enhancement and peripheral hypodense halo and was clinically less aggressive, and Type III ALA, the least common type, demonstrated a non-enhancing wall and a chronic indolent course (Figure 3). Patients who have bleeding symptoms from ileocolonic ulcerations or ruptured ALA into the gastrointestinal tract may require endoscopic evaluation (Figure 4).

More importantly, there is no clinical or imaging characteristic that can accurately diagnose ALA or distinguish it from a PLA. Such differentiation is crucial in order to prevent a delay in the implementation of appropriate therapy. Determination of an accurate diagnosis of ALA relies mainly on laboratory tests that use immunological, molecular, and parasitological methods to confirm EH in different specimens[55].

Stool examination

The utility of microscopic stool testing for cysts is limited because only 10%-40% of ALA patients have concurrent intestinal amebiasis, and EH cysts are morphologically identical to nonpathogenic strains of Entamoeba such as *E. dispar* and *E. Moshkovskii*[56]. Stool antigen assays based on enzyme immunoassay or PCR are highly sensitive in detecting EH and distinguishing it from nonpathogenic strains; however, they are neither widely available nor well standardised[57, 58]. Moreover, as EH trophozoites are only viable for a few minutes, fresh stool specimens must be examined for both stool microscopy and antigen testing.

Serum-based tests: The enzyme-linked immuno sorbent assay (ELISA) and hemagglutination assay are commonly used in conjunction with clinico-radiological to diagnose ALA[59-61]. Anti-amebic antibodies can be detected in about 95% of cases of ALA. Recently, ELISA, targeting the IgG1 subclass antibody to EH exhibited 100% sensitivity and 99.1% specificity in patients with ALA[62]. However, anti-amebic antibodies become detectable in serum only 5-7 d after infection and continue to exist for 6-12 months after the infection has been eradicated. As a result, they might be false negatives during the first week of illness and might not be useful for those living in highly endemic areas. Therefore, the current applications of serological testing are mostly limited to sero-epidemiological research and the diagnosis of ALA in travellers from endemic locations. Detection of circulating antigen or DNA of EH can be helpful tools for the diagnosis of acute infection and follow-up after therapy in endemic regions. Recently, circulating Gal/GalNAc lectin of EH was detected in the serum of ALA patients with high sensitivity (96%) prior to anti-amebic therapy[63]. Also, PCR can detect circulating DNA of EH in the serum of ALA patients with 89.5% sensitivity and 100% specificity[64]. However, the expense is a barrier to their regular usage in the poorly resourced endemic nations.

ALA aspirate-based tests: ALA aspirate appears as a thick, brown, odourless fluid that contains acellular proteinaceous debris and resembles an anchovy sauce. ALA is typically regarded as sterile lesion unless there is a secondary bacterial infection. Nonetheless, various anaerobes of gut microbiota, such as *Fusobacterium*, *Peptococcus* and *Prevotella*, have been found in the 37% of aspirate[13]. Demonstration of amebic trophozoites in the pus can be confirmatory for diagnosis, however, this is only observed in a small proportion of aspirates (7.2%-25%) and only when the cyst wall is sampled[65, 66]. In a study, amebic antigen was detected by ELISA in 97.6% of pus specimens from ALA patients[67]. However, the sample must be obtained before the beginning of the anti-amebic treatment, which often causes rapid loss of antigen.

Recently, a simple and efficient technique for DNA extraction has been developed. Consequently, molecular diagnostic assays based on PCR have emerged as the diagnostic gold standard[68,69]. Numerous commercial PCR assays, including conventional PCR, real-time PCR, nested PCR, and multiplex PCR have been designed to identify amebic DNA from both pus and stool samples. It has been shown in numerous studies that PCR has very high sensitivity (84%-100%) and specificity (100%) for detecting EH-DNA from ALA aspirate[70,71]. In a comparison study, the sensitivity of real-time PCR in detecting EH in ALA aspirate was significantly higher than that of an ELISA-based antigen detection test (97% *vs* 40%)[72]. It should be noted that the sensitivity of PCR for detecting EH-DNA reduces after initiation of anti-amebic treatment; hence, the test sample should be aspirated before initiation of therapy[66]. Currently, no commercial rapid diagnostic test is available to diagnose ALA. Nonetheless, a preliminary investigation has found that the IgG4-based rapid dipstick test, which detects anti-EH pyruvate phosphate dikinase antibody, has an excellent diagnostic performance for the rapid identification of ALA[73].

To summarize, serological testing in association with clinico-radiological features is still relied upon to diagnose ALA in most of the endemic regions due to the high cost and unavailability of molecular diagnostic tests. Since serological tests can be fallacious in early stages of disease and in endemic locations, efforts should be undertaken to develop a molecular diagnostic test that is rapid, sensitive, specific, and inexpensive so that it may be used in low-resource countries.

EVIDENCE-BASED MANAGEMENT AND CHANGING TREATMENT PARADIGM

Tissue amebicides form the mainstay of management of all patients with ALA. The need for interventional treatment is determined by a number of variables, including the abscess characteristics, persistence of symptoms, and existence of complications. Figure 5 shows the proposed treatment algorithm for ALA.

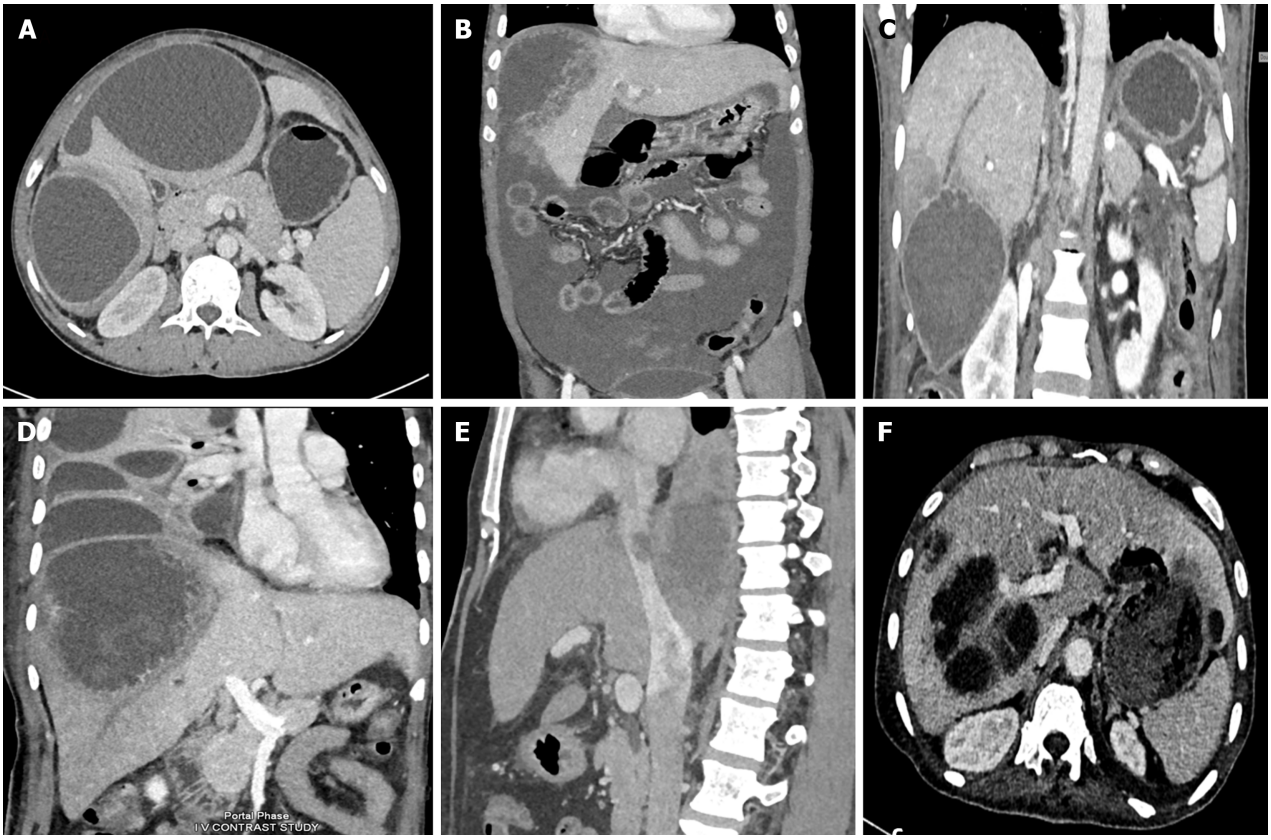

Figure 2 Computed tomographic scans showing various spectrum of complicated amebic liver abscess. A: Axial computed tomography (CT) scan showing a contained rupture of an abscess with localized fluid collection in the perihepatic area. There is a thin enhancing rim around the abscess, characteristic of a type II amebic liver abscess (ALA); B: Coronal CT scan of a 54-year-old male showing rupture of the abscess with intraperitoneal fluid collection diffusely spreading throughout the entire peritoneal cavity, indicative of free abscess rupture; C: Coronal contrast-enhanced CT image showing an amebic abscess located in segment VI with long-segment thrombosis in the peripheral branch of the right hepatic vein. Note the triangular hypodense area surrounding the abscess, indicating hypoperfused parenchyma; D: Coronal contrast-enhanced CT image showing a large amebic abscess that has ruptured into the thoracic cavity with loculated pleural fluid collections. Note that the abscess demonstrates ragged edges with indistinct enhancement, characteristic of a type I abscess; E: Sagittal contrast-enhanced venous phase CT image of a patient showing thrombus extending from the ALA into the inferior vena cava; F: Axial CT scan showing a communication of left lobe ALA with the gastric lumen forming a hepatogastric fistula.

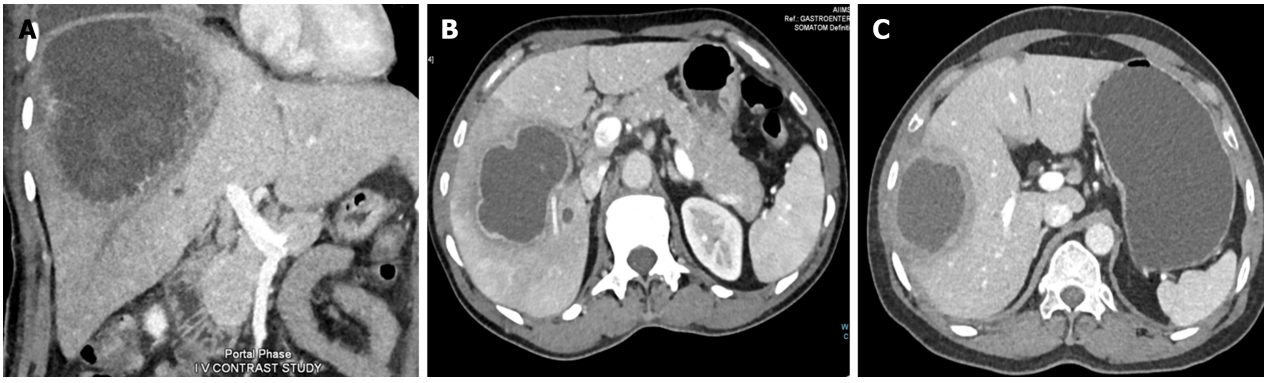

Figure 3 Computed tomographic classification of amebic liver abscess. A: Type I amebic liver abscess (ALA) shows incomplete wall and ragged edges with irregular enhancement; B: Type II ALA showing a complete rim enhancement with peripheral hypodense halo; C: Type III ALA showing a chronic non-enhancing wall.

Management of uncomplicated ALA

Two prospective studies that assessed the outcome of conservative therapy in uncomplicated ALA found that the majority of patients could be managed with an antiamebic drug alone[74,75]. After receiving pharmacotherapy for 72 h, only 13% of patients in one study and 18% of patients in another study needed drainage. Size of ALA (> 7.7 cm and 10.7 cm, respectively) was the most significant factor determining radiological intervention in both studies. Thus, a conservative strategy should be adopted for most patients with uncomplicated ALA.

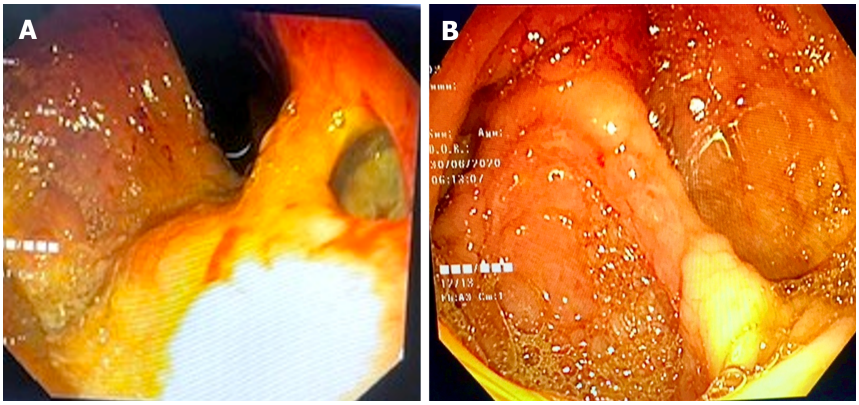

Figure 4 Endoscopic images of complicated amebic liver abscess patients. A: Shows an upper endoscopic view of hepatogastric fistula resulting from rupture of a left lobe amebic liver abscess (ALA) into the stomach near high lesser curve; B: Shows colonoscopic view of a complicated ALA patient presenting with lower gastrointestinal bleeding from multiple small ulcers from the cecum.

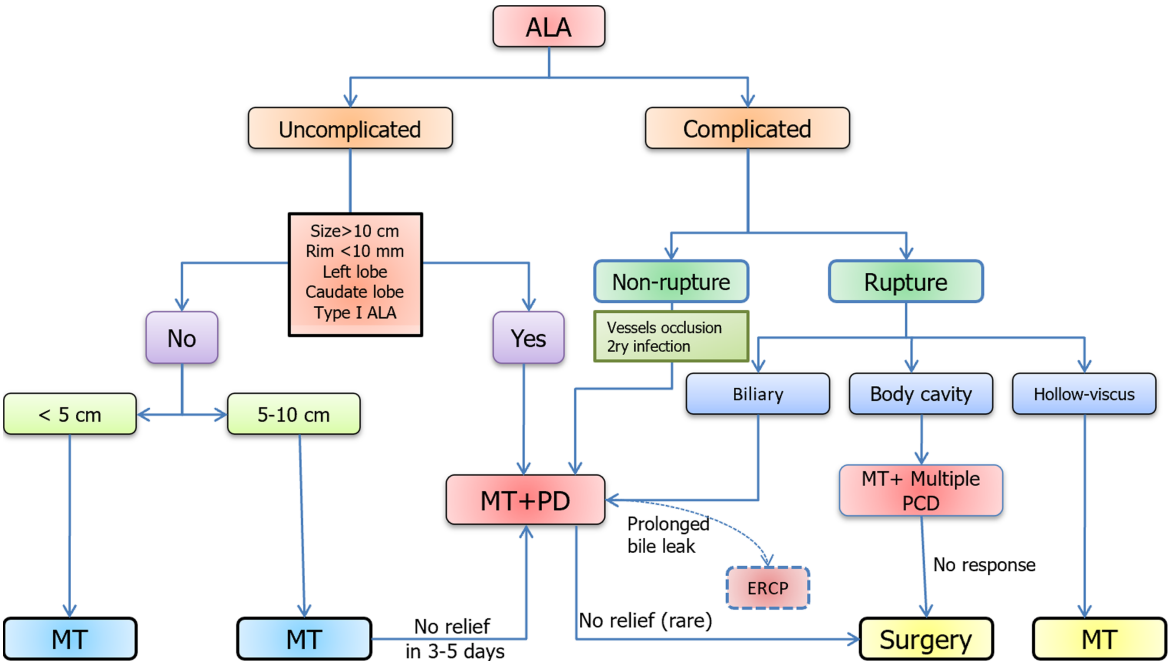

Figure 5 Proposed treatment algorithm for patient with amebic liver abscess. For uncomplicated amebic liver abscess (ALA): Upfront percutaneous drainage (PD) should be considered only in the presence of high risk signs; PD doesn't provide added benefit when ALA size is < 5 cm, and ALA with size > 5 should be treated initially with medical therapy (MT) consisting of anti-amebic drug for 3-5 d before considering PD in case of non-response. For PD, a percutaneous catheter drainage (PCD) is preferred over needle aspiration, particularly for larger and incompletely liquified ALA. For complicated ALA patients, some form of drainage procedure is always required. A large majority of such patients can be treated with PCD along with MT. ALA with biliary fistula can be treated with prolonged PCD, and only on rare occasion, an endoscopic retrograde cholangiopancreatography will be required. Finally, ALA with rupture into a hollow viscus can be treated with MT alone. MT: Medical therapy; PD: Percutaneous drainage; ALA: Amebic liver abscess; PCD: Percutaneous catheter drainage; ERCP: Endoscopic retrograde cholangiopancreatography.

Drug of choice

Metronidazole (MTZ), the most widely used nitroimidazole, is the cornerstone of treatment for uncomplicated ALA for more than four decades. MTZ has a good hepatic penetration, and when used at a dose of 500 mg to 750 mg three times a day for seven to ten days, it resolves symptoms within 72 h of treatment[76]. Since the parasites can linger in the colon, MTZ treatment should be followed with a luminal agent, such as paromomycin (500 mg 3 times a day for 7 d) or diloxanide furoate (500 mg three times a day for 20 d). The failure to take luminal medicines can result in relapse of infection in about 10% patients[77].

Emerging concerns with MTZ

Even though resistance to MTZ is uncommon, *in vitro* studies and sporadic reports of treatment failures and relapse indicate emergence of resistance to this drug[78,79]. The inhibitory concentration of nitroimidazoles against EH was found to be rising in a recent study from India[80]. The nim gene-encoded nitroimidazole reductase enzyme is commonly

associated with resistance to MTZ[81]. In a recent study, 22.2% of recurrent ALA patients revealed the presence of *nimE* gene[77]. Other issues related to MTZ include adverse symptoms such nausea, vomiting, disorientation, metallic taste, and peripheral neuropathy. Concerns have also been raised about its carcinogenic potential in animals and mutagenic potential in bacteria such as *H. pylori* and *E. coli*[82,83]. Moreover, MTZ is not very effective in treating asymptomatic intestinal amebiasis.

Alternative anti-amebic drugs

Although there is limited clinical experience, other nitroimidazole compounds that have been used in the treatment of ALA include tinidazole, ornidazole, and nitazoxanide. Both ornidazole and tinidazole have better tolerance and longer half-lives, making them suitable for a shorter course of treatment (3-5 d)[84]. In a small study, 2 g tinidazole once daily for 2 d resulted in completed recovery of ALA in all (n = 10) subjects[85]. Nitazoxanide has emerged as an effective agent against a variety of parasite infections. An in-vitro study has found it to be more active than metronidazole against amebiasis[86]. It also has the advantage of being a tissue as well as luminal amebicide. In a recent randomized controlled trial (RCT), nitazoxanide, at 500 mg twice daily for 10 d, was found more tolerable and as effective as MTZ in uncomplicated ALA patients[87]. However, clinical response was faster with MTZ compared to nitazoxanide.

Emerging anti-amebic molecules

Apart from nitroimidazole compounds, there has not been much progress made in the creation of alternative anti-amebic drugs thus far. Riluzole, a benzothiazole derivative, and andrographolide, a repurposing drug, have recently demonstrated strong anti-amoebic action against EH[80,88]. Apocynin, an NADPH oxidase enzyme inhibitor, was found to prevent ALA in animals[89]. Proton pump inhibitor, which has a benzimidazole nucleus resembling MTZ, was recently found to inhibit thioredoxin reductase of EH, an enzyme essential to the pathogen's virulence and survival[90,91]. Moreover, the inhibitory concentration of Rabeprazole and Pantoprazole was found to be much lesser than MTZ[90]. However, the utility of these newer and repurposing medications needs to be investigated in ALA patients in further studies.

Drainage of uncomplicated ALA

The role of upfront drainage of uncomplicated ALA is controversial. The results of multiple RCTs have produced mixed conclusions (Table 1). While some RCTs have reported no added benefits of percutaneous needle aspiration (PNA)[92, 93], others found minor to significant improvements in certain outcome parameters[94-100]. In a recent systematic review and meta-analysis of 570 ALA patients, the addition of PNA to MTZ therapy produced additional benefits in terms of early resolution of abdominal pain and tenderness in patients with ALA of size > 5 cm. However, there was no discernible effect on the fever, abscess size, and length of hospital stay[101]. Additionally, patients with small ALAs (< 5 cm) did not show any benefit after draining. It is noteworthy that most of the included studies employed PNA as a drainage technique, rather than the more effective percutaneous catheter drainage (PCD), making it difficult to draw firm conclusions on the efficacy of adjunct drainage.

Approximately 15% of uncomplicated ALA patients do not respond well to MTZ alone, necessitating a drainage procedure. Therefore, percutaneous drainage should be considered for patients who fail to respond within 3 to 5 d of medical therapy. This is the most common real-life scenario where drainage is needed irrespective of the imaging findings. Other indications that warrant consideration of early drainage are: (1) ALA in the left lobe or caudate lobe; (2) a thin rim (< 1 cm) of hepatic parenchyma; (3) Type I ALA, lack of mature wall or signs of impending rupture on imaging; (4) secondary bacterial infection; and (5) an unclear diagnosis between ALA and PLA[22,32,76,97].

Regarding the mode of drainage, PCD is frequently chosen over PNA. Multiple sessions are generally necessary for PNA to effectively drain the abscess cavity. However, PNA may be considered for draining completely liquefied multiple smaller abscesses. For draining larger ALAs, two RCTs have found PCD to be better than PNA[102,103]. Gupta *et al*[103] observed a higher success rate, faster clinical relief, and shorter duration of parenteral antibiotics with PCD *vs* PNA in 82 large (> 10 cm) ALA patients. Similarly, Jha *et al*[102] have observed a higher success rate, shorter hospital stays, and a faster abscess resolution time with PCD compared to PNA in patients with ALA > 5 cm.

Management of complicated ALA

All ruptured ALAs require urgent drainage, with the exception of those that rupture into the hollow viscus. Pleuropulmonary ruptures are successfully treated with PCD[19,22]. In the case of ALA with intraperitoneal rupture, surgical drainage is traditionally recommended[104,105]. Nonetheless, due to advancements in the PCD technique and growing data on its favourable result, there has been a paradigm shift over the past two decades from surgical drainage to catheter drainage for the management of most ruptured ALAs[16,19]. Currently, ultrasound-guided PCD is considered the standard of care for ALA with contained intraperitoneal rupture and localised peritonitis[19,22]. Still, when ALA rupture is associated with diffuse peritonitis, a real therapeutic challenge arises. Surgery is often recommended in such circumstances; nevertheless, it should be emphasized that the surgical mortality risk is high for this patient group due to the systemic toxaemia, hypoalbuminemia, and malnourishment[16]. Several studies show that for patients with amoebic peritonitis, non-surgical treatment is associated with significantly better outcomes than surgical treatment (Table 2)[104-109]. Most of the contents of ruptured ALA are sterile acellular debris, which can be easily drained by interventional radiologists. In a large series of 117 patients with ruptured ALA, Priyadarshi *et al*[19] have shown that they can be effectively treated with ultrasound-guided PCD. Compared to patients with controlled rupture, those with free rupture needed more catheterizations and a longer hospital stay. Notably, despite diffuse peritoneal spread in 27% of patients and complex septations, PCD was able to treat them with a 97% success rate[19]. In various other studies on diffuse amebic

Table 1 Randomised studies comparing treatment of amebic liver abscess patients with or without percutaneous aspiration

Ref./country	Treatment groups (*n*)	ALA size/volume (cm/mL)	Normalization of fever	Reduction of pain and/or local tenderness	Resolution in abscess size	TLC decline	Length of hospital stay
Sharma *et al*[98], India	MTZ (n = 20) and MTZ + PD (n = 19)	7.2 ± 0.20 cm and 5.4 ± 2.2 cm	Similar on day (84 *vs* 94%)	Similar on day 10 (67% *vs* 58%)	No significant change at day 10	Similar between the groups	NA
Blessmann *et al* [99], Germany	MTZ (n = 19) and MTZ + PD (n = 20)	169 ± 90 mL and 161 ± 49 mL	100% at day 6 in both groups	Significantly better at day 3 in MTZ + PD group: (60% *vs* 5%, P = 0.001)	Greater reduction in MTZ + PD at day 10 (109 mL *vs* 64 mL, P = 0.02)	Similar between groups	NA
Van Allan *et al* [94], United States	MTZ (n = 21) and MTZ + PD (n = 20)	8.5 ± 3.5 cm and 7.5 ± 2.4 cm	Similar between groups (3 d *vs* 4 d, P = 0.5)	Early reduction in MTZ + PD group (3 d *vs* 1 d, P = 0.05)	NA	NA	Similar between groups (5 d *vs* 6.2 d, P = 0.19)
Ghosh *et al*[95], India	MTZ (n = 98) and MTZ + PD (n = 96)	6.8 ± 2.6 cm and 6.8 ± 2.6 cm	Better at day 8 in MTZ + PD group (P < 0.001)	Significantly better at day 3 in MTZ + PD group (P = 0.03)	Greater reduction in MTZ + PD (P = 0.003)	Better in MTZ + PD at day 8 (P < 0.001)	NA
Freeman *et al* [93], Nigeria	MTZ (n = 17) and MTZ + PD (n = 19)	1.5 to 14.5 cm and 1.5 to 14.5 cm	Similar between groups at week-3 (19/19 *vs* 16/17)	Similar between groups at week-3	Greater reduction in MTZ + PD when ALA > 6 cm (P = 0.005)	NA	NA
Bammigatti *et al* [100], India	MTZ (n = 29) and MTZ + PD (n = 28)	148.4 ± 103 mL and 211.5 ± 119 mL	Similar between groups (30 h *vs* 17 h, P = 0.48)	Median time similar between groups (48 h *vs* 27 h, P = 0.16)	NA	Shorter in MTZ + PD group (2.4 d *vs* 3.7 d, P = 0.05)	Similar between groups (4.5 d *vs* 4.4 d, P = 0.62)
Widjaya *et al* [96], Indonesia	MTZ (n = 16) and MTZ + PD (n = 17)	NA	NA	NA	Shorter duration of abscess resolution in M + PA group	NA	NA
Tandon *et al* [92], India	MTZ (n = 14) and MTZ + PD (n = 15)	> 5 cm	Better in MTZ + PD group (5.6 d *vs* 3.8 d; P < 0.05)	Better in MTZ + PD group (P < 0.001)	NA	Similar between groups	Shorter in MTZ + PD group (7.4 *vs* 5.8, P < 0.001)
de la Rey Nel *et al*[97], South Africa	MTZ (n = 43) and MTZ + PD (n = 37)	NA	Similar between groups (P > 0.05)	Better in MTZ + PD group (6.9 ± 2.3 *vs* 4.5 ± 2.2, P < 0.05)	No significant difference between groups	NA	NA

ALA: Amebic liver abscess; PD: Percutaneous drainage; MTZ: Metronidazole; TLC: Total leukocyte count; NA: Not available (data).

Table 2 Mortality rates of surgical versus non-surgical therapy in patients with amebic liver abscess with diffuse peritoneal rupture

Ref.	*n*	Medical therapy (*n*)	Surgery (*n*)	Medical therapy[1]	Surgery
				Mortality (%)	Mortality (%)
Eggleston *et al*[104]	19	0	19	-	42
Memon *et al*[106]	36	20	16	5	37.5
Sarda *et al*[107]	16	8	8	0	50
Meng *et al*[40]	11	1	10	0	50
Baijal *et al*[108]	02	2	-	0	-
Bhatia *et al*[109]	43	43	0	-	26
Priyadarshi *et al*[19]	32	32	0	0.3	-
Greaney *et al*[105][2]	08	3	5	0	20

[1]Medical therapy included anti-amebic treatment along with percutaneous catheter drainage, with exception of one study.
[2]In which percutaneous drainage was not performed.

peritonitis, the mortality rate following surgical drainage ranged from 26%-30%, whereas it was only 0%-5% following ultrasound-guided PCD therapy (Table 2).

PCD treatment is also effective in the management of ALA with biliary communications. In a study, prolonged catheter drainage (12 to 50 d) was found to be an effective treatment for all ALA patients who had intrabiliary communication, and neither biliary sphincterotomy nor stenting was necessary[110]. Many vascular complications of ALA, including venous thrombosis and arterial pseudoaneurysm have shown to improve with PCD treatment[43,44,111]. Finally, ALA rupturing into hollow viscus such as stomach, bronchus, and intestine can be managed conservatively with antibiotics alone, as fistula itself provides natural drainage in such patients[38,39].

Role of surgery

The role of surgery in the treatment of ALA patients has drastically decreased lately[16,19,22]. Surgical intervention is taken into consideration only in cases when radiological intervention has failed or is difficult due to a challenging location or multiloculation. Whenever possible, a laparoscopic drainage should be preferred over the open surgery. Laparoscopic surgical drainage provides better cosmetics, a quicker recovery, a shorter hospital stay, fewer surgical site infections, and much lesser mortality risk[112]. ALA in the caudate lobe of the liver is often considered a challenging location for percutaneous drainage due to its proximity to major vessels, and a surgical drainage is often recommended for this location. However, in a recent study, 30 cases of caudate lobe ALA were managed with percutaneous interventions (PCD or PNA) with technical and clinical success rates of 100% and 96.7%, respectively[52]. In a recent systematic review and meta-analysis, in which data of 299 Liver abscess patients undergoing laparoscopic drainage were analysed, there were no reported deaths, but the post-operative rate of recurrence or residual liver abscess was 4.2%[112]. However, liver abscess patients included in that study had mixed etiology (both ALA and PLA), and the indication for laparoscopic drainage was quiet variable.

Post-treatment recurrence of ALA

In a recent 2-year follow-up study, recurrent ALA was noted in 9 (8.9%) of 101 ALA patients[77]. Large abscess sizes (> 10 cm), the presence of bacterial flora (Prevotella), the presence of resistance genes (nim), EH genotypes, and elevated levels of matrix metalloproteinase were all significantly associated with the recurrence. The genotype of EH was identical to that of the primary ALA in majority (78%) of recurrent ALA patients, and only two patients (22%) had infection with new genotype[77]. In a similar 2-year follow-up study from Bangladesh, post-treatment recurrence rate for ALA was 6.7%[28]. Even travellers from non-endemic nations who have not returned to endemic regions have been known to experience recurrent ALA[113]. Inadequate anti-amebic treatment, drug resistance, failure to use a luminal agent, continued alcohol usage, or immunological suppression can also result in the recurrence of ALA[114].

CONCLUSION

ALA is the most prevalent type of liver abscess in the tropical world. It has many peculiar characteristics, such as non-suppurative lesion, strong male predisposition, association with alcohol consumption, predilection for the right liver lobe, and potential for healing without drainage. Differentiating it from a pyogenic liver abscess can be challenging in the clinical practise. Role of serological test is limited in the endemic regions where microbiological evidence often requires molecular tests. Recent years have seen the development and refinement of newer molecular diagnostic techniques; however, high cost and availability remains an issue. Therefore, effort should be made to develop a molecular diagnostic test that is not only rapid, sensitive, and specific, but also affordable so that it can be implemented in poorly resourced countries. A clinico-radiological classification system has emerged during the recent times, which can assist clinicians in making treatment decisions. MTZ continues to be the preferred anti-amebic medication for ALA. However, it is necessary to investigate the alternative and newer medications in light of some growing concerns regarding the MTZ resistance. Unless high-risk features are present, an upfront percutaneous drainage should be avoided in patients with uncomplicated ALA, as majority of such patients can be treated successfully with anti-emebic drug alone. Nevertheless, further studies are needed to determine which patients with uncomplicated ALA could benefit from early drainage. When an ALA ruptures, upfront percutaneous drainage should be taken into consideration, unless the rupture has happened into a hollow viscus. When it comes to draining larger ALAs, PCD is better than PNA. In patients with ruptured ALA with diffuse peritonitis, surgery carries a high risk of mortality, nevertheless, evidence suggests that even such patients can be managed with percutaneous drainage with very low mortality risk. Direct comparison studies between laparoscopic surgery and percutaneous therapy can provide additional insight into the therapeutic modalities that should be chosen by clinicians for such patients.

FOOTNOTES

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MINIREVIEWS

Advances in discovery of novel investigational agents for functional cure of chronic hepatitis B: A comprehensive review of phases II and III therapeutic agents

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Abstract

Chronic hepatitis B virus (HBV) infection affects over 295 million people globally and an estimated 1.6 million people in the United States. It is associated with significant morbidity and mortality due to cirrhosis, liver failure, and liver cancer. Antiviral therapy with oral nucleos(t)ide analogues is associated with high rates of virologic suppression, which in turn has been associated with a decreased risk of liver complications. However, current antiviral regimens are limited by concerns with adverse effects, adherence, resistance, long-term treatment, and ongoing risk for liver events. Novel investigational agents are currently in development and are targeted at achieving functional cure with sustained hepatitis B surface antigen (HBsAg) loss and suppression of HBV DNA. Herein we review key evidence from phases II and III trials defining the efficacy and safety profiles for key investigational agents for functional cure of chronic hepatitis B, including core/capsid inhibitors, entry inhibitors, RNA interference (siRNA/ASO), HBsAg inhibitors, Toll-like receptor agonists, checkpoint inhibitors, and therapeutic vaccines.

Key Words: Hepatitis B virus; Treatment; Clinical trials; RNA interference; Entry inhibitors; Core inhibitors; Immunomodulators

Core Tip: Novel investigational agents targeting functional cure [sustained hepatitis B surface antigen (HBsAg) loss and undetectable hepatitis B virus (HBV) DNA] are currently in clinical trial development. Herein we review key evidence from phases 2 and 3 trials defining the efficacy and safety profiles for key investigational agents, including core/capsid inhibitors, entry inhibitors, RNA interference (siRNA/ASO), HBsAg inhibitors, Toll-like receptor agonists, checkpoint inhibitors, and therapeutic vaccines.

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INTRODUCTION

Chronic hepatitis B virus (HBV) infection is a global health problem with more than 295 million people infected worldwide and as many as 1.6 million people infected in the United States[1,2]. Complications of chronic HBV infection, including cirrhosis, hepatocellular carcinoma (HCC), and liver failure, may take years to develop and have led to more than 800000 deaths each year[3-5].

While the ideal goal of HBV therapy would be a complete sterilizing cure, such a therapy does not exist because it is difficult to directly target the covalently closed circular DNA (cccDNA) in the hepatocyte nucleus and the integration of HBV DNA into the host genome. The best that can be achieved with current therapies is a functional cure where there is loss of HBV surface antigen (HBsAg) with undetectable HBV DNA after 6 months off therapy. This is an important endpoint given its association with reduced liver necroinflammation, reduced risk of HCC, increased liver fibrosis regression, normalization of alanine aminotransferase (ALT) levels, reduced risk of liver cirrhosis, and increased survival [6-11].

Current FDA-approved therapies include pegylated interferons (PEGIFNα) and nucleos(t)ide analogues (NA)[12,13]. PEGIFNα are administered as subcutaneous injections on a once weekly dosing schedule for one year. They exert antiviral and immunomodulatory activities by enhancing cccDNA degradation and modifying cccDNA transcription. PEGIFNα therapy has higher rates of HBsAg loss and HBV e-antigen (HBeAg) seroconversion than NA, but are associated with poor tolerability and risk for depression[14,15]. In contrast, NA are administered orally every day. They suppress HBV replication by causing chain termination when incorporated into HBV DNA undergoing reverse transcription. Early NA such as Lamivudine and Adefovir had high rates of antiviral resistance with only a few years of treatment[16]. NA currently used in clinical practice, namely, Tenofovir disoproxil fumarate (TDF) and Entecavir (ETV), have potent antiviral activity and a high barrier to resistance[17]. Compared to PEGIFNα therapy, NA are well-tolerated, but require a long term duration of maintenance therapy[18]. Both PEG-IFNα and NA therapies are unable to eliminate the HBV because they do not directly target cccDNA and integrated HBV DNA. Consequently, cccDNA persists, which enables transcription of RNA and translation of viral HBV proteins, such as HBsAg, to continue[19,20].

Rates of a functional cure with PEGIFNα and NA therapies are low. With PEGIFNα treatment, HBsAg loss has only been reported in approximately 7% of both HBeAg positive and negative patients after a year of treatment[21]. HBsAg loss is even lower in patients receiving NA, with only 0.3%-5% of HBeAg negative patients and 1.4% of HBeAg positive patients achieving HBsAg loss after treatment for 5-7 years[22,23]. Given the limitations of existing HBV therapies, there is great interest in novel HBV therapeutics that can lead to the following outcomes: Functional cure, improvement in quality of life, and preventing progression of chronic HBV infection to cirrhosis, HCC, and HBV-related mortality. Herein, this review will focus on novel HBV therapies in active phases II and III clinical trial development.

METHODOLOGY

This paper is a narrative review. Investigational agents for treatment of chronic HBV infection under active phases II and III development were identified using the National Institutes of Health Clinical Trials directory[24]. This directory includes details regarding the study design, population, treatment arms, and sponsoring pharmaceutical company for all publicly supported clinical studies. Information from this website was incorporated in the development of Table 1, which summarizes information about investigational agents in phases II and III trials without published study results. A PubMed search was conducted for each investigational agent under active phases II and II development. Data was retrieved from published original research articles and conference abstracts. The sponsoring pharmaceutical company website for each investigational agent was reviewed for published presentation slides from international liver meetings.

FUTURE THERAPY FOR HEPATITIS B

A summary of the novel therapies in phases II and III development with study data are listed in Table 2, while therapies

Table 1 Novel therapeutic agents for treatment of chronic hepatitis B infection in phase II or III development

Drug name (therapeutic class)	Drug sponsor	Phase
Core/capsid inhibitors		
JNJ56136379 (JNJ-6379)	Janssen Pharmaceutics	II
ABI-H0731 (Vebicorvir)	Assembly Biosciences	II
Entry inhibitors		
Bulevirtide (Hepcludex, formerly Myrcludex)	Gilead Sciences	III
Small interfering RNA		
GSK3228836 (Bepirovirsen)	Ionis Pharmaceuticals	III
VIR-2218	Vir Biotechnology	II
VIR-3314	Vir Biotechnology	II
JNJ-73753989 (JNJ-3989, formerly ARO-HBV)	GlaxoSmithKline Pharmaceuticals	II
Arbutus-729 (AB-729 or Imdusiran)	Arbutus Biopharma	II
HBsAg inhibition		
REP 2139/REP 2165	Replicor	II
Toll-like receptor agonists		
GS-9620 (Vesatolimod)	Gilead Sciences	II
GS-9688 (Selgantolimod)	Gilead Sciences	II
Therapeutic vaccines		
GS-4774	Gilead Sciences	II
BRII-179	Brii Biosciences	II

Table 2 Summary of novel investigational agents in phase II trials for treatment of chronic hepatitis B infection

Drug name (therapeutic class)	Drug sponsor	Phase	Trial ID	Study design	Study population	Sample size	Intervention and control	Primary outcome
RG6346 (siRNA); *RO7020531* (TLR-7 agonist)	Hoffman-La Roche	II	NCT04225715	Randomized, open-label, parallel assignment	Chronic HBV infection patients on established NA monotherapy for ≥ 12 months, HBV DNA < 20 IU/mL, ALT ≤ 1.5 ULN	280	Control arm: NA; Experimental arms: (1) CpAM (RO7049389) + TLR-7 agonist (RO7020531) + NA; (2) siRNA (RG6346) + NA; (3) siRNA (RG6346) + PEG-IFN + NA; (4) siRNA (RG6346) + TLR (RO7020531) + NA; (5) siRNA (RG6346) + PD-L1 LNA (R07191863) + NA	Percentage of participants with HBsAg loss at 24 wk after end of treatment
GC1102 (HBsAg neutralizing antibody)	Green Cross Corporation	II	NCT03801798	Double-blind, randomized, placebo-controlled, parallel-group	Chronic HBV infection patients on NA ≥ 24 wk before screening	42	Control arm: NA + placebo; Experimental arm: NA + GC1102	Proportion of participants with ≥ 1 log IU/mL reduction in HBsAg titer

CpAM: Core Protein Allosteric Modulator; HBV: Hepatitis B Virus; HBsAg: Hepatitis B surface antigen; LLOQ: Lower limit of quantification; NA: Nucleos(t)ide therapy; TLR: Toll-like receptor; SiRNA: Short interfering RNA; ULN: Upper limit of normal.

without study data are listed in Table 1.

Core/capsid inhibitors

Capsid allosteric modulators directly target the destabilization of HBV core protein, resulting in the formation of abnormal capsids or morphologically normal capsids lacking genetic material[25]. This prevents further release and spread of HBV to other hepatocytes.

JNJ56136379 (JNJ-6379): JNJ56136379 (also known as JNJ-6379) targets the HBV capsid assembly process needed for HBV replication. It accelerates the rate of HBV capsid assembly to form empty, morphologically intact viral capsids and has a secondary mechanism of inhibiting *de novo* cccDNA[26].

The JADE study was a randomized, partially blinded, placebo-controlled phase II study evaluating the efficacy and safety of JNJ-6379 in 232 adults with non-cirrhotic, chronic HBV infection. Participants were virally suppressed or not on active treatment at the time of entry into the clinical trial. Participants were randomized to receive JNJ-6379, either as monotherapy or in combination with NA (ETV or TDF), and then compared to a control group of placebo plus NA. Dosing of JNJ-6379 at 75 mg and 250 mg daily was investigated. Overall, JNJ6379 did not show a clear benefit over NA monotherapy. The primary endpoint of a 1 log IU/mL mean decrease in HBsAg from baseline to week 24 for the JNJ-6379 treatment groups and control was not achieved. Specifically, the mean change in HBsAg compared to baseline for the JNJ-6379 treatment groups ranged from -0.41 to 0.11 log IU/mL. Among participants who were HBeAg-positive at the start of the study, there was also a limited reduction of HBeAg of 0.49 and 0.70 log IU/mL for JNJ-6379 75 mg and 250 mg plus NA treatment groups, respectively. Over the 24-wk follow-up period, the use of JNJ6379 both as monotherapy and in combination with NA led to a marked reduction in both HBV DNA (mean JNJ-6379 75 mg or 250 mg plus NA HBV DNA reduction of 5.53 and 5.58 log IU/mL, respectively, compared with 5.21 log IU/mL in the placebo plus NA group) and HBV RNA (mean JNJ-6379 75 mg and 250 mg plus NA HBV RNA reduction was 2.96 and 3.15 log IU/mL, respectively, compared with 1.33 log IU/mL in the placebo plus NA group). Both doses of JNJ6379 were safe and well-tolerated[26].

ABI-H0731 (Vebicorvir): ABI-H0731 (Vebicorvir/VBR) is an orally administered small molecule that disrupts HBV replication by inducing altered, non-functional core protein assembly[27].

One of the phase II studies evaluated the efficacy and safety of VBR in combination with ETV for treatment-naïve, non-cirrhotic, HBeAg positive study participants. In this double-blind, randomized, and placebo-controlled study, participants either received a combination of once daily VBR 300 mg daily and ETV 0.5 mg daily, or a combination of placebo and ETV 0.5 mg daily. The study revealed that the combination of VBR and ETV was safe and well-tolerated, and augmented a reduction of HBV DNA and RNA. The primary endpoint was achieved as there was a significantly greater mean log reduction in HBV DNA from baseline with VBR plus ETV combination therapy as compared to placebo plus ETV therapy at both treatment weeks 12 (-4.45 log IU/mL with VBR and ETV *vs* -3.3 log IU/mL with placebo and ETV) and 24 (-5.33 log IU/mL with VBR and ETV *vs* -4.2 log IU/mL with placebo and ETV). Furthermore, a greater proportion of patients had normalized ALT levels by treatment week 24 among the VBR and ETV combination therapy group (12/13 participants) as compared to the placebo and ETV therapy group (5/12 participants). No resistance breakthrough occurred with the use of VBR. The study demonstrated that VBR can be combined with current NA therapy to enhance anti-viral activity in treatment-naïve patients with chronic HBV infection[27].

Another phase II study evaluated the efficacy and safety of combination VBR and NA therapy as compared to NA monotherapy in non-cirrhotic, chronic HBV participants who were virally suppressed by NA for at least 6 months. The 73 enrolled study participants were randomized to receive VBR 300 mg daily plus NA or matching placebo plus NA for 24 wk. Results showed that there was no difference between the two groups for the change in HBsAg or HBeAg from baseline to treatment week 24. Of note, the combination of VBR plus NA led to a more marked reduction of HBV DNA and pregenomic RNA at week 24 from baseline compared to the placebo plus NA group, irrespective of HBeAg status. Among patients with detectable HBV DNA at baseline, there were a greater proportion of patients in the VBR plus NA group (29/35 HBeAg+ patients, 16/17 HBeAg- patients) compared to the placebo plus NA group (17/59 HBeAg+ patients, 10/14 HBeAg- patients) who achieved undetectable HBV DNA levels at week 24. VBR was found to be safe and well-tolerated. This clinical study provided further support that even greater levels of viral suppression can occur with the addition of a VBR core inhibitor to existing NA therapies, although the clinical significance of this is yet to be investigated[28].

Entry inhibitors

Entry inhibitors target the function of HBV surface proteins or host receptors to prevent HBV entry into the host cell required for infection[29].

Bulevirtide (formerly Myrcludex): Bulevirtide is a synthetic myristoylated peptide entry inhibitor that competitively binds and blocks a hepatocyte surface protein, sodium taurocholate cotransporting polypeptide (NTCP) receptor, such that HBsAg is unable to enter the hepatocyte[29]. Hepatitis D virus (HDV) uses the same NTCP receptor as HBV, so Bulevirtide has been also used to prevent co-infection by HDV[30]. Increases in bile acid level are expected since NTCP plays a role in bile transport[31].

The MYR-201 study was a phase Ib/IIa, randomized, open-label study investigating the safety and efficacy of Bulevirtide with regard to the HBV and HDV virologic response and tolerability. The study featured 24 participants randomized to receive either Bulevirtide for 24 wk followed by PEGIFNα-2a weekly for 48 wk (Bulevirtide cohort), 2 mg Bulevirtide daily plus PEGIFNα-2a weekly for 24 wk followed by 24 wk of PEGIFNα-2a alone (Bulevirtide-IFN cohort), or PEGIFNα-2a weekly alone for 48 wk (IFN cohort). Study results revealed that HBsAg levels remained unchanged compared to baseline throughout the study in all treatment groups. ALT normalized in 6/8 patients in the Bulevirtide cohort compared to only 1/15 patient in the Bulevirtide-IFN and IFN cohorts. Notably, mean HBV DNA was significantly reduced by $10^{1.28}$ copies/mL at week 24 from baseline in the Bulevirtide-IFN cohort, with 6/7 patients showing a ≥ 1 log decline. There was a non-significant decline of the HBV DNA from baseline in the IFN and Bulevirtide cohorts. This was the first proof-of-concept study showing that Bulevirtide was safe and well-tolerated, and could enhance viral suppression when used in combination with PEGIFNα-2a[32].

Another phase II, multicenter, open-label study, known as MYR-202, randomized patients into four groups: 2 mg subcutaneous Bulevirtide daily with TDF daily, 5 mg subcutaneous Bulevirtide daily with TDF daily, 10 mg subcutaneous Bulevirtide daily with TDF daily, or TDF alone for a total of 24 wk. Therapeutic impact on HBsAg was investigated as a secondary endpoint. There was no significant change in HBsAg concentration from baseline in any of the treatment groups throughout the treatment and follow-up period. Like the MYR-201 study, Bulevirtide in the MYR-202 study was well-tolerated. Common treatment-related adverse events included elevations in asymptomatic bile salt levels and ALT levels[33].

MYR-203 assessed the safety and efficacy of Bulevirtide alone or in combination with PEGIFNα for 48 wk. Treatment arms included PEGIFNα alone weekly, 2 mg Bulevirtide daily plus PEGIFNα weekly, 5 mg Bulevirtide daily plus PEGIFNα weekly, or 2 mg Bulevirtide daily for a total of 48 wk. This was then followed by a treatment free period of 24 wk. By weeks 48 and 72, there was a > 1 log reduction from baseline or undetectable HBsAg levels in the Bulevirtide plus PEGIFNα combination groups, but not in the monotherapy groups. Specifically, by 72 wk, 6/15 participants in the combination arm of the 2 mg Bulevirtide plus PEGIFNα group and 2/15 participants in the 5 mg Bulevirtide plus PEGIFNα group achieved either a > 1 log IU/mL decline or undetectable levels of HBsAg. MYR-203 study findings demonstrated a potential role of combination Bulevirtide and PEGIFNα therapy in future HBV cure given that it led to a large proportion of patients achieving HBsAg loss[34].

The MYR-204 multicenter, randomized phase II trial studied the safety and efficacy of Bulevirtide administered subcutaneously at 2 mg or 10 mg daily dosing in combination with PEGIFNα weekly compared to Bulevirtide 10 mg monotherapy over 48 wk. Interim data at the 24-wk mark showed that a > 1 log IU/mL decline in HBsAg levels from baseline was achieved only in the Bulevirtide and PEGIFNα combination groups (10/100 participants) and the PEGIFNα alone group (1/24 participants). There was a modest decline in HBV DNA from baseline in the groups that received Bulevirtide (mean HBV DNA change ranged from -0.3 to -0.7 log IU/mL)[35].

The MYR-301 trial was the first phase III multicenter, randomized, parallel design study of Bulevirtide monotherapy at 2 and 10 mg daily dosing compared to no active anti-HDV treatment for 48 wk, defined as delayed treatments. For HBV efficacy endpoints at week 48, no patient in any group experienced HBsAg loss and changes in HBsAg from baseline were minimal. Only a small decline in HBV DNA levels was observed with Bulevirtide treatment. No severe adverse effects were observed in patients receiving Bulevirtide that led to discontinuation of the drug[36].

Small interfering RNAs

Small interfering RNAs (siRNAs) are short RNA molecules that hybridize to specific viral mRNA sequences and target bound mRNA for degradation[37]. Effectively, siRNA prevents the expression of HBV proteins needed for replication.

Bepirovirsen (GSK3228836): Bepirovirsen is an antisense oligonucleotide that targets all HBV RNA, including mRNA and pregenomic RNA, and designates it for degradation[38].

One of the two phase II randomized controlled trials evaluated the safety, tolerability, and antiviral activity of Bepirovirsen. The study enrolled 24 treatment-naïve participants and 7 participants receiving stable NA therapy with chronic HBV infection. Patients who were treatment-naïve were randomized to receive placebo or Bepirovirsen at a dose of 150 mg or 300 mg. Patients on stable NA therapy were randomized to receive placebo or Bepirovirsen at a dose of 300 mg. Bepirovirsen was administered twice weekly for 2 wk and then once weekly for another 2 wk, after which patients were followed for 26 wk to assess for a change in HBsAg levels from baseline. After 4 wk of treatment with 300 mg Bepirovirsen for treatment-naïve patients, there was a significant decrease in HBsAg levels and HBV DNA from baseline compared to placebo; this was not observed in the Bepirovirsen 150 mg group. Specifically, among treatment-naïve subjects, there was a mean 1.56 log IU/mL reduction in HBsAg in the Bepirovirsen 300 mg group from baseline to day 29, as compared to a 0.5 log IU/mL reduction in the Bepirovirsen 150 mg group and < 0.07 log IU/mL reduction in the placebo group. The timing of HBsAg reduction in responders occurred rapidly after 4 wk of therapy. Bepirovirsen was found to have a favorable safety profile and treatment response, which encouraged its use in a larger study cohort[39].

The B-Clear Trial was a phase IIb randomized controlled study investigating the efficacy and safety of Bepirovirsen in 457 enrolled participants with chronic HBV infection when used for 12 and 24 wk. Results revealed that 6/68 participants and 7/70 participants who received 24 wk of Bepirovirsen once weekly with and without NA therapy, respectively, achieved HBsAg and HBV DNA loss that persisted for 24 wk following the end of the treatment period. While there were similar results of HBsAg loss irrespective of NA therapy use or HBeAg status, HBsAg loss among patients who were HBeAg-positive only occurred in those receiving NA therapy. The study also showed that levels of HBsAg at baseline can be predictive of response to therapy. Specifically, receiver operating characteristic curve analysis revealed that baseline HBsAg level < 3000 IU/mL was the cutoff level associated with functional cure when treated with Bepirovirsen. Common adverse events observed more commonly in the study cohort receiving Bepirovirsen compared to placebo included injection site reactions, pyrexia, fatigue, and increased ALT levels. A brief increase in HBV DNA observed after stopping Bepirovirsen raised potential concerns about the durability of treatment response; however, these blips in HBV DNA levels were postulated to be due to spontaneous release of virions from the hepatic reservoir. Durability of treatment response will be investigated in future studies with longer follow-up time[40].

VIR-2218: VIR-2218 is a triantennary N-acetyl galactosamine (GalNAc) conjugated siRNA that targets the X region of the HBV genome[41]. As the X region contains overlapping HBV gene templates, the use of a single siRNA can effectively silence all HBV RNA production in this region. VIR-2218 can also suppress the X-mediated upregulation of cccDNA transcription.

VIR-2218-1001 was a two-part, phase I/II, randomized, double-blind, and placebo-controlled study. The first part of the study evaluated the safety and tolerability of a single dose of VIR-2218 at six dosing levels administered to healthy

adult volunteers. The second part of the study evaluated the safety and therapeutic effect across various increase doses of VIR-2218 given 4 wk apart. Study participants were non-cirrhotic adults with chronic HBV infection on NA therapy for at least 6 months and HBV DNA < 90 IU/mL. In both parts of the study, VIR-2218 was well-tolerated across all dose levels with only mild adverse events, commonly headache, injection site reactions, and mild ALT elevations. The study found a dose-dependent reduction in HBsAg in all VIR-2218 treatment groups compared to placebo by the 48-wk follow-up. A total of 12/24 participants across the VIR-2218 cohorts as compared to none in the placebo group achieved a reduction of HBsAg levels to < 100 IU/mL. The greatest mean reduction of HBsAg (-1.65 log IU/mL) occurred at week 20 for those receiving the 200 mg VIR-2218. While no participants had serum HBsAg loss or anti-HBs seroconversion by week 48, an HBsAg level < 100 IU/mL has been associated with a significantly higher chance of HBsAg loss[42]. This study demonstrated that VIR-2218 is well-tolerated with antiviral effects that could potentially lead to functional cure[43].

Another phase II trial investigated the safety and efficacy of VIR-2218 alone and in combination with PEGIFNα in non-cirrhotic participants with chronic HBV infection. Inclusion criteria included NA therapy for at least 2 months, HBsAg > 50 IU/mL, and HBV DNA < 90 IU/mL. Preliminary data revealed that VIR-2218 was generally well-tolerated both alone and in combination with PEGIFNα. Adverse events that occurred were more consistent with known effects of PEGIFNα, such as mild ALT elevations and reductions in neutrophil and platelet levels. Four of 13 study participants treated with VIR-2218 combined with PEGIFNα for a longer duration of 48 wk achieved HBsAg seroclearance and anti-HBs seroconversion. Patients in this longer duration combination group also had the largest mean HBsAg reduction of 2.9 log IU/mL at the end of therapy. While the study is still ongoing with longer follow-up time, the preliminary results demonstrate that the antiviral effect of VIR-2218 may be potentiated by PEGIFNα and they show promise as a future combination therapy[44].

VIR-3434: VIR-3434 is a subcutaneously administered monoclonal antibody that targets an antigenic loop of HBsAg to block HBV cell entry[45]. In addition to clearing HBsAg, it can stimulate T cells for a vaccinal effect.

The MARCH trial was a phase II study that evaluated the safety, tolerability, and antiviral activity of VIR-2218 and VIR-3434 either as monotherapy or as combination therapy. Study participants were virally-suppressed, non-cirrhotic adults on NA therapy with chronic HBV infection. Both VIR-2218 and VIR-3434 were well-tolerated with mild adverse effects. The study was instrumental in showing that the combination of VIR-2218 and VIR-3434 Led to a marked mean HBsAg decline of > 2.5 log IU/mL in all cohorts. In fact, most participants were able to achieve an HBsAg level < 10 IU/mL. VIR-3434 has an additive effect to VIR-2218 in achieving a greater HBsAg reduction compared to monotherapy; this is consistent with their established complimentary mechanisms of action on HBV replication[46].

JNJ-73753989 (JNJ-3989, formerly ARO-HBV): JNJ-3989 is composed of two siRNAs which target both the S gene and X gene of the HBV. Consequently, it impairs the production of HBV RNA transcripts which are essential for replication[47].

A phase IIa clinical trial assessed the safety and efficacy of JNJ-3989 both with and without JNJ-6379 in 84 recruited participants with chronic HBV infection who were treatment-naïve or on chronic NA-suppressive therapy. All participants received an NA throughout the study. JNJ-3989 was well-tolerated across all doses throughout the study period. By day 112, there was an HBsAg reduction of ≥ 1 log IU/mL from baseline in 39/40 participants who received 100 to 400 mg of JNJ-3989 every 4 wk in combination with an NA daily. Also, 30/40 patients achieved HBsAg < 100 IU/mL by day 112. A dose-dependent relationship was seen with higher doses of JNJ-3989 achieving higher levels of HBsAg reductions. More frequent dosing intervals did not change the magnitude and rate of response compared to dosing of JNJ-3989 every 4 wk. All 12 patients in the triple combination of JNJ-3989, JNJ-6379, and NA therapy achieved a ≥ 1 log IU/mL HBsAg reduction from baseline to the nadir. The HBsAg reduction was also durable - 15/19 participants maintained a ≥ 1 log HBsAg reduction for nearly 336 d after their last JNJ-3989 dose. This trial provided support that JNJ-3989 can be used safely in combination with an NA and that JNJ-6379 is an efficacious and durable HBV therapy[48].

The REEF-1 study was a large multicenter, double-blinded, randomized, phase IIb clinical trial studying the efficacy and safety of combination therapies of JNJ6379, JNJ-3989, and NA at various doses. The study featured non-cirrhotic adults with chronic HBV infection who were either treatment-naïve or virologically suppressed on NA therapy. The primary endpoint was the proportion of patients who met NA stopping criteria, as defined as ALT < 3 × upper limit of normal, HBV DNA less than the lower limit of quantitation, HBeAg negativity, and HBsAg < 10 IU/mL by week 48. Over the course of 48 wk, JNJ-3989 in combination with NA therapy led to a robust, dose-dependent response for meeting NA stopping criteria as well as reducing HBsAg and HBV RNA levels. In fact, 94/96 patients in the combination JNJ3989 200 mg every 4 wk and NA group had a ≥ 1 log IU/mL HBsAg decline with a mean decline of 2.6 log IU/mL. Most patients did not reach the NA stopping criteria for two reasons: Failure to achieve HBsAg < 10 IU for those who were HBeAg-negative at baseline, or not achieving HBeAg seronegative status for patients who were HBeAg-positive at baseline. JNJ-3989 in combination with NA was safe and well-tolerated. Overall, REEF-1 showed that the combination of novel therapies, involving JNJ-3989 and/or JNJ6379, with established NA therapies is insufficient to achieve functional cure, but can achieve substantial HBsAg reductions[49].

Arbutus-729 (AB-729 or Imdusiran): AB-729 is a subcutaneously administered, GalNAc-conjugated RNA interference agent that blocks all RNA transcripts and reduces all HBV viral antigens[50]. It has an immunostimulatory component by enhancing HBV-specific T cell responses following repeat dosing[51].

In the AB-729-001 phase II study, healthy subjects and those with chronic HBV infection were subjected to single and repeat doses of AB-729 at various doses (60 or 90 mg of AB-729) and frequencies (every 4, 8, or 12 wk). ABI-729 with repeat dosing was found to be safe and well-tolerated. The most frequent adverse events included injection site events and asymptomatic ALT elevations which were Grade 2 in severity or lower. There was a robust and persistent decline in HBsAg in most subjects across cohorts regardless of dose, dosing interval, or HBeAg status; there was a mean reduction of HBsAg by 1.5 log IU/mL from baseline to 24 wk after the last dose. In fact, 26/34 participants achieved HBsAg < 100

IU/mL at some point in the study. As well, there was a sustained reduction in HBsAg and HBV DNA in 7 of 9 patients even after discontinuation of both AB-729 and NA-therapy. Only one subject seroconverted at week 48. These study findings demonstrated that AB-729 may be considered as a potential therapy for achieving functional cure of chronic HBV infection[52].

The AB-729-201 trial was a randomized, open-label, multicenter, phase IIa study which evaluated the safety, tolerability, and antiviral activity of AB-729 with PEGIFNα. The 43 non-cirrhotic, HBeAg-negative subjects had virally suppressed chronic HBV infection and were on stable NA therapy for at least 12 months prior. Patients received 4 doses of ABI-729 60 mg every 8 wk, and at week 24 were randomized to either of two treatment combinations (AB-729 + NA + PEGIFNα or NA + IFN) and at two treatment durations (12 wk *vs* 14 wk) followed by another 24 wk of follow-up where patients were evaluated to stop NA therapy. Preliminary results showed that by week 24 of treatment, there was a mean HBsAg decline of 1.6 log IU/mL across all cohorts. As well, 38 of 41 subjects achieved HBsAg levels < 100 IU/mL at some point during the treatment period. The interim data also showed that AB-729 with and without IFN was safe and well-tolerated with most treatment related adverse events unrelated to AB-729 therapy[53].

HBsAg inhibition

HBsAg is a main surface protein on the envelope of the new HBV virion and subviral particles that maintains chronic infection *via* immune exhaustion[54]. HBsAg loss is one primary component required for functional cure[55]. HBsAg inhibitors disrupt the secretory processes involved in translocating HBsAg to the surface and effectively decrease HBsAg availability[56].

REP 2139/REP 2165 (Replicor): REP2139 is a nucleic acid polymer (NAP) that stops the assembly of subviral particles in hepatocytes and blocks the release of HBsAg[57]. REP2165 is a biologically equivalent variant of REP2139 with equivalent HBV antiviral activity *in vivo*. However, it has accelerated clearance which may be useful in cases requiring high frequency dosing for patients with slow rates of HBsAg clearance[58].

REP401 was an open-label phase 2 study evaluating the safety and efficacy of the combination therapy TDF, PEGIFNα, and either REP2139 or REP2165. Participants had chronic HBV infection and were HBeAg-negative. Patients received 24 wk of TDF therapy, followed by 24 wk of a control backbone therapy (TDF and PEGIFNα) or combination triple therapy (TDF, PEGIFNα, and either REP2139 or REP2165). Then participants were monitored for a treatment-free period of 48 wk. The addition of either REP2139 or REP2165 to TDF and PEGIFNα was safe and well-tolerated. Use of REP2139/REP2165 did not affect PEGIFNα-induced thrombocytopenia and neutropenia. Notably, there was a significantly more frequent and greater increase in asymptomatic transaminase levels among patients receiving an NAP which correlated with an initial decrease in HBsAg levels. From weeks 25 to 48, the combination triple therapy led to a rapid 4 to 6 log IU/mL decline in HBsAg in 15/20 patients by week 35. By week 48, HBsAg was not detected in 10 of 20 patients and HBsAg seroconversion was achieved in 11/20 patients, all with HBsAg < 1 log IU/mL. In contrast, the control backbone therapy group had an HBsAg decline > 1 log IU/mL in only 3 of 20 patients with no HBsAg seroconversion observed. Both the triple combination group and control group achieved a similar HBV DNA decline with 18 of 40 participants achieving HBV DNA less than the lower limit of quantification by week 48. In the 48-wk follow period, functional cure persisted in 14 of the 40 patients. Within the triple combination therapy group, there was no difference in response between REP2139 and REP2165 with regards to HBsAg, hepatitis B surface antibody (anti-HBs), and HBV DNA levels. REP401 showed that the addition of REP2139 or REP2165 to TDF and PEGIFNα therapy did not affect tolerability and increased rate of functional cure both during and after therapy[59].

REP301 was an open-label, nonrandomized, phase II trial investigating the use of REP2139 with PEGIFNα-2a in adults with chronic HBV infection. These participants were HBeAg-positive, anti-hepatitis D antigen-positive, and HDV RNA-positive, and had an HBsAg levels > 1000 IU/mL. Study subjects received intravenous (IV) REP2139 once weekly for 15 wk, followed by a combination of IV REP2139 and subcutaneous PEGIFNα-2a once weekly for another 15 wk, and then finally, PEGIFNα-2a for 33 wk. By the end of treatment, 6/12 subjects had HBsAg < 50 IU/mL, 6/12 subjects had HBsAb > 10 mIU/mL, and 9/12 subjects had suppressed HBV DNA < 10 IU/mL. The response was durable to 1 year of follow-up: 5/6 patients maintained HBsAg suppression < 50 IU/mL, all 6/6 patients maintained HbsAb > 10 mIU/mL, and 7/9 patients had HBV DNA < 10 IU/mL. Use of both REP2139 and PEGIFNα-2a was safe and well-tolerated. The most frequent adverse events with REP2139 monotherapy were pyrexia and chills, while the introduction of PEGIFNα-2a led to asymptomatic transient elevations in ALT and aspartate aminotransferase (AST). REP301 underscored that combination REP2139 therapy with PEGIFNα-2a has robust and durable HBV and HDV antiviral effects even after completion of therapy[60].

Toll-like receptor agonists

Toll-like receptor (TLR) agonists act as immunomodulators to enhance the immune response against chronic HBV infection[61]. They induce the production of interferons, cytokines, and chemokines which upregulate antiviral effects [62].

Vesatolimod (GS-9620): Vesatolimod selectively activates TLR-7 found in gut-associated plasmacytoid dendritic cells and B lymphocytes to upregulate T and B cell responses[63].

The first phase II double-blind, randomized, placebo-controlled study evaluated the safety, efficacy, and pharmacodynamics of Vesatolimod in virally-suppressed, non-cirrhotic patients with chronic HBV infection. The 162 participants were randomized to receive weekly dosed placebo or Vesatolimod (1 mg, 2 mg, or 4 mg) for various treatment durations (4, 8, 12, and 48 wk). Vesatolimod was safe and well-tolerated at all doses with no clinically significant adverse events or lab derangements in the cohorts. Although the biological activity of Vesatolimod was verified with a dose-dependent

pharmacodynamic induction of the biomarker ISG15, no significant HBsAg decline from baseline was observed in any of the cohorts by week 48[64].

The second phase II study evaluated the safety and efficacy of Vesatolimod on patients with non-cirrhotic, chronic HBV infection who were not on oral antiviral treatment for at least 3 months. Additionally, patients had HBV DNA ≥ 2000 IU/mL. In this multicenter, double blind, randomized, placebo-controlled study, patients were randomized to receive weekly placebo or oral Vesatolimod (1 mg, 2 mg, or 4 mg) for 12 wk. All subjects also received TDF of 300 mg daily for 48 wk. Vesatolimod was safe and well-tolerated. None of the patients achieved HBsAg loss or HBsAb seroconversion in any of the cohorts, and there was no significant difference in the decline of HBsAg among the Vesatolimod treatment groups compared to placebo. Only three total patients in the Vesatolimod groups had HBeAg loss and HBeAb seroconversion at week 48. There was no significant difference in HBV DNA decline among the Vesatolimod groups compared to placebo. Like the first study, a pharmacodynamic response was verified with a consistent dose-dependent induction of ISG15 biomarker level[65].

Selgantolimod (GS-9688): Selgantolimod is a selective TLR-8 agonist with antiviral activity against chronic HBV infection. It leads to the production of proinflammatory cytokines, chemokines, and interferons that initiate an innate and adaptive immune response against HBV[66].

A phase II, randomized, double-blind, placebo-controlled, multicenter study investigated the safety and efficacy of Selgantolimod in virally suppressed individuals on antiviral therapy with chronic HBV infection. Patients were randomized to receive once weekly placebo or oral Selgantolimod dosed at 1.5 mg or 3 mg for a total of 24 wk while continuing oral NA agents. Only one of the 48 participants in the 1.5 mg Selgantolimod group achieved the primary endpoint of a ≥ 1 log IU/mL decline in HBsAg from baseline to week 24. As compared to placebo where no participants achieved HBsAg or HBeAg loss, 2 of the 39 subjects with HBeAg negative status achieved HBsAg loss and 3 of the 39 subjects had HBeAg loss in the Selgantolimod groups. The largest HBsAg reductions during the study occurred in patients who received Selgantolimod. In fact, HBsAg declines persisted even after treatment cessation. Selgantolimod was safe and generally well-tolerated with the most common adverse events including nausea, vomiting, and headache[67].

Therapeutic vaccinations

Therapeutic vaccinations present HBV vaccine antigens in a non-infective form to antigen presenting cells to stimulate a CD4 and CD8-mediated T cell response against HBV[68]. In comparison to preventative vaccines, therapeutic vaccinations are given during ongoing infection.

GS-4774: GS-4774 is a vaccine composed of heat-inactivated yeast cells expressing HBsAg, hepatitis B core antigen, and HBV-encoded oncogene X protein as a single fusion protein. Inoculation of individuals with GS-4774 as a subcutaneous injection elicits a significant T cell response[69].

A phase II study evaluated the safety, tolerability, and efficacy of GS-4774 in non-cirrhotic patients with chronic HBV infection who were virally suppressed with oral antiviral therapy for at least a year. Subjects were randomized to receive either oral antivirals alone or a combination therapy of oral antivirals plus GS-4772 (dosed as 2, 10, or 40 yeast units) subcutaneously every 4 wk until week 20. Subjects continued oral antivirals for the remainder of the study to week 24 and then followed to week 48. No significant difference in mean HBsAg decline was found from baseline to week 24 or week 48 between any of the GS-4772 combinations therapy groups compared to oral antivirals alone. No patient experienced loss of HBsAg. Combination therapy of GS-4774 and antivirals was found to be safe and well-tolerated - there was no virologic breakthrough or treatment discontinuations in any patient and injection site reactions were the most common adverse event. The study showed that GS-4774 has limited efficacy for functional cure of chronic HBV infection among virally suppressed patients[70].

Another phase II, open-label, multicenter study evaluated the safety and efficacy of GS-4774 in combination with TDF in patients who were treatment-naïve. Inclusion criteria included positive HBsAg serology for at least 6 months, HBV DNA levels ≥ 2000 IU/mL, and no use of antiviral therapy within 3 months of study screening. Subjects were randomized to receive oral TDF alone or in combination with GS-4774 (dosed 2, 10, or 40 yeast units) every 4 wk until week 20. GS-4774 was safe and well-tolerated. There was no significant decrease in levels of HBsAg from baseline to weeks 24 and 48 among treatment groups despite a strong immune stimulatory effect on CD8+ T cells[71].

BRII-179: BRII-179 is a virus-like therapeutic vaccine expressing Pre-S1, Pre-S2, and S HBV surface antigens which stimulates an HBV specific T and B cell-mediated response[72].

In a randomized, open-label phase Ib/IIa study, the safety, antiviral activity, and immunogenicity of subcutaneously-administered BRII-179 at 20 mcg and 40 mcg doses with and without PEGIFNα was evaluated in subjects with non-cirrhotic, chronic HBV infection. Subjects did not have detectable levels of HBsAg and were on NA for at least 6 months prior to the study. Results showed that both doses of BRII-179 were safe and well-tolerated with no severe adverse events. Limited HBsAg reductions (< 0.2 log HBsAg IU/mL) from baseline were observed after 4 doses of BRI-179 in both dosing groups. BRII-179 was found to be immunogenic: All BRII-179 treatment groups had increased HBsAb levels by at least > 30%, as compared to NA therapy alone which elicited no detectable anti-HBs response[72].

BRII-179 was also studied in combination with VIR-2218 for treating chronic HBV infection. An ongoing phase II study with interim results compared the combination of BRII-179 and VIR-2218 to VIR-2218 alone. Subjects were virally suppressed on an NA for at least 12 months and had HBV DNA less than the lower limit of quantification. Patients were followed to week 40. Interim results showed that BRII-179 in combination with VIR-2218 was safe and well-tolerated with mild adverse events, most commonly an injection site reaction. Although no significant difference in mean HBsAg reduction from baseline was found between combination therapy and VIR-2218 alone, the combination of BRII-179 and VIR-2218 led to a potent increase in anti-HBs level of more than 100 IU/L in more than 40% of the subjects compared to

none in the VIR-2218 alone. Final results will evaluate the long-term therapeutic and immune response to BRII-179 and VIR-2218 combination therapies[73].

Anti-programmed cell death ligand-1

In chronic HBV infection, there is upregulation of programmed cell death ligand-1 (PD-L1) which is responsible for T-cell exhaustion and persistence of HBV viral disease[74]. The goal of checkpoint inhibitor therapy that blocks PD-L1 is to restore the function of HBV-specific T cells[75].

ASC22 (Envafolimab): ASC22 is a subcutaneously administered immunotherapy that blocks the programmed cell death protein 1 (PD-1)/PD-L1 pathway to restore T cell function. A phase IIb, randomized, single-blind, multicenter clinical trial was conducted to assess the efficacy and safety of ASC22 in subjects with chronic HBV infection who were virally suppressed on NA. Included subjects had HBsAg ≤ 10000 IU/mL, HBV DNA < 20 IU/mL, and ALT/AST less than 2 × upper limit of normal, and were HBeAg-negative. Subjects were randomized to recieve either 1 mg/kg or 2 mg/kg subcutaneously-administered ASC22 every 2 wk in combination with an NA for 24 wk or placebo with NA. Both groups then received an additional 24 wk of NA therapy. Interim results of the combination therapy group with 1 mg/kg ASC22 and NA showed a more significant HBsAg reduction as compared to placebo and NA therapy, especially among patients with a baseline HbsAg level ≤ 100 IU/mL. This response was durably sustained - 3 of the 7 patients with baseline HBsAg ≤ 100 IU/mL in the ASC22 treatment group was able to sustain an HBsAg loss lower than the lower limit of quantification (0.05 IU/mL) by the end of the follow-up period. ASC22 1 mg/kg combined with NA for up to 24 wk was also safe and well-tolerated. Low-grade ALT flares were observed in 10/48 patients from the ASC22 group compared to none in the placebo group; these ALT flares also tended to occur more frequently in patients with a more significant HBsAg reduction. Thus, ALT flares may be a marker to monitor treatment response[76,77].

CONCLUSION

Current antiviral therapy with PEGIFNα and NA have low rates of functional cure and have limitations with regards to adverse effects, adherence, resistance, long-term treatment, and ongoing risk for liver events. Innovative clinical trials have been key in the development of novel therapies with a diverse range of mechanisms that strive to achieve the goal of functional cure (sustained HBsAg loss and undetectable HBV DNA 24 wk post-treatment). Based on available phases 2 and 3 data, it appears that single agent approaches (*e.g.*, RNAi alone) are unlikely to result in HBsAg loss and therefore agents combining HBsAg lowering antivirals (*e.g.*, RNAi and monoclonal antibody) +/- immunomodulator +/- NA may be required. Combination regimens with two drug (RNAi plus NA with bepirovirsen) or three drug approaches (RNAi plus immunomodulator plus NA with VIR-2218/ PEGIFNα/NA) have demonstrated proof of principle that functional cure can be achieved. Future randomized controlled trials in larger representative cohorts (HBeAg-positive/negative, NA-naïve/experienced, low *vs* high HBsAg titer) are needed to further confirm the efficacy/safety profiles of functional curative regimens and predictors of virologic response.

FOOTNOTES

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MINIREVIEWS

Protein succinylation, hepatic metabolism, and liver diseases

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Abstract

Succinylation is a highly conserved post-translational modification that is processed *via* enzymatic and non-enzymatic mechanisms. Succinylation exhibits strong effects on protein stability, enzyme activity, and transcriptional regulation. Protein succinylation is extensively present in the liver, and increasing evidence has demonstrated that succinylation is closely related to hepatic metabolism. For instance, histone acetyltransferase 1 promotes liver glycolysis, and the sirtuin 5-induced desuccinylation is involved in the regulation of the hepatic urea cycle and lipid metabolism. Therefore, the effects of succinylation on hepatic glucose, amino acid, and lipid metabolism under the action of various enzymes will be discussed in this work. In addition, how succinylases regulate the progression of different liver diseases will be reviewed, including the desuccinylation activity of sirtuin 7, which is closely associated with fatty liver disease and hepatitis, and the actions of lysine acetyltransferase 2A and histone acetyltransferase 1 that act as succinyltransferases to regulate the succinylation of target genes that influence the development of hepatocellular carcinoma. In view of the diversity and significance of protein succinylation, targeting the succinylation pathway may serve as an attractive direction for the treatment of liver diseases.

Key Words: Protein succinylation; Hepatic metabolism; Fatty liver; Hepatitis; Hepatocellular carcinoma

Core Tip: Succinylation is the process of transferring succinyl groups through enzymatic and non-enzymatic means using succinyl CoA as a direct substrate. The succinylation degree could be promoted by succinyltransferases (*e.g.*, lysine acetyltransferase 2A, histone acetyltransferase 1, α-ketoglutarate dehydrogenase complex, and carnitine palmitoyltransferase 1A). Desuccinylases including CobB, sirtuin 5, and sirtuin 7 negatively regulate protein succinylation. Several proteins and enzymes in glucose, amino acid, and lipid metabolisms are succinylated in the liver. Succinylation is associated with the progression of several liver diseases. Proteins with varied levels of succinylation may be potential targets for the treatment of fatty liver, hepatitis, and hepatocellular carcinoma.

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INTRODUCTION

Introduction to protein succinylation

Post-translational modification is an important mechanism that affects protein function, integrating metabolism with physiological and pathological processes. Succinylation is an important post-translational modification of proteins *via* both enzymatic and non-enzymatic manners[1].

Process of succinyl modification: Succinylation is the process by which a succinyl donor transfers a negatively charged four-carbon succinyl group to the amine of lysine residues by enzymatic or non-enzymatic means[2,3] (Figure 1). The succinyl group binding to the lysine residue has a relatively larger molecular weight (approximately 100.02 Da), which significantly changes the protein structure. Additionally, the charge carried by the lysine residues changes from +1 to -1, resulting in alterations to the physical and chemical properties as well as the functions of the proteins[1-4].

Succinyl modification is widespread in both the cytoplasm and nucleus[5]. In the cytoplasm, succinylation is highly concentrated in mitochondria and may be involved in regulating the tricarboxylic acid cycle, amino acid metabolism, and fatty acid metabolism[6-9]. In the nucleus, lysine succinylation is present in more than one-third of nucleosomes, and the succinylation sites are mainly enriched in the gene promoter region, suggesting that succinylation may be involved in the transcriptional regulation of genes[6,8,9]. Succinylated lysine residues have greater structural changes and charge differences than other typical covalent lysine modification groups such as acetyl and dimethyl[1,5]. Therefore, the influence and mechanism of succinylation on the target proteins and its potential application for the treatment of metabolic diseases have received increasing research attention.

Mechanisms for succinylation: On one hand, succinylation could be processed *via* non-enzymatic manners, which relies on succinyl-CoA or succinate from mitochondrial and peroxisome sources[4,5,10-14]. Succinylation would occur if provided with sufficient succinyl-CoA[10]. It has been established that mixing succinyl-CoA with albumin or isocitrate dehydrogenase increases succinylation and mitochondrial pH in a pH-dependent and dose-dependent manner[4,5]. Sreedhar *et al*[11] showed that nicotinamide adenine dinucleotide phosphate-specific isocitrate dehydrogenase mutation results in a 280% increase in cellular succinyl-CoA levels and mitochondrial hyper-succinylation. Succinate dehydrogenase inactivation induces excessive succinylation *via* increasing the accumulation of succinyl-CoA[12]. Notably, tissues with high levels of succinyl-CoA also show a strong extent of succinyl modification, such as in the heart and liver[13]. Succinate entering the cells could be converted to succinyl-CoA to enhance lysine succinylation[1]. A study has shown that dietary succinate increases the succinylation of intestinal and hepatic proteins with a molecular weight of 25-35 kD in zebrafish[14].

On the other hand, the extent of succinylation could be positively regulated by several enzymes that play succinyl-writer roles (Figure 1)[15-19], even though no specific succinyltransferases have been identified to date. For example, lysine acetyltransferase 2A (KAT2A) was found to be a succinyltransferase[15,16], which can reportedly upregulate H3K79 succinylation and β-catenin stabilization, thereby promoting glycolysis[20]. Zhou *et al*[21] confirmed that KAT2A promotes the succinylation of K46 and K280 of C-terminal binding protein 1 and mediates the transcription-suppressing activity. In addition, histone acetyltransferase 1 (HAT1) was identified as a succinyltransferase of both histone and non-histone proteins[17,22]. HAT1 mediates the succinylation of histones, and quantitative proteomic analysis revealed five succinylation sites on 45 histones[17]. Research has shown that HAT1 is necessary for the regulation of epigenetic and gene expression by H3K122 succinylation[17].

Wang *et al*[22] and Yang *et al*[17] demonstrated that phosphoglycerate mutase 1 (PGAM1), a critical enzyme in glycolysis, is succinylated by HAT1 at K99. The later report also mentioned that aspirin downregulates HAT1 by targeting NF-kappaB to induce PGAM1 K99 desuccinylation, which suppresses the glycolytic process[22]. Furthermore, the α-ketoglutarate dehydrogenase complex (α-KGDHC) regulates succinylation either by regulating succinyl-CoA levels or by directly catalyzing succinylation[4,18]. Inhibition of α-KGDHC reduces succinylation levels of both cytoplasmic matrix and mitochondrial proteins[4]. The E2k subunit of α-KGDHC was demonstrated to be essential for its trans-succinylase activity. The absence of the E2k subunit reduces succinylation, while the presence of alpha-ketoglutaric acid

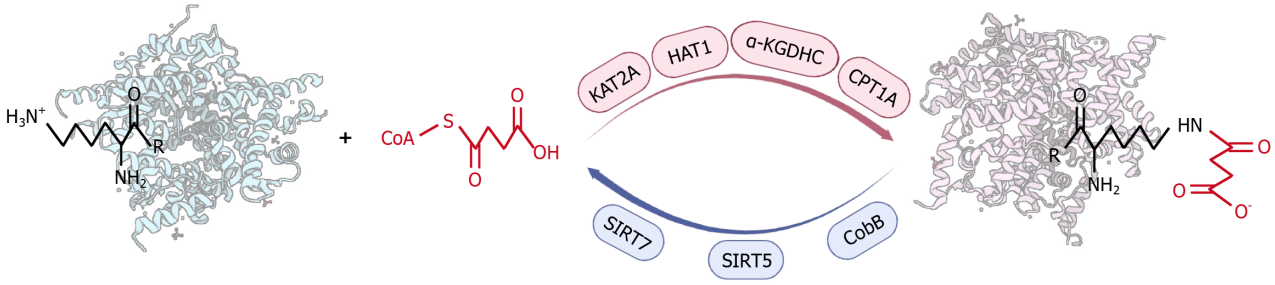

Figure 1 Mechanisms for succinylation. Succinylation is the process of transferring negatively charged four-carbon succinyl groups to amines of lysine residues through enzymatic and non-enzymatic manners using succinyl-CoA as a direct substrate. The succinylation degree can be promoted by succinyltransferases, such as lysine acetyltransferase 2A, histone acetyltransferase 1, α-ketoglutarate dehydrogenase complex, and carnitine palmitoyltransferase 1A. Meanwhile, desuccinylases, including CobB, sirtuin 5, and sirtuin 7 negatively regulate the extent of protein succinylation. KAT2A: Lysine acetyltransferase 2A; HAT1: Histone acetyltransferase 1; α-KGDHC: α-ketoglutarate dehydrogenase complex; SIRT5: Sirtuin 5; SIRT7: Sirtuin 7; CPT1A: Carnitine palmitoyltransferase 1A.

increases succinylation[4].

Another lysine succinyltransferase in mammalian cells is carnitine palmitoyltransferase 1A (CPT1A)[19]. Kurmi *et al*[19] demonstrated that CPT1A can play the role of a succinyltransferase both *in vivo* and *in vitro* to regulate substrate proteins and related metabolic processes. Wang *et al*[23] discovered that CPT1A-mediated succinylation of S100A10 (a protein that is overexpressed in gastric cancer) increases human gastric cancer invasion. Moreover, CPT1A promotes the succinylation of mitochondrial fission factor at K302 and enhances the development of ovarian cancer[24].

In addition, significant progress has been made in the exploration of desuccinylases that negatively regulate succinylation (Figure 1). CobB was the first desuccinylase discovered in prokaryotes with both deacetylation and desuccinylation activities[25]. A high-performance liquid chromatography assay showed that CobB could deacetylate and desuccinylate a histone H3K9 peptide with similar efficiency, whereas the desuccinylation activity of CobB might be induced when cells are treated with succinate[25].

Sirtuin 5 (SIRT5) and sirtuin 7 (SIRT7) are currently known as important desuccinylases in eukaryotes[26-32]. SIRT5 acts in all cell compartments. The activity of SIRT5 is dependent on NAD^+, which is influenced by the availability of NAD^+ (substrate) and the amount of nicotinamide (product)[26]. In SIRT5 knockouts, more than 80% of proteins are succinylated in the tricarboxylic acid cycle to enhance cell respiration, and 60% of proteins in fatty acid metabolism are succinylated[27]. At least 2565 succinylation sites on 779 proteins in mammalian fibroblasts and liver tissues were found to be regulated by SIRT5[27]. Novel targets for SIRT5 in regulating the mitochondrial lysine succinylome such as uncoupling protein 1 in mouse brown adipose tissue were recently identified[28,29].

SIRT7 is a member of the sirtuin family proteins that are described as NAD (+)-dependent class III histone deacetylases [30,31]. Research indicated that SIRT7 catalyzed the desuccinylation of H3K122, which promoted chromatin condensation and DNA double-strand break repair[30]. Yu *et al*[31] showed that SIRT7 restricted chronic hepatitis B virus (HBV) transcription and replication through catalyzing desuccinylation of H3K122 that is associated with covalently closed circular (ccc) DNA minichromosome. SIRT7 mediates the desuccinylation of arginine methyltransferase 5 (PRMT5) K387, which is involved in lipid reprogramming, tumor growth, and metastasis[32].

Collectively, succinylation is the process of transferring negatively charged four-carbon succinyl groups to amines of lysine residues through enzymatic and non-enzymatic manners using succinyl-CoA as a direct substrate. The succinylation degree could be promoted by succinyltransferases, such as KAT2A, HAT1, α-KGDHC, and CPT1A. Meanwhile, desuccinylases, including CobB, SIRT5, and SIRT7 negatively regulate the extent of protein succinylation. To date, the characterization of succinyltransferases and desuccinylases, their target specificity, the function of succinylation, and their clinical application still need to be further investigated, given their significance for proteomic analysis.

Effects of succinylation on hepatic metabolism

The liver is a crucial metabolic organ through which major metabolic processes including glucose, amino acid, and lipid metabolisms occur[33]. The overall abundance of lysine succinylation in the liver is higher relative to other tissues, with proteins and enzymes in several metabolic pathways being succinylated[34].

Influence of protein succinylation on glucose and amino acid metabolism: Glucose homeostasis is largely regulated by hepatic glycogen synthesis, gluconeogenesis, and glycolysis[35,36]. Enhancement of glycolysis contributes to the growth of tumor cells. Yang *et al*[17] performed a Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis on HAT1-targeted non-histone proteins and found that HAT1 mediates the succinylation of glycolytic-related proteins, including seven key enzymes including GPI, TPI, GAPDH, PGK, PGAM, enolase, and PKM. The authors further demonstrated that the HAT1-induced K99 succinylation of PGAM1 increased its activity, which further promoted tumorigenesis[17]. Wang *et al*[22] showed that aspirin reduced HAT1 expression, which decreased the K99 succinylation level of PGAM1, thereby restricting PGAM1 activity and inhibiting glycolysis in liver cancer (Figure 2).

The liver is also a major tissue for the conversion of ammonia[37], which is a toxic metabolite derived from amino acid metabolism under physiological conditions[38]. For the conversion of ammonia to non-toxic urea *via* the urea cycle, carbamoyl phosphate synthase 1 (CPS1) is the first enzyme that is highly abundant in mitochondria, and it is expressed

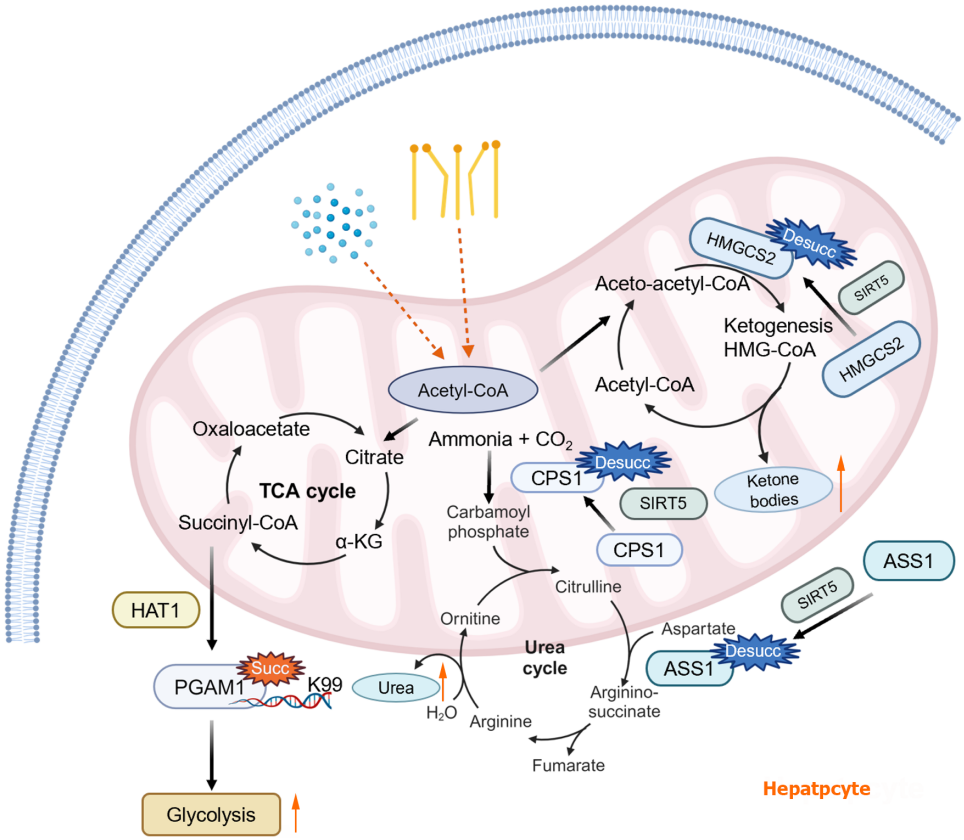

Figure 2 Effect of succinylation on hepatic metabolic pathways. The influence of succinylation on hepatic glucose metabolism occurs in the following ways: (1) Under the stimulation of succinyl-CoA. Histone acetyltransferase 1 causes the K99 site of phosphoglycerate mutase 1 to be succinylated and promotes its enzyme activity, thus promoting glycolysis; (2) the influence of succinylation on hepatic amino acid metabolism. Sirtuin 5 promotes urea production by regulating the desuccinylation of arginine succinate synthetase 1 and carbamoyl phosphate synthase 1; and (3) the influence of succinylation on hepatic lipid metabolism. Sirtuin 5 induces desuccinylation of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthetase 2 and promotes ketone body formation. HAT1: Histone acetyltransferase 1; SIRT5: Sirtuin 5; TCA: Tricarboxylic acid; HMGCS2: 3-hydroxy-3-methylglutaryl-CoA synthetase 2; PGAM1: Phosphoglycerate mutase 1; ASS1: Arginine succinate synthetase 1.

mainly in hepatocytes[39]. Polletta *et al*[40] demonstrated that mitochondrial SIRT5 not only promotes ammonia detoxification by catalyzing desuccinylation of CPS1, but it also regulates glutamine homeostasis and ammonia levels by inhibiting glutaminase activity to reduce ammonia release and the conversion of glutamine to glutamate (Figure 2). Additionally, Zhang *et al*[41] conducted stoichiometry of lysine succinylation in mouse liver and found several highly succinylated lysine sites in arginine succinate synthetase (ASS1-a key enzyme in the urea cycle), which were regulated by SIRT5. Metabolomic analysis confirmed that SIRT5 deficiency reduced liver urea cycle activity, and more importantly, SIRT5 deficiency affected ammonia tolerance.

Influence of protein succinylation on lipid metabolism: The liver serves as an important regulator of lipid homeostasis [42], which includes lipid uptake, lipogenesis, fatty acid oxidation, ketogenesis, and lipid secretion[43]. When lipid synthesis exceeds lipolysis or export, it causes the accumulation of lipids in hepatocytes, ultimately leading to hepatic steatosis[32,44]. PRMT5 is a type II arginine methyltransferase that affects a variety of metabolites including phospholipids, fatty acids, and steroid hormones. Yuan *et al*[32] demonstrated that SIRT7-mediated desuccinylation of PRMT5 at K387 increases its methyltransferase activity, thereby upregulating lipid metabolism-related factors, such as sterol-regulatory element binding protein 1a (SREBP1a), FASN, ACACA, PPARγ, SCD, *etc*. Moreover, SIRT5 is also involved in the regulation of fatty acid β-oxidation[45]. When SIRT5 is deficient, fatty acid β-oxidation is reduced, which leads to fat accumulation in the liver[13].

Ketone bodies, which are comprised of acetoacetic acid, β-hydroxybutyrate, and acetone[46], are produced by the liver through fatty acid catabolism during glucose deficiency[47,48]. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthetase 2 (HMGCS2) is a key enzyme required for ketogenic biosynthesis, which is regulated by succinylation[49]. Early studies on ketogenic regulation have shown that the accumulation of succinyl-CoA is the main process leading to enzyme inactivation in the liver. It was reported that glucagon drastically reduced succinyl-CoA levels and HMGCS2 succinylation, which led to strong ketogenic activation[4]. SIRT5 induces desuccinylation of HMGCS2 and promotes ketone body formation (Figure 2). Among the 15 succinylated lysine residues identified on HMGCS2, several sites appear to be highly targeted by SIRT5 including K83, K310, K350, K354, and K358[50]. Studies have shown that lysine adjacent to the HMGCS2 substrate binding site was strongly succinylated, suggesting that succinyl-CoA may interact with lysine residues around the catalytic pocket, resulting in non-enzymatic modification of these lysines[51,52].

At present, the discovery that various enzymes involved in liver glucose, amino acid, and lipid metabolisms were regulated by succinylation is only the tip of the iceberg, and whether other enzymes in the liver are modulated by succinylation remains to be ascertained.

Influence of succinylation on hepatic glucose metabolism: Under the stimulation of succinyl-CoA, HAT1 causes the K99 site of PGAM1 to be succinylated and promotes its enzyme activity, thus promoting glycolysis. The influence of succinylation on hepatic amino acid metabolism is shown by SIRT5 promotion of urea production by regulating the desuccinylation of ASS1 and CPS1. The influence of succinylation on hepatic lipid metabolism is shown by SIRT5-induced desuccinylation of HMGCS2 that promotes ketone body formation.

Succinylation in the progression of liver diseases

Several studies have established that succinylation is strongly associated with the progression of liver diseases, primarily for fatty liver disease, hepatitis, and hepatocellular carcinoma (HCC). Succinylation not only regulates fat deposition and thus fatty liver degeneration[45,53], but it also promotes HBV transcription and replication[31]. In addition, succinylation stimulates immune escape and tumor growth in HCC[54]. Therefore, the specific roles of succinylation in liver diseases are discussed herein.

Succinylation is involved in fatty liver disease: Fatty liver, which is caused initially by excessive fat accumulation in the liver, is a common chronic disease with a high prevalence worldwide[55,56]. As one of the metabolism-related post-translational modifications, the succinylation degree is enhanced in fatty liver samples[45,57]. Cheng *et al*[57] conducted quantitative succinylated proteome analysis using the livers of nonalcoholic fatty liver disease (NAFLD) rat models and identified 178 differentially succinylated proteins, which were involved in various metabolic and cellular processes and could promote the progression of NAFLD to varying degrees. Another study[45] also indicated that overexpression of SIRT5 in the liver resulted in decreased succinylation, enhanced fatty acid oxidation, and attenuated fatty liver degeneration. SREBP1, one of the transcription factors regulating hepatocellular lipogenesis, induces the expression of several lipogenic genes[58]. Guo *et al*[53] found that histone deacetylase 1 stabilized by P50 maintains SREBP1c activity through desuccinylation and promotes hepatic steatosis (Figure 3A). Yuan *et al*[32] also verified that SIRT7-mediated desuccinylation of PRMT5 at K387 promoted fatty liver by inducing arginine methylation of SREBP1a (Figure 3A). In summary, proteins with varied levels of succinylation may be potential targets for the treatment of fatty liver.

Succinylation promotes hepatitis virus replication: Viral hepatitis is an infectious disease threatening human health, with a growing number of incidences in recent years[59]. HBV is a hepatotropic DNA virus that encodes multiple gene products for viral replication[60-62]. cccDNA plays an important role as a template for HBV transcription[63]. In the nucleus of HBV-infected cells, SIRT7 catalyzes the desuccinylation of cccDNA-bound histone H3K122, thereby limiting HBV transcription and replication[31]. KAT2A is identified as an important host factor for HBV replication[16]. Wang *et al*[15] confirmed that KAT2A is coupled to nuclear α-KGDHC, which acts as a histone H3 succinyltransferase. Later research[64] found that KAT2A can bind to cccDNA by interacting with the HBV core protein and catalyzing the succinylation of H3K79 on cccDNA (Figure 3B), thus promoting cccDNA transcription. Interestingly, Yuan *et al*[65] discovered that IFN-α restrains HBV cccDNA by downregulating KAT2A-mediated histone H3K79 succinylation. Collectively, targeting succinyl-modification enzymes and the succinylated proteins may provide new perspectives for the treatment of HBV.

Succinylation degree is associated with the progression of HCC: HCC is a common and highly lethal cancer, which ranks fourth in cancer incidence and second in cancer mortality[66,67]. In liver cancer patients, the expression of SIRT7 is significantly higher than that in normal liver tissues, and this initially increases at the first and middle stages of HCC but tends to decrease at the later stages[68]. Moreover, deficiency of SIRT5 promotes HCC and is associated with oxidative damage response[54]. Sun *et al*[54] showed that SIRT5 depletion led to increased lysine succinylation of acyl-CoA oxidase 2 (ACOX2) (Figure 3C), resulting in the synthesis of primary bile acids, which further promoted immune escape and tumor growth in HCC. In addition, Yang *et al*[17] confirmed that HAT1 promoted cell proliferation in HCC by catalyzing H3K122 succinylation (Figure 3C). Aspirin inhibits the succinylation level of PGAM1 at K99 by downregulating the expression of HAT1 and decreasing the level of glucose consumption and lactic acid production in liver cancer cells, thereby attenuating the glycolytic pathway in HCC[22,69]. In view of the complex roles of the succinylation signaling pathway in HCC, further studies are necessary to distinguish the pleiotropic effects of succinylation for its application in treating liver cancers.

CONCLUSION

Through delineating the pleiotropic relationships between succinylation and hepatic metabolism, protein succinylation is involved in various physiological and pathological processes in the liver. Despite the significant progress in understanding this kind of post-translational modification, many issues remain unresolved, providing opportunities for future studies.

Succinylation is site-specific, and some proteins have several succinylation sites to make the substrate perform varied biological functions. Research has found that the 252 identified succinylated proteins have 1190 SuK sites and a total of 6579 lysines, with at least 18% of lysines on these proteins being modified by succinylation[49]. Whether these lysine succinylation sites overlap with known enzyme active sites may be an important sign to examine the function of

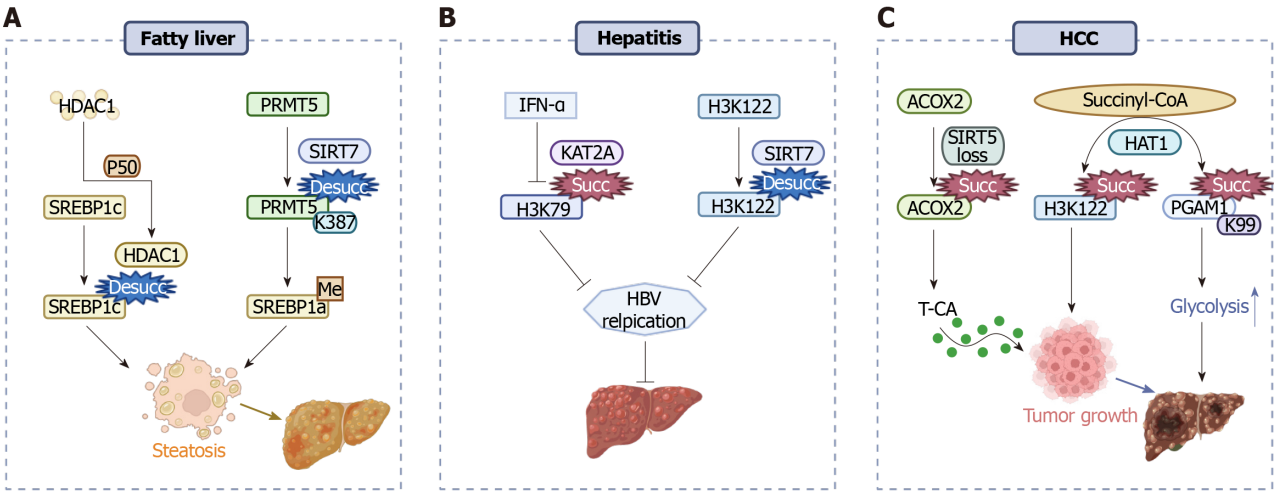

Figure 3 Succinylation affects the progression of fatty liver, hepatitis, and hepatocellular carcinoma. A: P50 stabilizes histone deacetylase 1 protein to keep desuccinylation of sterol-regulatory element binding protein 1c, thereby promoting fatty liver. Sirtuin 7 (SIRT7)-mediated desuccinylation of SIRT7 mediates the desuccinylation of arginine methyltransferase 5 at K387 promotes fatty liver by inducing arginine methylation of SREBP1a; B: IFN-α inhibits lysine acetyltransferase 2A-mediated succinylation of histone H3K79 and SIRT7 promotes desuccinylation of histone H3K122, which restrains viral replication and hepatitis; C: Sirtuin 5 deficiency activates acyl-CoA oxidase 2 succinylation, leading to elevated bile acid levels and promoting hepatocellular carcinoma (HCC). Histone acetyltransferase 1 not only promotes hepatocellular carcinogenesis by activating H3K122 succinylation but also promotes the glycolytic pathway by promoting succinylation of phosphoglycerate mutase 1 at K99, thereby promoting HCC. HBV: Hepatitis B virus; PRMT5: SIRT7 mediates the desuccinylation of arginine methyltransferase 5; SREBP: Sterol-regulatory element binding protein; ACOX2: Acyl-CoA oxidase 2; HAT1: Histone acetyltransferase 1; HCC: Hepatocellular carcinoma; PGAM1: Phosphoglycerate mutase 1.

succinylation regulation. Therefore, further research on the exact influences and mechanisms for succinylation on different proteins and/or different lysine sites of one target protein is of great importance.

Some specific succinylases regulate glycolysis and amino acid and lipid metabolisms by modifying the succinylation degree of critical enzymes. Are there other succinylases that are crucial for hepatic metabolism? Histone deacetylase 1 maintains SREBP1c activity through desuccinylation and promotes hepatic steatosis[53]. Similarly, some succinylation-modifying enzymes also exert other enzymatic activities. For instance, the demalonylation activity of SIRT5[27] and the acetylation activity of KAT2A are likely to contribute to regulating the biological processes of the liver. This suggests that some enzymes with other functions can also exert succinylation or desuccinylation activity, and some identified succinylases may act as other enzymes to participate in varied metabolic reactions.

In addition, succinylation-regulated metabolic processes could affect the progression of fatty liver, hepatitis, and HCC. In some cases, the effect of succinylation on disease development may not be common between histone and non-histone proteins. For instance, Yuan *et al*[32] verified that SIRT7-mediated desuccinylation of PRMT5 at K387 promoted fatty liver. Meanwhile, SIRT7 catalyzes the desuccinylation of cccDNA-bound histone H3K122, thereby limiting HBV transcription and replication[16]. This indicates that the roles of succinyltransferase/desuccinylase are not consistent with different metabolic environments or reactions. Therefore, we ask that the following scientific questions be resolved. What are the differences in succinylation levels and regulatory mechanisms during the occurrence and development of various metabolic diseases at different stages? How can we modulate more succinylation-related pathways in target tissues to improve human health?

In conclusion, the in-depth study of these issues would greatly enhance our understanding of protein succinylation, which further supports the theoretical basis for the treatment of metabolic diseases and the development of related drugs.

FOOTNOTES

Co-first authors: Shuang Liu and Rui Li.

Author contributions: Liu S and Li R wrote the original draft, created the figures, and revised the manuscript; Sun YW wrote the original draft and created the figures; Lin H supervised and verified the paper; Li HF supervised, conceived, verified, reviewed, and edited the manuscript; All authors were involved in the critical review of the results and have contributed to reading and approving the final manuscript. Liu S and Li R contributed equally to this work as co-first authors. The reasons for designating Liu S and Li R as co-first authors are twofold. First, the review was prepared as a collaborative effort with Liu S and Li R contributing equally to literature searching, draft writing, figure drawing, and manuscript revising. The designation of co-first authors authorship reflects the distribution of responsibilities and burdens associated with the time and effort required to complete the review and ensure effective communication and management of post-submission matters. Second, Liu S and Li R are skilled in different fields, which promotes the most comprehensive and in-depth discussion of the review topic, ultimately enriching reader understanding by offering various expert perspectives.

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Hepatocellular carcinoma immune microenvironment and check point inhibitors-current status

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary tumor of the liver and has a high mortality rate. The Barcelona Clinic Liver Cancer staging system in addition to tumor staging also links the modality of treatment available to a particular stage. The recent description of the tumor microenvironment (TME) in HCC has provided a new concept of immunogenicity within the HCC. Virus-related HCC has been shown to be more immunogenic with higher expression of cytotoxic T lymphocytes and decreased elements for immunosuppression such as regulatory T cells. This immunogenic milieu provides a better response to immunotherapy especially immune checkpoint inhibitors (ICIs). In addition, the recent data on combining locoregional therapies and other strategies may convert the less immunogenic state of the TME towards higher immunogenicity. Therefore, data are emerging on the use of combinations of locoregional therapy and ICIs in unresectable or advanced HCC and has shown better survival outcomes in this difficult population.

Key Words: Hepatocellular carcinoma; Tumor immune microenvironment; Immune checkpoint inhibitor; Atezolizumab; Bevacizumab; Pembrolizumab

Core Tip: Hepatocellular carcinoma (HCC) is a prototype of inflammation-associated cancer. Its varied etiology from viral to alcohol and non-alcoholic steatohepatitis, tumor extent, intrahepatic spread, vascular invasion and metastases along with the underlying severity of liver dysfunction make it a complex scenario for adequate management. The recent elaboration of the tumor microenvironment revealing an immunogenic milieu and bringing the concept of "Cold" and "Hot" tumor opened the way for evaluation of immunotherapy in HCC. In recent years, with use of immune checkpoint inhibitors, there is a paradigm shift in the management of advanced and unresectable HCC. With the use of combination regimens including immune checkpoint inhibitors and transarterial chemoembolization/ablation/tyrosine kinase inhibitors, there is an ongoing effort to improve disease outcomes and minimize adverse events.

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INTRODUCTION

Primary as well as metastatic carcinoma can be found in the liver. Liver cancer is the sixth most common cancer worldwide and ranks fourth in the list of cancer-related deaths. It has a dismal 5-year survival of 18%[1]. Hepatitis B which is a carcinogenic virus has remained among the most common causes of hepatocellular carcinoma (HCC), especially in China. However, with universal immunization programs and hepatitis C elimination programs, alcohol and metabolic dysfunction associated steatotic liver diseases are emerging etiologies of HCC worldwide.

The Barcelona Clinic Liver Cancer (BCLC)[2] guidelines, first proposed in 1999, are the most accepted and practiced guidelines for prognostication and management of HCC. The recent BCLC 2022 update[3] has further clarified the grey areas in different stages of HCC, downstaging of tumors, treatment stage migration and progression of HCC within the same stage. "Untreatable progression" represents failure of the selected treatment strategy or progression of disease but remains in the same stage resulting in the need for consideration of therapy for a more advanced stage. This led to new staging upon progression after initial diagnosis, which includes 3 groups BCLCp-B defined as initially stage B and progressed but remained in stage B, BCLCp-C1 shows growth of the existing lesion or new lesions in the liver only. If there is new vascular invasion or new extrahepatic sites of metastases this is considered BCLCp-C2.

The pathophysiology of HCC is intricately linked to chronic liver diseases, which are characterized by prolonged hepatocytic injury and inflammation resulting in repair and regeneration of hepatocytes. These repeated cycles of injury and repair lead to genetic mutations such as in telomerase reverse transcriptase, catenin beta-1, tumor protein 53 (TP53), axis inhibition protein 1, AT-rich interaction domain 1A (ARID1A) and ARID2[4,5]. These mutations affect the cell cycle control and wingless-related integration site (WNT)-beta-catenin pathway which in addition to epigenetic mechanisms result in activation of hepatocarcinogenic pathways. Unlike other solid tumors, no single gene mutation is attributable to HCC development. Systemic therapies have been an integral part of the management of advanced HCC (BCLC stage C), especially tyrosine kinase inhibitors (TKIs) [Sorafenib 2007 and lenvatinib (LEN) since 2018]. They have improved outcome in HCC[6-8] and other TKIs such as regorafenib and cabozantinib are used in the second-line treatment of advanced HCC.

Tumor growth and regression also depend on interaction of the immune system with cancer cells, where cancer cells employ mechanisms to evade the immune system such as by downregulating the major histocompatibility complex (MHC) or expressing the immune checkpoint proteins like programmed death receptor ligand-1 (PD-L1) and programmed cell death protein-1 (PD-1). This has led to the development of therapies targeting these molecular and immune mechanisms.

TUMOR IMMUNE MICROENVIRONMENT

HCC is almost a prototype of inflammation-associated cancer. The tumor microenvironment (TME) has both cellular and non-cellular components. The cellular component has damaged hepatocytes, hepatic progenitor cells and different types of immune cells. The non-cellular component has tumor stroma with growth factors, inhibitory factors, proteolytic enzymes and both pro-inflammatory and anti-inflammatory cytokines. The TME is also dependent upon and modulated by the etiology of chronic liver disease, genetics, epigenetics and other factors related to cellular metabolism.

The liver plays a pivotal role in immune regulation with its large reservoir of immunocompetent cells including neutrophils, monocytes, Kupffer cells, natural killer (NK) cells, dendritic cells (DCs) and lymphocytes (B lymphocytes, $CD4^+$, $CD8^+$). To maintain homeostasis, the liver environment always has a balance between pro-inflammatory [Interleukin (IL)-2, IL-7, IL-12, IL-15, and interferon γ (IFN-γ)] and anti-inflammatory mechanisms [IL-10, IL-13, and transforming growth factor β (TGF-β)][9]. In chronic liver diseases, there is an inclination towards pro-inflammatory signals due to necroinflammation in hepatocytes. Also, the abnormal gut-microbiota-liver axis increases the risk of bacterial infections in patients with cirrhosis leading to the production of anti-inflammatory cytokines such as IL-10 by Kupffer cells and DCs in the liver which suppress the co-stimulatory molecules on antigen presenting cells preventing

CD4+ T cells activation[10,11]. T cell mediated immunity is also decreased in chronic hepatitis B whereas hepatitis C evades the immune system of the host due to its high mutational rates and through viral factors that counteract DNA sensors[12,13]. Hence, the microenvironment in cirrhosis is a combination of inflammation and immunosuppression forming a safe niche for cancer cells to grow and counteract the immune mechanisms.

Immune activation

Due to tumor cell proliferation, necrosis and lately due to treatment, cancer cell antigens are released continuously. These antigens are captured by DCs through interaction with toll-like receptor (TLR)2 and TLR4. The DCs undergo maturation and under the influence of chemokines migrate to the lymph nodes[14,15]. Following the activation of co-stimulatory molecules CD40 on DCs, these antigens are presented to CD8+ cytotoxic T lymphocytes (CTLs) in lymph nodes. Additionally, CTLs are also activated by IFN-γ released from NK cells, TH1 cells and tumor necrosis factor (TNF)-α and IL-12 released from macrophages and chemokines (CXCL-9, CXCL-10, CCL-5). With the interaction between lymphocyte function associated antigen 1 on activated T lymphocytes and intercellular adhesion molecule 1, tumor cells can be infiltrated by CTLs. Subsequently with recognition of cancer cells by T cell receptors along with co-stimulatory receptors, activated CTLs kill the cancer cells[16] (Figure 1).

On the contrary, various check point molecules such as CTL-associated protein 4 and PD-1 bind to the CD80/86 molecule and interact with PD-L1 on DCs, respectively, and suppress the immune response. Immune-inhibiting cytokines such as IL-10, TGF-β, prostaglandin E2 (PGE2), and indoleamine 2,3-dioxygenase influence the expression of PD-1 on T cells and PD-L1 on DCs[17]. Additionally, vascular endothelial growth factor (VEGF) phosphatase and tensin homolog deleted on chromosome 10 produced by cancer cells activate the phosphotidylinositol 3/AKT pathway to suppress T cell infiltration[18].

Immune suppression

Tumor associated neutrophils: Neutrophils are a vital component of the immune system playing important roles during infection, injury and tumorigenesis. In the TME, tumor associated neutrophils (TANs) are recruited through the release of CXCL-5 and CXCL-6[19]. These neutrophils have a key role in tumor initiation, proliferation, progression and metastasis. The location of the neutrophils can be predominantly at the tumor periphery initially, and later within the tumor with different phenotypes initially anti-tumorigenic (N1) and later pro-tumorigenic (N2). Cancer associated fibroblasts (CAFs) modulate the expression of CXCL6 and TGF-β through cardiotrophin-like cytokine factor 1 by polarizing the TANs towards the pro-tumoral phenotype (N2). The N2 phenotype form neutrophil extracellular traps (NETs) which are released by a process of cell death called NETosis[20]. These NETs support tumor growth and increase invasiveness through activation of TLR-4/9-COX2. N2 TANs inhibit the activation or migration of neutrophils into the tumor through the PD-1/PD-L1 pathway[21]. Expression of CD66b, PD-L1, CCL-2, CXCL8, TNF-α, and elevation of CD66b+ neutrophils in the peritumoral region has shown decreased survival in HCC patients. Many studies have shown blocking NETs (by inhibiting COX2, inhibiting NETosis by inhibiting cathepsin G) decreased invasion and metastasis *in vitro*[22]. Studies have shown that TANs cause recruitment of macrophages and Treg cells within the tumor by secreting CCL-2 and CCL-17, resulting in resistance to sorafenib[23]. TME neutrophils act as a principal source for the production of prometastatic Oncostatin M and matrix metalloproteinase which promote angiogenesis by releasing pro-angiogenic factors leading to migration of cancer cells. Evasion of autophagy or delay in apoptosis of neutrophils in the TME is also associated with tumor growth and angiogenesis[24]. The extensive role of TANs reveals new horizons in our understanding of the cancer microenvironment and potential therapeutic options.

DCs-the initiator: DCs are unique cells for capturing pathogens or antigens from tumor cells and presenting them to naive T cells which leads to their differentiation into effector T cells marking the initiation of immune response. Based on the stage of differentiation and development, physiological and pathological environment, DCs are divided into (1) Conventional DCs (cDCs) also known as myeloid DCs (CD141+/CD14- type 1 cDCs and CD1c+/CD14- type 2 cDCs); (2) Plasmacytoid DCs (pDCs) (CD303+ CD304+, secreting type I IFN); and (3) Inflammatory DCs[25]. The interaction of DCs with other immune cells occurs in a sequential manner; DCs presenting antigen to CD4+Th cells through MHC class II and CD8+T cells through MHC class I, which results in a co-stimulatory molecular interaction leading to cytokine production that stimulates CD8+T cells differentiation and expansion[26]. Studies have observed that in patients with HCC, there is lowered expression of co-stimulatory molecules and decreased levels of cDCs and pDCs making the TME appropriate for tumor growth. In HCC, the presence of BDCA2+ pDCs increase infiltration of T regulatory cells, which secrete IL-10, and IL-17 producing cells into the tumor. In addition, pDCs and tumor cDCs express Gal9 (ligand of TIM3), PD-L1, MHC-II (for LAG3), and CD80 (for CTLA 4) inducing an immunosuppressive environment in the TME. Newer subsets of DCs (DC-c1-CD1C, DC-c3-CLEC9A, and DC-c4-LAMP3) have been found in treatment naive HCC patients with LAMP3+ DCs having a strong association with exhaustion/regulation of T cells.

The TME also diverts the process of dendropoiesis (DCs generation) and tends to polarize the phenotype of DCs which creates an immunosuppressive environment by acting against anti-tumor immunity[27]. Anti-tumor immunity enhancing strategies such as DC based vaccines or immunotherapies are under clinical trials and have shown better outcomes and an enhanced CD4+T cells/CD8+T cells ratio[28]. The profound impact of DCs on immune modulation may lead to the development of new immunotherapies.

Tumor associated macrophages – a double edged sword: Liver parenchyma has a high macrophage density. Cytokines influence macrophage differentiation into classically activated M1 (CD86+) macrophages performing pro-inflammatory functions and M2 (CD163+, CD206+) macrophages which suppress the immune system and perform tissue repair. Liver tumor associated macrophages (TAMs) are commonly associated with CD68+ as their marker[29]. In HCC, studies have

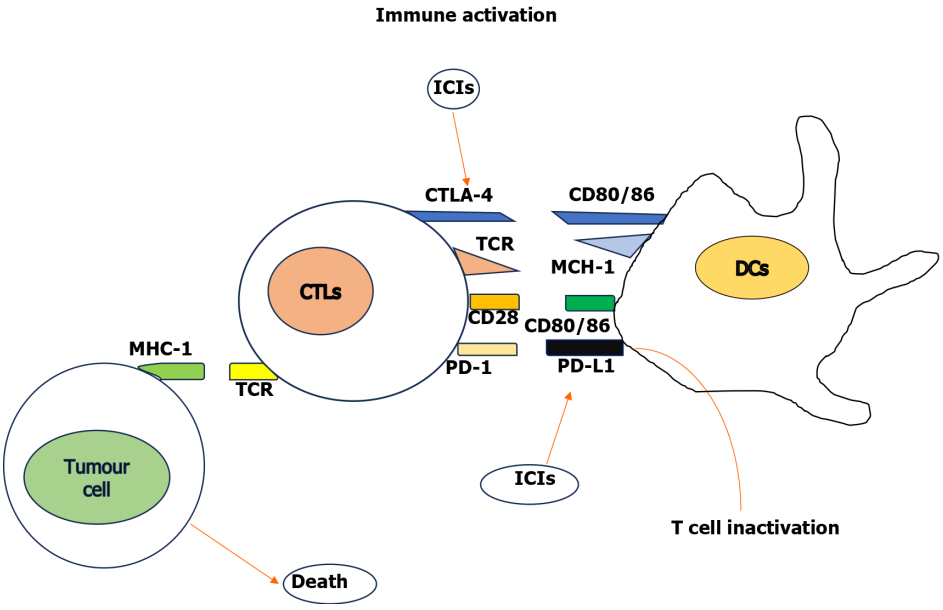

Figure 1 Immune activation in the tumor microenvironment. Interaction between tumor cells and the immune system is demonstrated in this figure. Antigen presentation by dendritic cells (antigen presenting cells) leads to activation of cytotoxic T lymphocytes which eventually leads to death of tumor cells by the release of granzymes and perforins. Activation of cytotoxic T cells require additional co-stimulatory signals during the interaction between dendritic cells and cytotoxic $CD8^+$ T lymphocytes. PD-1: Programmed death receptor-1; PD-L1: Programmed death receptor ligand-1; ICI: Immune checkpoint inhibitor; TCR: T-cell receptor; MHC-1: Major histocompatibility complex I.

shown that joint analysis of high level $CD206^+$ M2 macrophages and low level $CD86^+$ M1 macrophages is associated with aggressive HCC phenotype thus indicating their utility as a prognostic tool for HCC[30]. TAMs promote metastasis, anti-tumor immunity suppression, angiogenesis and drug resistance. The TME contains two different TAMs *i.e.* resident macrophages and infiltrating macrophages. Osteopontin/CSF1/CSF1R pathways are other mechanisms leading to the infiltration of macrophages and drug resistance[31]. Activation of M2 macrophages through the Wnt/β-catenin pathway may pose an increased risk for tumor progression in HCC. TAMs modulate the tumor structure, migration, invasion and metastasis through various cytokines such as IL-6, IL-10, TNF-α, VEGF and other signals inhibiting T cells, NK cells cytotoxicity, and differentiation of Tregs. Studies have documented an association between high levels of TAMs in the peri-tumoral region and poor prognosis of HCC[32]. TAMs with actions of M1 macrophages cause immune activation, phagocytosis, and apoptosis of tumor cells. Many newer immune combination therapies targeting these immune suppressive mechanisms are under trials.

Monocytes and Myeloid derived suppressor cells: Recruited through tumoral CCL-2 production, monocytes have antitumoral effects in the early stages of HCC and later the tumor cells evade monocyte induced death and cause progression of the tumor. In the TME monocytes are classified as $CD14^+$ monocytes, $CCR1^+$ $CD14^+$ monocytes, and Myeloid derived suppressor cells-M type. In advanced stages of HCC, $CD14^+$ monocytes due to the expression of PD-L1/2^+, IL-10, and CCL-1 promoted an immunosuppressive environment in the TME[33]. The CCL-15 chemokine recruits the suppressive phenotype of monocytes and promotes immune escape of HCC by increased angiogenesis and metastasis [34].

Monocytes and Myeloid derived suppressor cells (MDSCs) are immature immune cells that suppress the antitumor immunity in tumors. Phenotypically, MDSCs are classified into two types - polymorphonuclear (PMNs) MDSC and monocytic MDSC[35]. They differ in their mechanism in which they mediate the immune suppression. PMN-MDSCs mediate through PGE2, arg-1, and ROS while M-MDSCs facilitate their action through immunosuppressive cytokines (IL-10 and TGF-β), nitric oxide and immunomodulatory molecules such as PD-L1. In HCC, the proportion of M-MDSCs is high, inhibiting NK cell cytotoxicity and inducing Tregs[36]. The TME in HCC is also modulated by MDSC through the production of angiogenic factors and other enzymes promoting angiogenesis and growth of the tumor.

T regulatory cells-suppressors of anti-tumor immunity: Tregs are a specialized subset of T lymphocytes having a distinctive role in the suppression of excessive immune response and mitigating inflammation. Tregs are classified into (1) Natural Tregs possessing (nucleus FOXP3, CD25 and CTLA-4 surface markers); and (2) Induced Tregs (FOXP3 and CD4+ markers)[37]. These cells modify the T cell activation, differentiation, proliferation and function of effector T cells by various genetic mechanisms. Infiltration of Tregs into the TME is influenced by chemokines CCL-17, 22 through the CCR4 receptor. Most CCR4+ Tregs are more immunosuppressive than CCR4- Tregs[38]. Many studies have shown that although Checkpoint inhibitors show a good response in HCC, a few individuals with resistance to immune therapy can be attributed to Treg cells (Figure 2).

CAF: These are Fibroblast-specialized cells with a role in the synthesis and maintenance of the extracellular matrix. CAFs

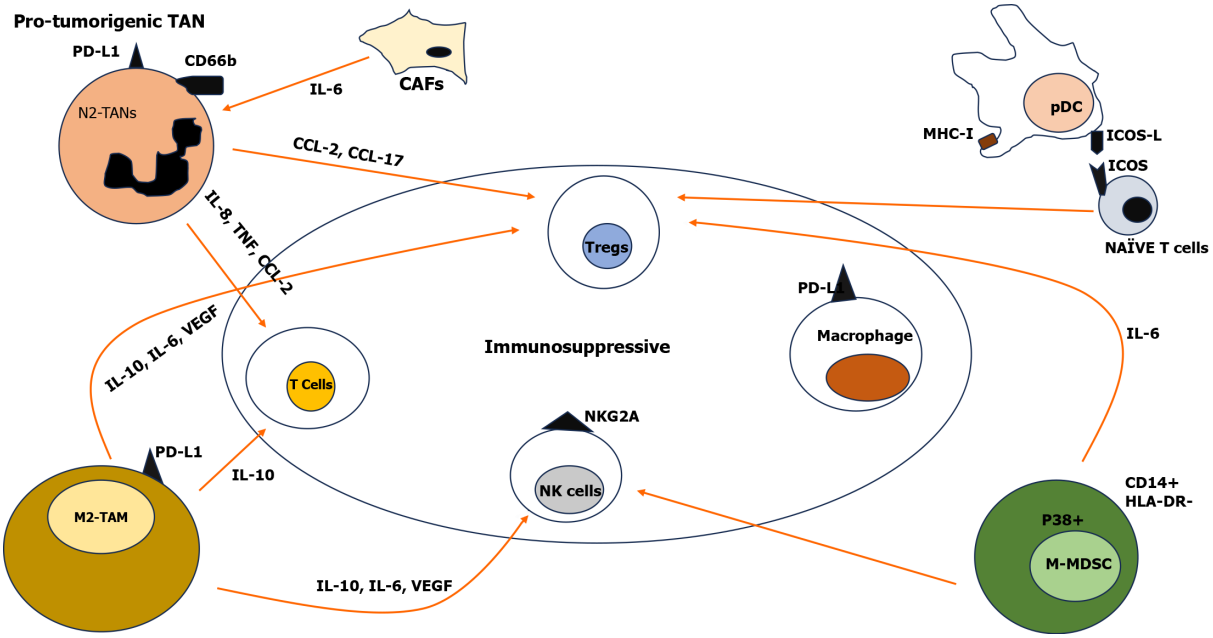

Figure 2 Immune suppression in the tumor microenvironment. An immunosuppressive environment is brought about by the interaction of various immune cells in the tumor microenvironment (TME). N2 pro-tumorigenic tumor associated neutrophils (TANs) influence Tregs and T cells by various chemokines such as CCL-2, CCL-17 and interleukin (IL)-8, tumor necrosis factor, respectively. M2 tumor associated macrophages modulate the TME by influencing Tregs, natural killer (NK) cells and T lymphocytes through IL-6, vascular endothelial growth factor and IL-10, respectively. M type myeloid derived suppressor cells inhibit the NK cells and influence Tregs through IL-6. Cancer associated fibroblasts modulate the effect of N2 TANs *via* IL-6. Dendritic cells also play a role in the TME by regulating Tregs. CAFs: Cancer associated fibroblasts; PD-1: Programmed death receptor-1; PD-L1: programmed death receptor ligand-1; pDC: Dendritic cell; M-MDSC: Monocytic myeloid derived suppressor cells; NK cell: Natural killer cell; VEGF: Vascular endothelial growth factor; TAN: Tumor associated neutrophils; TAM: Tumor associated macrophages; Tregs: Regulatory T cells; IL: Interleukin.

are derived from mesenchymal lineage and contribute to tumor promoting inflammation and fibrosis. CAFs can differentiate from blood vessels, epithelial cells, pericytes, adipocytes *via* endothelial to mesenchymal transition[39]. In HCC, these fibroblasts can differentiate from cancer cells or vascular cells or from mesenchymal stem cells in bone marrow. Based on the expression of α-smooth muscle actin (α-SMA) and IL-6, two major phenotypes of CAFs are present (1) Myofibroblastic (myCAF); and (2) Inflammatory type (iCAF). The myCAF are more matrix-secreting, TGF-β–responsive with high α-SMA expression, and low-cytokine IL-6 and IL-11 production, and are localized in dense stroma near cancer cells. The iCAF exhibit high IL-6 and IL-11 production with low α-SMA expression and are localized in stroma away from cancer cells[40,41].

CAFs induce changes in the tumor by (1) Angiogenesis through production of VEGF, platelet derived growth factor and CXCL-12; (2) Invasion and metastasis; (3) Immune modulation by recruitment of immune suppressors and suppressors of anti-tumor immunity; and (4) Resistance to therapeutic modalities. CAFs in the TME shape the milieu of the tumor by generating pro-inflammatory cytokines including IL-1β and IL-6 and by expressing ligands CXCL12 and CXCL1 resulting in tumor promotion. The interaction of CAFs with other immune cells such as T cells, NK cells, MDSCs, DCs, TANs, and TAMs in the TME result in immunosuppression. The CAFs have a key role in promoting carcinogenesis of epithelial cells and inducing the generation of MDSC through the IL-3/STAT3 axis and SDF-1α which suppress antitumor immunity[42,43].

Cold and hot tumors concept

Based upon the inflammatory milieu of the TME, no T cell infiltrate, presence of regulatory cytokines, no PD-L1, and increased CAFs, increased MDSC population, the tumor is labeled as "COLD" and poorly responsive to immunotherapy [44]. On the other hand, increased T cell infiltrate, pro-inflammatory cytokines, high PD-L1, increased $CD8^+$T cells, and increased TAMs, the tumor is labeled as "HOT" and is amenable to immunotherapy (Figure 3).

IMMUNOTHERAPY IN HCC

The primary treatment options for each HCC stage depends not only on the stage but also on the patient characteristics and profile of the patient. According to BCLC update 2022, systemic therapy is treatment of choice in patients with advanced stage (BCLC-C) HCC and in patients with stage A and B where other treatment options are not feasible or failed[3]. Immune therapy utilizes the body's natural defense mechanisms to combat tumor cells in any cancer. Immune checkpoints (ICs) are molecules present on lymphocytes which regulate the functions of T lymphocytes and influence tumor autoimmunity. Immune cells such as T cells, NK cells, and Tregs express PD-1 checkpoint molecules whereas

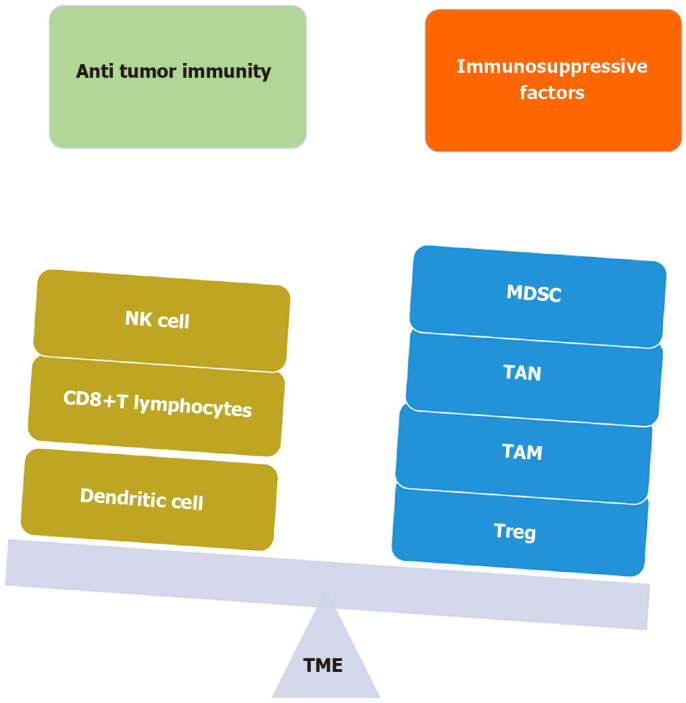

Figure 3 Tumor microenvironment balance. Depicted is the inclination of the tumor microenvironment towards an immunosuppressive environment and its components. NK cell: Natural killer cell; TAN: Tumor associated neutrophils; TAM: Tumor associated macrophages; Treg: Regulatory T cells; MDSC: Myeloid derived suppressor cells; TME: Tumor microenvironment.

stromal cells, myeloid cells, and tumor cells express PD-L1/PD-L2 which inhibit the functions of effector T cells and create an immunosuppressive environment. ICIs target these molecules expressed on immune cells to enhance autoimmunity in the TME.

Current status

Atezolizumab (Atez) is a monoclonal antibody against PD-1 and in combination with Bevacizumab (Bev) monoclonal antibody against the VEGF receptor has been approved as first-line therapy for advanced stage HCC (BCLC stage C). The IMbrave150 study[45] showed that the combination arm (Atez 1200 mg/Bev 15 mg/kg) resulted in overall survival (OS) at 12 months of 67.2% *vs* 54.6% in the sorafenib (400 mg BD) arm. Further analysis showed that the Atez/Bev combination resulted in an OS of 19.2 months *vs* 13.4 months and progression free survival (PFS) of 6.9 months *vs* 4.8 months in the sorafenib arm in unresectable HCC (uHCC), respectively[46]. The beneficial effects of the Atez/Bev combination arm were persistent across BCLC stage B or C, extrahepatic metastases and portal vein invasion. Grade 3 or 4 adverse events related to treatment occurred in 43% and 46% patients in the Atez/Bev combination and sorafenib, respectively. This was a landmark trial and led to FDA approval of this combination (Atez/Bev) in advanced HCC[47]. In a multi-centric retrospective real world evaluation of data, the Atez and Bev combination was well tolerated with no evidence of treatment related deaths or new adverse events across CP-A and CP-B patients with an OS of 14.9 months and PFS 6.8 months[48]. A systematic review of studies from 2002-2020 on systemic therapies in HCC (including all disease stages) examined the association between etiology of HCC and therapy outcomes. The results revealed that immunotherapies were more effective in viral etiologies as compared to non-viral etiologies as compared to TKIs/anti-VEGFs[49]. The viral etiology related HCC is more immunogenic and therefore, ICIs are more effective due to their favorable TME. On the other hand, non-alcoholic steatohepatitis (NASH) related HCC has been shown to accumulate exhausted $CD8^+PD1^+$T cells in the TME, and in preclinical models, anti-PD-1 therapy instead of tumor regression led to tumor progression in terms of size as well number of nodules[50]. Another systematic review[51] showed the non-inferiority of LEN to Atez/Bev in achieving an objective response rate (ORR) and disease control rate (DCR) in advanced HCC; however, data were insufficient for evaluation of OS.

The Himalaya trial evaluated the STRIDE regimen *i.e.* anti-CTLA4 inhibitor tremelimumab single dose (T300 mg) and anti-PD-L1 durvalumab (D1500 mg every 4 wk) in uHCC and found a significant increase in median OS by 2.5 months (16.4 months *vs* 13.8 months) as compared to sorafenib (400 mg BD) alone. They showed that a single priming dose of T was sufficient to enhance the efficacy of D in uHCC patients with no increased adverse drug events[52]. This groundbreaking trial led to the recommendation of tremelimumab and durvalumab as first-line therapy for uHCC.

If first-line therapies are not feasible or contraindicated for some reason, monotherapy with TKIs sorafenib/LEN or durvalumab (anti PD-L1) can be considered.

The KEYNOTE 224 phase II trial[53] documented the antitumor activity and safety of pembrolizumab therapy in patients with HCC previously treated with sorafenib. Subsequently, the multi-centric KEYNOTE 240 phase II trial[54] in HCC patients (previously treated with sorafenib) showed no significant difference in OS and PFS after pembrolizumab therapy. The recent KEYNOTE 394 multi-centric trial[55] from Asia, in HCC patients (post sorafenib or progression/

intolerance on sorafenib) demonstrated significantly increased OS (14.6 months *vs* 13 months, $P = 0.01$) and PFS (2.6 months *vs* 2.3 months, $P = 0.003$) in the pembrolizumab group compared with placebo, respectively.

The CheckMate 040 phase I/II, non-comparative trial[56] showed safety data in patients with advanced HCC treated with nivolumab (anti-PD-1 inhibitor). The phase 3 trial CheckMate 459 compared nivolumab and sorafenib in advanced HCC and both groups had similar OS and PFS with no significant differences. The CheckMate 040 phase III RCT[57] showed improved ORR and durable response with the combination of nivolumab and ipilimumab therapy in advanced HCC (Table 1).

Predictors of response to immunotherapy

Pre-existing immunity *i.e.* intra-tumoral $CD8^+$ Tcell density, high expression of CD274, low Treg to T effector cell ratio, low expression of oncofetal genes such as GPC3 and AFP, high expression of VEGF receptor 2 and myeloid inflammation signatures are predictors of response to the Atez/Bev combination regimen. Viral etiology related HCC is more immunogenic and therefore, is more responsive to ICIs.

Combination therapies

HCC is a complex tumor where multiple factors such as size of the primary tumor, intrahepatic spread, vascular invasion and metastatic disease need to be addressed. Additionally, liver dysfunction and its severity affect the feasibility of locoregional as well as systemic therapies. The combination of locoregional and systemic therapies has been evaluated in recent trials to improve overall outcomes (Table 2).

ICI and transarterial chemoembolization

A propensity score matched study compared the combination of transarterial chemoembolization (TACE) and Atez/Bev against the combination of TACE and LEN (LEN-TACE). They found that both groups showed comparable safety and efficacy in uHCC patients[58]. Another recent Chinese study[59] investigated the combination of TACE and Atez/Bev which resulted in significantly better ORR, OS and PFS as compared to Atez/Bev. The rationale for this, is that TACE decreases the primary tumor load and therefore, the burden of immunosuppressive Treg cells *etc.* and induces hypoxia in the TME. Therefore, CTLs increase in the TME and hypoxia induces an increase in VEGF expression and ICIs (Atez/Bev) have better action due to favorable conditions in the TME. For BCLC stage B, the recommended treatment modality is TACE. Switching to ICIs before deterioration of liver function in patients with BCLC stage B could improve their prognosis and survival. The REPLEC study[60] included HCC patients with BCLC stage B beyond up to seven criteria (unsuitable for TACE) UT-7/multiple/Child Pugh A treated with Atez/Bev who showed an ORR and DCR of RECIST and mRECIST of 17.7%/84.7% and 42.5%/86.2%, respectively.

ICIs and TKIs

The phase 1 KEYNOTE-524 trial[61] demonstrated that the LEN + pembrolizumab (PEM) combination resulted in a median PFS of 9.3 months, ORR of 46% by mRECIST, and median OS of 22 months in patients with uHCC in 29% of BCLC stage B (not suitable for TACE) and in 71% stage C patients. The rationale behind this combination was that LEN, due to its immunomodulatory effects, inhibits proangiogenic and immunosuppressive mechanisms in the TME and enhances the antitumor effects of pembrolizumab. However, the recent LEAP-002 phase 3 trial[62] failed to show any benefit of LEN + PEM combination therapy in uHCC.

ICIs and Ablation

A recent study demonstrated that tremelimumab (anti-CTLA4 Ab) combined with ablation achieved good anti-tumor activity due to enhanced $CD8^+$T cells in the tumor periphery after ablation[63].

ICIs and surgical resection of HCC

Surgical resection in HCC is confined to BCLC very early stage and early stage of HCC (stage A). In advanced stages of uHCC, ICIs are used as bridging therapy for tumor downstaging, negative selection and as neoadjuvant therapy[64]. Downstaging of HCC refers to a shift in tumor stage to a lower level after immunotherapy when surgical intervention can be considered. Negative selection refers to a new concept of "absence of appearance of new lesions after immunotherapy with steady response." This can be treated as localized disease and surgical options can be tried as definitive management. Neoadjuvant therapy is used to shrink the tumor size and allow wider safety margins during surgery. Neoadjuvant immunotherapy is administered for patients with either early resectable tumor or initially unresectable tumor for downstaging[65].

In a study involving 54 patients with uHCC, who received combination immunotherapy followed by radical surgery, pathological evaluation of postoperative specimens confirmed 21.4% ($n = 3$) of patients achieved a complete response and 78.6% ($n = 11$) achieved PR[66]. Zhang *et al*[67] reported 10 patients with HCC and major vascular invasion who achieved a 12-month recurrence-free survival rate of 75% after combinations of ICI and TKI with subsequent salvage surgery[67].

Immune therapy related adverse events

Tolerance of the immune system is the ability to prevent an immune response against a particular antigen. Immune therapy (ICIs) breaks the tolerance of the body's immune system which produces immune related adverse events. Based on common terminology criteria for adverse events grading, the severity of immune therapy related adverse events (irAEs) are Grade 1 mild, Grade 2 moderate, Grade 3 severe, Grade 4 life threatening, Grade 5 death[68]. Inhibition of checkpoint molecules which prevent the tumor cells escaping the immune system can cause disruption in tolerance of the

Table 1 Clinical studies on immunotherapy

No.	Ref.	Study characteristics	Intervention	Patient characteristics	Outcome
1	IMbrave 150[45]	Phase III, Open label RCT	Group I Atez/Bev. Group II SOR	501 BCLC-C, uHCC CP-A, ECOG 0/1	OS: Gp I *vs* II-67% *vs* 54%. PFS: Gp I *vs* II-6.8 months *vs* 4.3 months
2	Multi-centric (Real world data)[48]	Retrospective	Atez/Bev	216 BCLC-C, uHCC m-HCC	mOS-14.9 months; mPFS-6.8 months; ORR (RECIST)-25%; DCR-73%
3	Himalaya[52]	Phase III RCT	STRIDE regime Treme/Durva. Durva alone. Treme alone. SOR alone	1171, BCLC-C, uHCC	mOS: STRIDE *vs* SOR (16.3 months *vs* 13.7 months). ORR: STRIDE *vs* Durva (20.1 *vs* 17%). DCR: STRIDE *vs* Durva *vs* SOR (60.1% *vs* 54.8% *vs* 60.7%)
4	Keynote 224[53]	Phase II, Open label	Pembrolizumab	104, BCLC-C, CP-A ECOG 0/1	ORR-17%
5	Keynote-240[54]	Phase III, Double blind RCT	PEM *vs* Placebo	413, BCLC-C	mOS: PEM *vs* Placebo (13.8 months *vs* 10.6 months). mPFS: PEM *vs* Placebo (3 months *vs* 2.8 months)
6	Keynote-394[55]	Phase III, Double blind RCT	PEM *vs* Placebo	453, BCLC-C	mOS: PEM *vs* Placebo (14.6 months *vs* 13 months). mPFS: PEM *vs* Placebo (2.6 months *vs* 2.3 months)
7	Check-Mate 040 [56]	Phase I/II Open label Noncomparative	Nivolumab	262, BCLC-C CP-A/B ECOG 0/1	ORR: Dose expansion phase 20%. Dose escalation phase 15%

Atez/Bev: Atezolizumab + Bevacizumab; SOR: Sorafenib; RCT: Randomized control study; uHCC: Unresectable HCC; ECOG: Eastern Cooperative Oncology Group; CTP: Child-Turcotte-Pugh; BCLC: Barcelona Clinic Liver Cancer; mHCC: Metastatic hepatocellular carcinoma; OS: Overall survival; PFS: Progression free survival; mOS: Median overall survival; mPFS: Median progression free survival; Treme: Tremelimumab; Durva: Durvalumab; PEM: Pembrolizumab; NIV: Nivolumab; ORR: Objective response rate; DCR: Disease control rate

Table 2 Clinical studies on combination therapies of immunotherapy with locoregional/tyrosine kinase inhibitors

No.	Ref.	Study characteristics	Intervention	Patient characteristics	Outcome
1	Propensity score matched study[58]	Retrospective study	Group I: TACE + Atez/Bev. Group II: TACE + LEN	98, BCLC-C, uHCC	ORR: 12 months. Group I-75%; Group II-79%. mPFS: Group I-7.03 months; Group II-6.03 months. No significant difference in ORR and DCR
2	Chinese study[59]	Retrospective study	Group I: TACE + Atez/Bev. Group II: Atez/Bev only	139, BCLC-C	ORR: Group I-38%; Group II-16.9%. mOS: Group I-14 months; Group II-10 months. mPFS: Group I-10 months; Group II-6 months
3	Keynote-524[61]	Open label Multi-centric study	LEN + PEM	104, uHCC	ORR: mRECIST-46%; RECIST-36%. mPFS: mRECIST-9.3 months; RECIST-8.6 months
4	LEAP 002[62]	Double blind, Randomized control study	Group I: LEN + PEM. Group II: LEN + Placebo	uHCC, CP-A ECOG 0/1	mOS: Group I-21.2 months; Group II-19.0 months. mPFS: Group I-8.2 months; Group II-8.0 months
5	REPLEC study[60]	Multi-centric analysis	Atez/Bev	52, uHCC classified as UT-7, CP-A ECOG0/1	ORR at 6 wk: RECIST-17.7%; mRECIST-42.5%. DCR at 6 wk: RECIST-84.7%; mRECIST-86.2%; mPFS-8 months

TACE: Transarterial chemoembolization; Atez/Bev: Atezolizumab + Bevacizumab; SOR: Sorafenib; RCT: Randomized control study; uHCC: Unresectable hepatocellular carcinoma; ECOG: Eastern Cooperative Oncology Group; CTP: Child-Turcotte-Pugh; BCLC: Barcelona Clinic Liver Cancer; mHCC: Metastatic hepatocellular carcinoma; OS: Overall survival; PFS: Progression free survival; mOS: Median overall survival; mPFS: Median progression free survival; LEN: Lenvatinib; Treme: Tremelimumab; Durva: Durvalumab; PEM: Pembrolizumab; ORR: Objective response rate; DCR: Disease control rate.

immune system (mainly peripheral T cells) leading to proliferation of immune cells and high inflammation and autoimmunity. Hence, most common sites involved would be skin and colon as they predominantly depend on peripheral T cell tolerance for maintaining immune homeostasis[69]. Other mechanisms of irAEs involve autoreactive B cells, cytokines and other host factors.

In HCC patients receiving ICIs, the incidence of irAEs is not higher than that in other carcinomas. The most common irAEs are skin manifestations, followed by gastrointestinal effects such as diarrhea. Hepatic irAEs include raised liver enzymes; however, they are grade 1 to 3 and are not life threatening. Patients with hepatitis B or C seropositive status are not prone to developing a flare if antiviral therapy is started and continued during ICI administration. However, CP-B patients due to their underlying severe liver dysfunction are more prone to severe irAEs. Nivolumab and pembrolizumab monotherapy in HCC patients resulted in rash and pruritus, which were the most common manifestations with an incidence of 11%-23% and 13%-19%, respectively. The incidence of colitis was 1% in patients treated with PD-L1 antibodies and 2.6% in patients treated with the combination of PD-L1 antibody and CTLA-4 antibody. Pneumonia was the main irAE with an incidence of 3% in patients treated with nivolumab[70]. Following tremelimumab therapy, grade 3 or higher encephalopathy was observed, but this may have been attributed to underlying cirrhosis than immunotherapy. Hypertension was observed to be the most common adverse event in patients treated with Atez/Bev with an incidence of 29.8%. Hypertensive encephalopathy, nephrotic syndrome, bleeding, myelosuppression and infection are severe irAEs in patients receiving Atez/Bev and these are influenced mostly by Bev. There is a need for vigilant monitoring to identify adverse events related to immunotherapy and prompt intervention is required for optimal patient outcomes.

CHALLENGES WITH ICIs

Tumor resistance to ICIs is a major challenge which is multifactorial and includes the following: (1) HCC mutational burden-total somatic mutations in HCC responsible for immune cells regulation; (2) Genetic pathways[71] (overactivation of beta-catenin) leading to decreased $CD8^+$T cells infiltration and low PD-L1 expression in the TME; (3) TP53 inactivating mutations leading to ICIs resistance and tumor progression[72]; and (4) T cell exhaustion due to interaction of LAG 3 molecules and overexpressed FGL-1 in the TME[73].

CONCLUSION

ICIs have resulted in a paradigm shift in the management of advanced HCC. However, there is still a long way to go. There is a need to evaluate ICIs use in early HCC and to evaluate their role in downstaging of tumors for curative therapies such as resection or liver transplantation. Future strategies regarding other targets may overcome the ICI resistance seen in clinical practice. With upcoming NASH and obesity epidemics and NASH-HCC being less immunogenic with ICI resistance, it is necessary to determine how this low immunogenicity may be converted or reverted back to the immunogenic state to achieve ICI response. Cell based therapies or vaccines are other areas requiring research.

FOOTNOTES

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ORIGINAL ARTICLE

Case Control Study

Associations of *PNPLA3* and *LEP* genetic polymorphisms with metabolic-associated fatty liver disease in Thai people living with human immunodeficiency virus

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Abstract

BACKGROUND

The prevalence of metabolic-associated fatty liver disease (MAFLD) is a growing public health issue in people living with human immunodeficiency virus (PLWH). However, the pathophysiology of MAFLD is still unknown, and the role of genetic variables is only now becoming evident.

AIM

To evaluate the associations of gene-polymorphism-related MAFLD in PLWH.

METHODS

The study employed transient elastography with a controlled attenuation parameter ≥ 248 dB/m to identify MAFLD in patients from a Super Tertiary Hospital in central Thailand. Candidate single-nucleotide polymorphisms (SNPs) were genotyped using TaqMan® MGB probe 5' nuclease assays for seven MAFLD-related genes. Statistical analyses included SNP frequency analysis, Fisher's Exact and Chi-square tests, odds ratio calculations, and multivariable logistic regression.

RESULTS

The G-allele carriers of *PNPLA3* (rs738409) exhibited a two-fold rise in MAFLD, increasing by 2.5 times in MAFLD with human immunodeficiency virus infection. The clinical features and genetic patterns imply that *LEP* rs7799039 A-allele carriers had a nine times (P = 0.001) more significant chance of developing aberrant triglyceride among PLWH.

CONCLUSION

The current study shows an association between *PNPLA3* rs738409 and *LEP* rs7799039 with MAFLD in PLWH.

Key Words: *PNPLA3*; *LEP*; Metabolic-associated fatty liver disease; People living with HIV; Thai

Core Tip: The prevalence of metabolic-associated fatty liver disease (MAFLD) in people living with human immunodeficiency virus (PLWH) is increasing, becoming a public health concern. The current evidence suggests that aspartate transaminase, fasting plasma glucose, triglyceride, total cholesterol, low-density lipoprotein, and the genetic factors *PNPLA3* rs738409 and *LEP* rs7799039 indicate genetic susceptibility for PLWH, leading to improvements in MAFLD.

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INTRODUCTION

Metabolic-associated fatty liver disease (MAFLD) related to systemic insulin resistance is defined as an accumulation of fat in the hepatocytes of more than 5%, consisting of steatosis, non-alcoholic steatohepatitis, fibrosis, and cirrhosis[1,2]. Nowadays, the pathogenesis of MAFLD remains unclear. Furthermore, the prevalence of MAFLD in people living with human immunodeficiency virus (PLWH) was reported to be 40%-55%, based on different MAFLD phenotype-proven techniques in multiple ethnicities[3,4]. Additionally, liver disease is the second leading cause of death in PLWH[5]. Since 2005, several studies have suggested that MAFLD is common in human immunodeficiency virus (HIV) patients, and its prevalence appears to be increasing. Therefore, HIV infection remains a contributing factor of MAFLD that directly activates insulin resistance in adipose tissue and generates mitochondrial toxicity and reactive oxygen species in hepatocytes, which worsens the MAFLD prognosis[6].

Genetic differences in the risk of MAFLD or NASH progression in the general population are well described[7]. One of the strongest and most consistent associations with the presence and progression of MAFLD in certain populations is associated with the single-nucleotide polymorphism (SNP) on the *PNPLA3* rs738409[8-12]. However, only limited studies exist regarding the role of rs738409 SNP on the *PNPLA3* gene among people living with HIV. A previous report analyzed the association between *PNPLA3* rs738409 polymorphism and the severity of liver disease, insulin resistance, and obesity in patients co-infected with HIV/hepatitis C virus, which was not associated with the duration of the HIV infection or antiretroviral therapy (ART), specific antiretroviral drugs, a history of opportunistic infection, the patient's immune status, or the duration of the aminotransferase elevation[13,14]. Additionally, other studies indicate that genes involved in the lipid metabolism pathway, such as *APOC3, APOB, APOA5,* and *LIPC*, are associated with MAFLD[15-22]. Moreover, *GHRL* and *LEP* also exhibit an association with MAFLD pathogenesis[23-25]. In a previous study, it was shown that the *APOC3* rs2854116 is associated with elevated serum levels of triglycerides, while this genotype did not affect the incidence of lipoatrophy after adjusting for gender and stavudine (d4T)-containing regimens in Thai people living with HIV[26]. Importantly, *GHRL* gene polymorphism was significantly correlated with insulin resistance, which is a hallmark of MAFLD and increased type 2 diabetes mellitus risk, particularly among Chinese people and in other populations[27-29]. However, the genome-wide associations replicated in people living with HIV and MAFLD remain inconclusive. A previous study showed both positive and negative associations between candidate SNP and MAFLD, making it difficult to determine the significance of these findings[7,30]. Hence, our study aimed to evaluate the association between several genes related to MAFLD in Thai people living with HIV.

MATERIALS AND METHODS

Study subjects

We enrolled patients from a Super Tertiary Hospital in central Thailand and classified them into 4 groups: 83 PLWH and MAFLD (Group 1); 94 people living with HIV and without MAFLD (Group 2); 145 with NAFLD without HIV infection (Group 3), and 93 Chinese Dai genotyping data from the 1000 Genome Project phase (http://www.1000genomes.org) used to represent the Thai ethnicity (Group 4). The presence of MAFLD was confirmed *via* transient elastography with a controlled attenuated parameter ≥ 248 dB/m, as prescribed by our colleague in a previous study[31]. The Infectious Disease Clinic enrolled PLWH who were on ART with full viral suppression and had no history of alcohol consumption in the trial. The key inclusion criteria were as follows: PLWH receiving ART with an undetectable HIV viral load for at least 6 months. Patients co-infected with hepatitis B or C virus, other known liver disorders such as cirrhosis or hepatocellular carcinoma, and critical liver disease were all excluded. The study protocol is shown in Figure 1.

Genotyping analysis

The genotyping of seven genes related to MAFLD was performed using an allele-specific TaqMan® MGB probe 5′ nuclease assay with a real-time polymerase chain reaction (PCR) ViiA7™ system (Applied Biosystems, Life Technologies). The allele-specific TaqMan® MGB probe 5′ nuclease chain reaction assay was performed with primers of *PNPLA3* (rs738409); *APOC3* (rs2854116); *APOA5* (rs662799); *APOB* (rs10495712); *LIPC* (rs1800588); *LEP* (rs7799039); and *GHRL* (rs27647). Each 6 μL of the PCR mixture contained 2 μL of genomic DNA in a concentration of 5 ng/μL, 2.5 μL of the TaqMan® Genotyping Master mix, 0.25 μL of allele-specific TaqMan® MGB probe and a sequence-specific primer kit, and 1.25 μL of DNase-free water. The thermal cycler program started with 10 min at 95°C, followed by 50 cycles of 15 s at 92°C and 90 s at 60°C. The allelic discrimination plot was analyzed using ViiA7™ software (Applied Biosystems, Life Technologies). Allele 1 was labeled with VIC® dye fluorescence, and allele 2 was labeled with FAM® dye fluorescence.

Statistical analysis

The frequencies of all SNPs were checked for Hardy-Weinberg equilibrium using the R statistic, version 3.6.1, from the R Foundation for Statistical Computing. Fisher's Exact and Chi-square tests were used to determine the statistical difference between the minor alleles between MAFLD patients and control patients using SPSS version 22.0 for Windows, SPSS Inc., Chicago, IL, United States. The association of the candidate genes' polymorphisms with MAFLD was assessed by calculating the odds ratios and the corresponding 95% confidence intervals. Backward stepwise multivariable logistic regression analysis was used to assess whether one or more genetic factors predicted MAFLD. A *P* value of less than 0.05 was considered significant.

RESULTS

Characteristics of people living with HIV and with MAFLD (Group 1) and of non-MAFLD patients (Group 2) and MAFLD patients (Group 3)

When comparing the people living with HIV and with and without MAFLD, we enrolled a higher proportion of males with a higher BMI. The levels of fasting glucose, HbA1C, triglyceride, aspartate transaminase (AST), alanine transaminase (ALT), and gamma-glutamyl transferase were significantly higher in the MAFLD group compared to the non-MAFLD group in the metabolic profiles and liver function tests. When comparing the HIV treatment regimens, the proportion of non-nucleoside reverse transcriptase inhibitor, nucleoside reverse transcriptase inhibitor (NRTI), protease inhibitor (PI)-

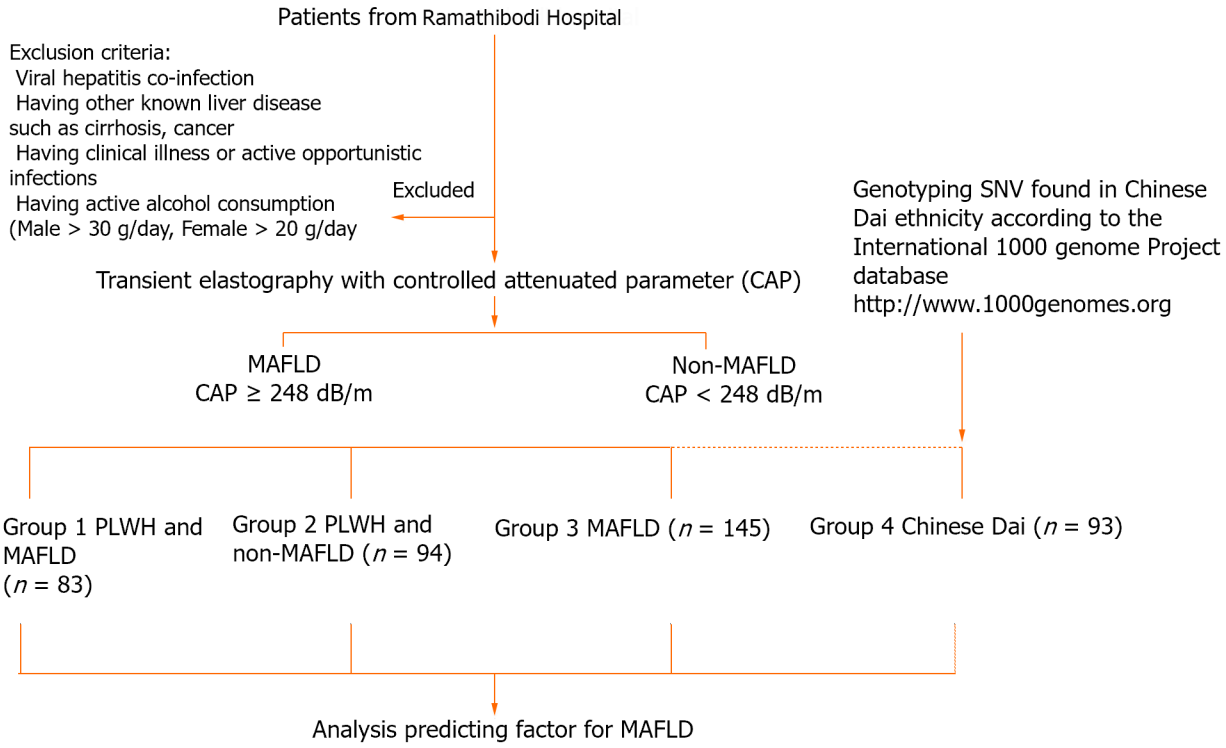

Figure 1 Protocol flowchart. MAFLD: Metabolic-associated fatty liver disease; PLWH: People living with HIV; SNV: Single nucleotide variant.

based, or alternative regimens did not differ between the two groups (P = 0.573), but comorbidities of dyslipidemia, hypertension, and diabetes mellitus were higher in people living with HIV and MAFLD (P = 0.002; P = 0.001 and P = 0.005, respectively) (Table 1).

Distribution of SNPs in people living with HIV and MAFLD (Group 1) or without MAFLD (Group 2), with MAFLD (Group 3), and Chinese Dai (Group 4)

All the SNP genotyping experiments were successful. Throughout the entire study, the genotype frequencies of each SNP did not deviate from Hardy-Weinberg equilibrium (P > 0.05). Table 2 shows the genotype distribution and minor allele frequency of the investigated SNPs in people living with HIV with or without MAFLD, MAFLD patients, and Chinese Dai. All potential SNP genotype distributions (*PNPLA3* rs738409, *APOC3* rs2854116, *APOA5* rs662799, *APOB* rs10495712, *LIPC* rs1800588, *LEP* rs7799039, and *GHRL* rs27647) in people living with HIV with MAFLD were similar to those seen in patients living with HIV without MAFLD. In comparison to Chinese Dai, patients with MAFLD had a higher frequency of the *PNPLA3* G-allele (P = 0.035). The frequencies of the other SNPs were not significant in persons living with HIV and those with or without MAFLD.

Association between PNPLA3 and other candidate SNPs with MAFLD

The data given in Tables 3 and 4 show the well-established *PNPLA3* rs738409 gene, which is found on chromosome 22 and has a function related to lipid droplet formation in the hepatocytes; the G-carrier patients had an approximately two-fold higher risk of developing MAFLD when compared to MAFLD with Chinese Dai (P = 0.012). Importantly, people living with HIV and MAFLD exhibited a 2.5-fold increased risk (P = 0.002) when compared to Chinese Dai (Group 4). In addition, *GHRL* (Ghrelin) rs27647 is a promising susceptibility gene for insulin regulation; the C-allele carrier has exhibited a protective effect in MAFLD in people living with HIV. The odds of having MAFLD is 53% lower if the people living with HIV are C-carriers of *GHRL* rs27647 than if they are not C-carriers (P = 0.047). However, there was no statistically significant association with *GHRL* in the other groups. Furthermore, *APOC3* rs2854116 C-allele carriers were also statistically significant in the MAFLD group, exhibiting a six-fold higher risk in the dominant model with Chinese Dai as the comparison (P < 0.001).

Association between candidate SNPs and the lipid profile, liver function, and glucose metabolisms

We performed a subgroup analysis of people living with HIV in terms of their metabolic profiles and compared the genotypes of candidate genes. As shown in Table 5, the mean or median values of the lipid profile [triglyceride, total cholesterol, LDL]; liver function (AST, ALT); and glucose metabolisms (HbA1C and fasting plasma glucose) were higher in the people living with HIV and MAFLD than in the control group (people living with HIV and non-MAFLD). The association between the genotypes of the *APOA5* rs662799 SNP and serum lipid parameters in the control group is presented in Table 5 and Supplementary Table 1. Serum total cholesterol levels in control patients differed between the AA and AG/GG genotypes (P < 0.05). The *APOA5* rs662799 G allele carriers had a lower proportion of total cholesterol

Table 1 Baseline characteristics of metabolic-associated fatty liver disease patients and controls

Characteristics	PLWH and MAFLD (*n* = 83)	PLWH and non-MAFLD (*n* = 94)	*P* value	MAFLD (*n* = 145)	*P* value	Chinese Dai (*n* = 93)	*P* value
Age (yr)	51.99 ± 7.65	49.55 ± 8.27	0.044[a]	65.00	< 0.001[b]	N/A	N/A
Gender							
Male	54 (65.10)	48 (51.10%)	0.060	68 (46.90%)	0.008[b]	44 (47.30%)	0.950
Female	48 (34.90%)	46 (48.90%)		77 (53.10%)		49 (52.70%)	
BMI (kg/m^2)	25.42	21.79	< 0.001[a]	27.67	< 0.001[b]	N/A	-
CD4 (cells/mm^3)	619.00	570.50	0.033[a]	N/A	-	N/A	-
%CD 4	26.10 ± 0.83	25.76 ± 0.81	0.853	N/A	-	N/A	-
Hb (g/dL)	14.36 1.62	13.84 1.90	0.015[a]	N/A		N/A	
Platelets (-/mm^3)	259063 77332	261694 66966	0.744	N/A		N/A	
AP (U/L)	85.00	86.00	0.821	72.00	0.001[b]	N/A	-
AST (U/L)	33.00	28.00	< 0.001[a]	38.00	0.015[b]	N/A	-
ALT (U/L)	38.00	25.00	< 0.001[a]	50.00	0.004[b]	N/A	-
GGT (U/L)	47.00	35.50	< 0.001[a]	51.50	0.853	N/A	-
Total protein (g/L)	79.60 ± 0.56	78.28 ± 0.47	0.071	75.96 ± 0.42	< 0.001[b]	N/A	-
Albumin (g/L)	40.80	38.35	< 0.001[a]	40.65	0.255	N/A	-
Total bilirubin (mg/dL)	0.60	0.50	0.759	0.70	0.10	N/A	-
Direct bilirubin (mg/dL)	0.20	0.20	0.862	0.30	0.007[b]	N/A	-
HbA1C (mmol/L)	5.68	5.38	< 0.001[a]	6.36	< 0.001[b]	N/A	-
Fasting plasma Glucose (mg/dL)	98	93	0.026	108	< 0.001[b]	N/A	-
Triglyceride (mg/dL)	169	109	< 0.001[a]	123	< 0.0001[b]	N/A	-
Total Cholesterol (mg/dL)	206.78 ± 5.57	199.10 ± 3.57	0.212	183	0.428	N/A	-
HDL (mg/dL)	44	49	0.004[a]	49	0.10	N/A	-
LDL (mg/dL)	130.67 ± 4.27	122.46 ± 2.98	0.086	114.94 ± 2.83	0.001[b]	N/A	-
Drug-regimen, *n* (%)							
NRTI+NNRTI	62 (74.7)	71 (75.5)	0.573	N/A	-	N/A	-
NRTI+PI	19 (22.9)	18 (19.1)		N/A	-	N/A	-
Alternative	2 (2.4)	5 (5.3)		N/A	-	N/A	-
Co-morbidities							
Dyslipidemia	30 (36.1)	15 (16.0)	0.002[a]	82 (56.9)	0.002[b]	N/A	-
Hypertension	21 (25.3)	6 (6.4)	0.001[a]	64 (44.1)	0.003[b]	N/A	-
Diabetes mellitus	16 (19.3)	5 (5.3)	0.005[a]	63 (43.4)	< 0.001[b]	N/A	-

[a]*P* value < 0.05 compared between people living with human immunodeficiency virus (PLWH) and metabolic-associated fatty liver disease (MAFLD) *vs* PLWH and non-MAFLD.

[b]*P* value < 0.05 compared between PLWH and MAFLD *vs* MAFLD.

Data represent as mode, mean ± standard deviation or *n* (%), differences between groups were tested by Chi-square test or one-way ANOVA as appropriate. AP: Alkaline phosphatase; AST: Aspartate aminotransaminase; ALT: Alanine aminotransaminase; ALP: Alkaline phosphatase; Hb: Henoglobin; TB: Total bilirubin; MAFLD: Metabolic-associated fatty liver disease; N/A: Not available; PLWH: People living with human immunodeficiency virus; GGT: Gamma-glutamyl transferase; HbA1C: Hemoglobin A1C; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; NRTI: Nucleoside reverse transcriptase inhibitor; NNRTI: Non-nucleoside reverse transcriptase inhibitor; PI: Protease inhibitor.

Table 2 Genotype distributions and Minor allele frequency of candidates single-nucleotide polymorphisms

Polymorphism	PLWH and MAFLD (*n* = 83)	PLWH and non-MAFLD (*n* = 94)	*P* value	MAFLD (*n* = 145)	Chinese Dai (*n* = 94)	*P* value
PNPLA3 rs738409						
MAF = G	57 (34.33)	56 (29.78)	0.413	92 (31.73)	43 (23.12)	0.035[a]
APOC3 rs2854116						
MAF=T	81 (47.09)	87(46.28)	0.691	128 (44.14)	88 (47.31)	< 0.001[b]
LEP rs7799039						
MAF=G	57 (33.14)	57 (30.32)	0.738	85 (29.31)	48 (25.81)	0.674
GHRL rs27647						
MAF = C	13 (7.56)	25 (13.30)	0.055	28 (9.66)	18 (9.68)	0.702
LIPC rs1800588						
MAF = T	70 (42.17)	70 (37.33)	0.421	101 (34.83)	67 (36.02)	0.872
APOB rs10495712						
MAF = A	15 (8.06)	14 (7.45)	0.853	22 (7.59)	8 (4.30)	0.339
APOA5 rs662799						
MAF = G	41 (22.04)	51 (27.13)	0.766	75 (25.86)	52 (27.96)	0.834

[a]*P* value < 0.05 compared between people living with human immunodeficiency virus (PLWH) and metabolic-associated fatty liver disease (MAFLD) *vs* PLWH and non-MAFLD.

[b]*P* value < 0.05 compared between PLWH and MAFLD *vs* MAFLD MAF minor allele frequency, Chinese Dai was represented as general population. Data represented as *n* (%), PLWH and MAFLD *vs* other groups, differences between groups were tested by Chi-square test. MAFLD: Metabolic-associated fatty liver disease; PLWH: People living with human immunodeficiency virus; MAF: Metabolic-associated fatty.

levels in the normal range (< 200 mg/dL) than the A allele non-carriers and indicated the protective effect of *APOA5* rs662799 in an abnormal range of total cholesterol (> 200 mg/dL); these results showed statistical significance ($P = 0.045$). Furthermore, *LEP* rs7799039 AG and AA carriers exhibited a significant nine-fold higher risk in an abnormal range of triglyceride (> 150 mg/dL) when compared with non-carriers ($P = 0.001$). Unfortunately, none of the individual SNPs were associated with LDL. Moreover, in men, *APOC3* rs2854116 TT alleles also showed a protective effect on the high-density lipoprotein (HDL) profile (Table 5 and Supplementary Table 1). Furthermore, AST is known to be a reliable surrogate marker for outcome measures in MAFLD. Table 6 shows that *PNPLA3* rs738409 G-carrier patients have an approximately 2.5 times higher chance of AST abnormality (> 34 U/L) when compared with non-carriers (statistically significant at $P = 0.010$).

Association between the genetic profile, clinical factors, and MAFLD

A stepwise multiple logistic regression was performed to investigate the relationship between the genetics profiles, clinical factors, and MAFLD. Sixteen variables, including gender, AST, ALT, total cholesterol, triglycerides, HDL, LDL, fasting plasma glucose, HbA1C, and the genetic profiles of *PNPLA3* rs738409, *APOC3* rs2854116, *APOA5* rs662799, *APOB* rs10495712, *LIPC* rs1800588, *LEP* rs7799039, and *GHRL* rs27647 were entered into the original equation. The results showed that seven variables, namely, AST, total cholesterol triglycerides, LDL, fasting plasma glucose, *APOB* rs10495712, and *APOA5* rs662799, were significantly associated with MAFLD (Table 7).

DISCUSSION

Risk factors for MAFLD in people living with HIV (PLWH) include the normal factors seen in the general population, such as components of metabolic syndrome (obesity, diabetes, hypertension, dyslipidemia, a sedentary lifestyle, and excessive dietary intake)[1]. Hepatic steatosis and mitochondrial oxidative stress are pivotal to MAFLD pathogenesis. In PLWH with MAFLD, HIV-specific factors such as lipodystrophy, ART, and HIV infection itself are strongly linked to the development of MAFLD[31,32]. However, our study did not find NRTI-based and PI-based regimens to be predictive factors for MAFLD.

The strongest and most consistent associations with the presence and progression of MAFLD in the studied populations are related to the SNP on the *PNPLA3* rs738409, which was discovered by the first GWAS in 2003[8]. Our study demonstrated the significance of *PNPLA3* rs738409 in MAFLD when compared to the general population, indicating the impact of genetic factors. Moreover, we evaluated the effect of both HIV infection and genetic factors by conducting a comparison between people living with HIV and Chinese Dai, finding that it increased the chance of the

Table 3 Genotype and allele frequencies of the single-nucleotide polymorphisms in the people living with human immunodeficiency virus and metabolic-associated fatty liver disease compared with people living with human immunodeficiency virus and non-metabolic-associated fatty liver disease group

Gene	SNP	B allele	Dominant model				Recessive model			
			PLWH and MAFLD *vs* PLWH and non-MAFLD		PLWH and MAFLD *vs* Chinese Dai		PLWH and MAFLD *vs* PLWH and non-MAFLD		PLWH and MAFLD *vs* Chinese Dai	
			OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
PNPLA3	rs738409	G	1.476 (0.809-2.694)	0.204	2.539 (1.382-4.665)	0.002[b]	0.94 (0.276-3.202)	0.921	0.929 (0.273-3.166)	0.907
APOC3	rs2854116	C	1.117 (0.543-2.297)	0.764	1.203 (0.588-2.462)	0.613	0.798 (0.411-1.550)	0.506	0.828 (0.425-1.614)	0.579
LEP	rs7799039	G	0.704 (0.287-1.727)	0.422	0.408 (0.146-1.142)	0.080	0.886 (0.491-1.601)	0.689	0.730 (0.403-1.322)	0.298
GHRL	rs27647	G	0.466 (0.217-1.001)	0.047[a]	0.704 (0.317-1.566)	0.388	2.146 (1.832-2.514)	0.469	2.134 (1.823-2.499)	0.472
LIPC	rs1800588	T	1.506 (0.806-2.815)	0.198	1.676 (0.898-3.128)	0.104	1.053 0.452-2.456)	0.905	1.040 (0.446-2.427)	0.928
APOB	rs10495712	A	1.264 (0.557-2.871)	0.575	2.156 (0.855-5.436)	0.098	1.134 (0.070-18.422)	1.000	2.134 (1.823-2.499)	0.472
APOA5	rs662799	G	0.914 (0.505-1.654)	0.766	0.960 (0.330-2.790)	0.940	0.629 (0.177-2.231)	0.470	0.734 (0.200-2.697)	0.751

Data expressed as OR odds ratio, CI confidence interval.

[a]*P* value < 0.05 compared between people living with human immunodeficiency virus (PLWH) and metabolic-associated fatty liver disease (MAFLD) *vs* PLWH and non-MAFLD.

[b]*P* value < 0.05 compared between PLWH and MAFLD *vs* Chinese Dai was represented as general population, B-allele expressed risk allele. MAFLD: Metabolic-associated fatty liver disease; PLWH: People living with human immunodeficiency virus; SNP: Single-nucleotide polymorphism.

Table 4 Genotype and allele frequencies of the single-nucleotide polymorphisms in the metabolic-associated fatty liver disease compared with Chinese Dai

Gene	SNP	B allele	Dominate model		Recessive model	
			OR (95%CI)	*P* value	OR (95%CI)	*P* value
PNPLA3	rs738409	G	1.970 (1.160-3.345)	0.012[a]	1.074 (0.377-3.061)	0.894
APOC3	rs2854116	C	6.109 (2.490-14.986)	< 0.001[a]	0.485 (0.259-0.907)	0.022[b]
LEP	rs7799039	G	0.840 (0.300-2.355)	0.740	0.790 (0.469-1.332)	0.376
GHRL	rs27647	G	0.953 (0.491-1.850)	0.888	1.646 (1.486-1.823)	1.000
LIPC	rs1800588	T	0.889 (0.525-1.504)	0.661	1.042 (0.494-2.199)	0.914
APOB	rs10495712	A	1.799 (0.762-4.251)	0.176	1.642 (1.486-1.823)	1.000
APOA5	rs662799	G	0.854 (0.507-1.438)	0.552	0.960 (0.330-2.790)	0.940

[a]*P* value < 0.05 compared between metabolic-associated fatty liver disease (MAFLD) *vs* Chinese Dai in dominant model.

[b]*P* value < 0.05 compared between MAFLD *vs* Chinese Dai in recessive model, B-allele expressed risk allele. Data expressed as *n* (%). MAFLD: Metabolic-associated fatty liver disease; PLWH: People living with human immunodeficiency virus; SNP: Single-nucleotide polymorphism; OR: Odds ratio.

development of MAFLD between 2 and 2.5 times when compared to the genetic factor alone. Moreover, our results agree with previous studies that demonstrated the significant association with *PNPLA3* rs738409 and biopsy-proven fibrosis or steatosis among HIV/hepatitis C virus or HBV co-infected patients, HIV-mono infection, and the group with no viral infection[33-35].

Insulin resistance has been characterized as the crucial pathophysiological factor in MAFLD. The advanced reports found that insulin resistance is associated with the reduction of circulating ghrelin level[21,36,37]. Interestingly, our study has shown that the G/A genotype and G/G genotype of *GHRL* rs27647 were associated with a 53% decreased risk of MAFLD in people living with HIV when compared with non-MAFLD patients. Moreover, a previous study observed higher levels of ghrelin in patients with hypertriglyceridemia, as well as a positive correlation between ghrelin and trigly-

Table 5 Association between genetic polymorphism and Lipid profile

Genetic polymorphisms	Lipid parameters							
	Triglyceride (mg/dL), (*n* = 177)		Total cholesterol (mg/dL), (*n* = 177)		LDL-cholesterol (mg/dL), (*n* = 177)		HDL-cholesterol (mg/dL), (*n* = 66)	HDL-cholesterol (mg/dL), (*n* = 111)
	< 150	≥ 150	< 200	≥ 200	< 130	≥ 130	Men ≥ 40 mg/dL, women ≥ 50 mg/dL	Men < 40 mg/dL, women < 50 mg/dL
PNPLA3 rs738409 CC *vs* CG+GG								
OR (95%CI)	0.699 (0.383-1.277)		1.053 (0.580-1.912)		1.088 (0.597-1.981)		0.9967 (0.538 -1.846)	
P value	0.243		0.865		0.784		0.992	
APOC3 rs2854116 TT *vs* CT+CC								
OR (95%CI)	0.796 (0.387-1.635)		1.611 (0.780-3.328)		1.173 (0.568-2.423)		0.4696 (0.253-0.873)	
P value	0.534		0.195		0.666		0.017[a]	
APOA5 rs662799 AA *vs* AG+GG								
OR (95%CI)	1.021 (0.562-1.855)		0.543 (0.299-0.989)		0.595 (0.326-1.084)		0.739 (0.374-1.461)	
P value	0.946		0.045[a]		0.089		0.385	
APOB rs10495712 (GG *vs* AG+AA)								
OR (95%CI)	0.749 (0.322-1.743)		0.719 (0.315-1.639)		0.807 (0.351-1.855)		0.816 (0.343-1.938)	
P value	0.501		0.431		0.613		0.645	
LIPC rs1800588 CC *vs* CT+TT								
OR (95%CI)	0.870 (0.467-1.621)		0.732 (0.393-1.363)		0.607 (0.326-1.132)		0.911 (0.482-1.722)	
P value	0.661		0.325		0.115		0.774	
LEP rs7799039 GG *vs* AG+AA								
OR (95%CI)	9.316 (2.064-40.428)		1.623 (0.655-4.017)		1.518 (0.602-3.825)		1.317 (0.507-3.419)	
P value	0.001[a]		0.292		0.374		0.572	
GHRL rs27647 (AA *vs* AG+GG)								
OR (95%CI)	0.570 (0.265-1.224)		0.997 (0.483-2.058)		0.905 (0.436-1.878)		0.889 (0.417-1.895)	
P value	0.147		0.993		0.788		0.761	

[a]*P* < 0.05 compare between normal level *vs* abnormal level. OR: Odds ratio; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

ceride levels in patients with hypertriglyceridemia[38,39]. Unfortunately, our study failed to detect the association between SNP and triglyceride levels in people living with HIV.

The *APOC3* gene plays a crucial role in the circulation and clearance of very-low-density lipoprotein, HDL, and chylomicron remnants[40,41]. The polymorphism in the promotor region of the *APOC3* rs2854116 (-455T>C) gene has been extensively studied and has been found to be related with insulin resistance at the transcriptional level. Consequently, the overexpression of *APOC3*, which functions to inhibit lipoprotein lipase and the cellular uptake of triglyceride-rich lipoprotein particles, may result in hypertriglyceridemia, as has been confirmed by *in vivo* and clinical studies[23,24,42-44]. In this study, we showed that *APOC3* rs2854116 C-allele carrier patients have a six-fold higher risk of developing MAFLD in a dominant model. Our findings are also consistent with previous reports that the *APOC3 rs2854116* genetic variant leads to increased plasma concentrations of apolipoprotein C3, resulting in hepatic insulin resistance and MAFLD in multiethnic populations[23,45,46].

Our results show a similar trend to those of a previous report, which demonstrated a positive correlation between AST levels and the accumulation of intrahepatic triglyceride[47]. Interestingly, our results indicate a robust association in *LEP* rs7799039 with the lipid profile, especially with triglyceride levels. According to a subgroup analysis of patients infected with HIV, a patient who is a carrier of the A-allele (AG and AA) has a nine-times-higher risk of exhibiting abnormal triglyceride levels (> 150 mg/dL). Further information suggests that *LEP* rs7799039, located on chromosome 7, encodes 167 amino acid peptide variants with a molecular weight of 16 ku, which may subsequently affect the biological functions of *LEP*[48]. In recent years, *LEP* has been found to regulate the energy balance in coordination with the regulation of the glucose and lipid metabolisms. Thus, it plays a vital role in the development of MAFLD. This finding aligns with that of previous reports that evaluated the association between *LEP* rs7799039 and diabetes mellitus, metabolic syndrome, MAFLD, and cardiovascular disease[49,50]. Our findings should be interpreted while bearing in mind several potential limitations. First, the small sample size of each group may have limited the study's ability to detect a significant

Table 6 Association between genetic polymorphism and metabolic traits

Genetic polymorphisms	Metabolic traits							
	FPG (mg/dL), *n* = 175		HbA1C (mmol/L), *n* = 159		AST (U/L), *n* = 175		ALT (U/L), *n* = 177	
	< 100	≥ 100	< 6.5	≥ 6.5	< 34	≥ 34	< 40	≥ 40
PNPLA3 rs738409 (CC *vs* CG+GG)								
OR (95%CI)	0.354 (0.063-1.984)		1.055 (0.437-2.543)		2.568 (1.243-5.305)		1.679 (0.713-3.953)	
P value	0.219		0.906		0.010[a]		0.232	
APOC3 rs2854116 (TT *vs* CT+CC)								
OR (95%CI)	0.956 (0.923-0.991)		1.553 (0.495-4.897)		0.735 (0.335-1.614)		0.630 (0.253-1.570)	
P value	0.342		0.448		0.442		0.318	
APOA5 rs662799 (AA + AG+GG)								
OR (95%CI)	1.167 (0.229-5.946)		1.213 (0.509-2.893)		0.823 (0.421-1.611)		0.730 (0.320-1.664)	
P value	1.000		0.663		0.570		0.453	
APOB rs10495712 (GG *vs* AG+AA)								
OR (95%CI)	1.100 (0.123-9.802)		1.150 (0.356-3.720)		2.063 (0.879-4.840)		1.255 (0.431-3.651)	
P value	0.923		0.815		0.092		0.774	
LIPC rs1800588 (CC *vs* CT+TT)								
OR (95%CI)	0.947 (0.907-0.989)		1.709 (0.636-4.592)		1.476 (0.719-3.027)		2.208 (0.844-5.776)	
P value	0.093		0.284		0.287		0.100	
LEP rs7799039 (GG *vs* AG+AA)								
OR (95%CI)	0.268 (0.046-1.561)		1.008 (0.272-3.746)		1.329 (0.462-3.827)		2.016 (0.444-9.155)	
P value	0.166		1.000		0.579		0.535	
GHRL rs27647 (AA *vs* AG+GG)								
OR (95%CI)	1.045 (1.009-1.083)		0.700 (0.222-2.206)		0.676 (0.285-1.605)		0.795 (0.280-2.256)	
P value	0.345		0.541		0.373		0.666	

[a]*P* value < 0.05. AST: Aspartate aminotransaminase; ALT Alanine aminotransaminase; OR: Odds ratio; FPG: Fasting plasma glucose; HbA1C: Hemoglobin A1C.

relationship. Second, the patients included in this study were exclusively Thai, so our findings may not apply to patients of other ethnic origins. Further, long-term studies are still needed to confirm these findings in other ethnicities. Although the results of the available research are satisfactory, they have not been proven in randomized control trials. Further studies of genetic predispositions for MAFLD with the absence or presence with MAFLD will certainly provide a better understanding of the molecular mechanisms of MAFLD.

CONCLUSION

The prevalence of MAFLD in people living with HIV is increasing, representing a public health concern. The existing evidence suggests that AST, fasting plasma glucose, triglyceride, total cholesterol, LDL, and the genetic factors *PNPLA3* rs738409 and *LEP* rs7799039 indicate genetic susceptibility for PLWH, leading to improvements in the treatment of MAFLD.

Table 7 Logistic regression analysis of factors associated with metabolic-associated fatty liver disease (people living with human immunodeficiency virus and metabolic-associated fatty liver disease *vs* people living with human immunodeficiency virus and non-metabolic-associated fatty liver disease)

Factor	Exp(B)	95%CI	*P* value
AST	4.615	1.081-19.709	0.039[a]
Fasting Plasma glucose	21.5	5.327-86.767	< 0.001[a]
Triglyceride	6.747	1.747-26.047	0.006[a]
Total cholesterol	0.125	0.019-0.819	0.030[a]
LDL	12.97	1.983-84.827	0.007[a]
*APOB rs*10495712	4.195	1.304-18.532	0.019[a]
APOA5 rs662799	0.012	0.002-0.770	< 0.001[a]
LEP rs7799039	0.321	0.070-1.469	0.143[a]

[a]*P* value < 0.2. AST: Aspartate aminotransaminase; LDL: Low-density lipoprotein.

ARTICLE HIGHLIGHTS

Research background

Metabolic-associated fatty liver disease (MAFLD), which is characterized by hepatocyte fat accumulation, poses substantial health risks; it affects a significant number of people globally, especially those living with obesity, diabetes, dyslipidemia, hypertension, and metabolic syndrome. Despite its prevalence, the precise mechanisms underlying MAFLD, which involve factors including viral hepatitis, human immunodeficiency virus (HIV), antiretroviral treatment, and genetics, remain unclear.

Research motivation

MAFLD is prevalent among individuals with HIV, with rates ranging from 40% to 55%; it is influenced by both antiretroviral medications and specific genetic variants. Notably, the *PNPLA3* rs738409 variant, a genetic factor, plays a significant role in the development of MAFLD.

Research objectives

The present investigation sought to assess the correlation between gene polymorphisms and MAFLD in individuals living with HIV.

Research methods

We employed transient elastography and set a threshold for the controlled attenuated parameter at ≥ 248 dB/m for the identification of MAFLD. All participants underwent genotyping for candidate single-nucleotide polymorphisms.

Research results

Individuals carrying the G-allele of *PNPLA3* (rs738409) demonstrated a two-fold increased risk of developing MAFLD; this risk rose to 2.5 times in cases of MAFLD with HIV infection. The clinical characteristics and genetic profiles suggested that carriers of the A-allele of *LEP* rs7799039 had a nine-fold higher likelihood of developing abnormal triglyceride levels among individuals living with HIV.

Research conclusions

The present research reveals a connection between *PNPLA3* rs738409 and *LEP* rs7799039 and MAFLD in individuals with HIV.

Research perspectives

Genetic factors play a crucial role in the pathophysiology of MAFLD. In upcoming research, targeting the *PNPLA3* gene in clinical trials may emerge as a promising direction for precision medicine in the treatment of MAFLD.

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FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Cohort Study

Comparison of fungal *vs* bacterial infections in the medical intensive liver unit: Cause or corollary for high mortality?

Sarah Khan, Hanna Hong, Stephanie Bass, Yifan Wang, Xiao-Feng Wang, Omar T Sims, Christine E Koval, Aanchal Kapoor, Christina C Lindenmeyer

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Abstract

BACKGROUND

Due to development of an immune-dysregulated phenotype, advanced liver disease in all forms predisposes patients to sepsis acquisition, including by opportunistic pathogens such as fungi. Little data exists on fungal infection within a medical intensive liver unit (MILU), particularly in relation to acute on chronic liver failure.

AIM

To investigate the impact of fungal infections among critically ill patients with advanced liver disease, and compare outcomes to those of patients with bacterial infections.

METHODS

From our prospective registry of MILU patients from 2018-2022, we included 27 patients with culture-positive fungal infections and 183 with bacterial infections. We compared outcomes between patients admitted to the MILU with fungal infections to bacterial counterparts. Data was extracted through chart review.

RESULTS

All fungal infections were due to *Candida* species, and were most frequently blood isolates. Mortality among patients with fungal infections was significantly worse relative to the bacterial cohort (93% *vs* 52%, $P < 0.001$). The majority of the fungal cohort developed grade 2 or 3 acute on chronic liver failure (ACLF) (90% *vs* 64%, $P = 0.02$). Patients in the fungal cohort had increased use of vasopressors (96% *vs* 70%, $P = 0.04$), mechanical ventilation (96% *vs* 65%, $P < 0.001$), and dialysis due to acute kidney injury (78% *vs* 52%, $P = 0.014$). On MILU admission, the fungal cohort had significantly higher Acute Physiology and Chronic Health Evaluation (108 *vs* 91, $P = 0.003$), Acute Physiology Score (86 *vs* 65, $P = 0.003$), and Model for End-Stage Liver Disease-Sodium scores (86 *vs* 65, $P = 0.041$). There was no significant difference in the rate of central line use preceding culture (52% *vs* 40%, $P = 0.2$). Patients with fungal infection had higher rate of transplant hold placement, and lower rates of transplant; however, differences did not achieve statistical significance.

CONCLUSION

Mortality was worse among patients with fungal infections, likely attributable to severe ACLF development. Prospective studies examining empiric antifungals in severe ACLF and associations between fungal infections and transplant outcomes are critical.

Key Words: Fungal; Infection; Sepsis; Acute on chronic liver failure; Intensive care

Core Tip: In the critical care setting, patients with advanced liver disease who develop fungal infections have significantly higher mortality than those who develop bacterial infections. These patients require greater support with vasopressors, mechanical ventilation, and dialysis than their counterparts with bacterial infections. Patients who developed fungal infections appeared more acutely ill on admission to the intensive care unit, with higher Acute Physiology and Chronic Health Evaluation, Acute Physiology Score, and Model for End-Stage Liver Disease scores. In such patients, fungal infection development is closely associated with development of severe acute-on-chronic liver failure. Further work elucidating this relationship will allow for better prognostication and development of predictors for acute on chronic liver failure in this population.

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INTRODUCTION

Advanced liver disease predisposes patients to acquisition of infections. This vulnerability is best described in cirrhosis, through development of a cirrhosis-associated immune dysfunction (CAID). Intestinal dysbiosis and disruption of the gut barrier leads to gut inflammation, causing portal and systemic inflammation in cirrhosis patients[1]. Despite persistent immune activation[2-5], converse immunodeficiency develops due to immune exhaustion and senescence in advanced cirrhosis[6]. Immune dysfunction through impaired phagocytosis, complement deficiency, and Kupffer cell disruption mediates this vulnerability to invasive fungal infections[7-9]. Vulnerability due to immune dysfunction is further compounded by management practices that heighten the risk of infections, such as need for invasive monitoring, use of proton-pump inhibitors, frequent procedures such as paracenteses, cardiopulmonary support, and use of corticosteroids [10]. This model of CAID has been extrapolated to other forms of advanced liver disease, including acute states such as acute liver failure and severe alcohol-associated hepatitis[11,12]. This innate immunodeficiency predisposes patients to infections and increased mortality[13,14]. Infections have been shown to be the most common cause of acute on chronic liver failure (ACLF), and development of ACLF in patients with cirrhosis contributes significantly to infection-related mortality[14-16]. Further, infection-triggered ACLF is associated with higher mortality than that triggered by non-infectious causes[17].

This theorized immunodeficient phenotype also predisposes patients to other types of opportunistic pathogens[14,16], including fungal infections. The existing literature on ACLF has predominantly focused on bacterial infections due to their prevalence as the primary triggers of ACLF in Western countries[18]. Invasive fungal infections, however, are emerging as under-recognized significant causes of mortality, particularly in the critical care setting[7,11,16,19]. Recent

studies have demonstrated an association between fungal infections with the development of severe ACLF, increased rate of intensive care admission among infected patients, and higher mortality[15,19], when compared with bacterial infections.

Due to this significant impact, there has been a growing interest in further characterizing the impact of fungal infections in cirrhosis[19]. There is limited data comparing outcomes between patients with fungal and bacterial infections among patients with advanced liver disease in the critical care setting, though studies have characterized these for general hospitalizations[20,21]. We aimed to compare mortality and clinical characteristics including laboratory markers, illness severity indices and degree of shock, between patients with fungal and bacterial infections within our Medical Intensive Liver Unit (MILU). Furthermore, we characterized epidemiology of such infections within our MILU.

MATERIALS AND METHODS

Study design and definitions

The Cleveland Clinic MILU is a multi-disciplinary care setting designed for daily co-management of patients by hepatology and critical care teams, with a special focus on bridging critically ill patients to transplant. We designed a cohort study comparing patients with fungal and bacterial infections, who were admitted to our MILU between January 2018 to September 2022. To identify a study sample of patients with culture-confirmed infections, we queried our prospectively-curated, longitudinal MILU database for patients with positive cultures. Diagnostic criteria for infections were: positive blood cultures/cultures from sterile sites in combination with clinical symptoms of infection, which were usually treated with antimicrobials in consultation with our infectious disease department[22]. Fungal infections were deemed present if fungi were isolated from blood (candidemia) or other sterile sites (peritoneal fluid), or urine in certain cases. Positive cultures from urinary sources were included as infection if there were clinically associated symptoms and were treated with targeted antifungal agents. One case of tracheitis was included following isolation from tracheal biopsy due to complicated wound infection at a tracheostomy site. For patients with multiple positive sites of fungal culture including blood and non-sterile sites, infection was classified as fungemia. Among patients with bacterial isolates, 15 patients had 2 separate culture-positive instances of infection within the same MILU stay. In such cases, the second instance of infection was used in the mortality analysis. All infection parameters were defined in consultation with our transplant infectious disease department. Multi-drug resistant organisms (MDRO) were defined using previously established guidelines for each isolated organism: resistance to two or more classes of antibiotics for the majority of bacterial pathogens; and resistance to two or more classes of antifungals for fungal pathogens[23-27].

Furthermore, patients were included if they had clinically significant advanced liver disease, as defined by the presence of cirrhosis, acute liver failure, severe alcohol-associated hepatitis, or severe acute liver injury. Cirrhosis was defined either as biopsy-proven bridging fibrosis of the liver or as a composite of clinical signs, laboratory tests, endoscopy and radiologic imaging. Acute liver failure and severe alcohol-associated hepatitis were defined in accordance with the American Association for the Study of Liver Diseases guidelines[28,29]. Severe acute liver injury was taken as clinically significant hepatic impairment with composite radiologic and laboratory abnormalities not meeting criteria for acute liver failure or alcohol-associated hepatitis. ACLF and organ failures were defined by the European Foundation for the Study of Chronic Liver Failure (CLIF) Consortium[30]. Exclusion criteria included culture from contaminants or clinically mild liver disease, such as transient liver injury. The Cleveland Clinic Foundation's institutional review board approved the study protocol as a non-interventional, anonymized study waiving the need for informed consent.

Outcomes

The primary outcome of interest for this study was mortality from time of onset of infection, which was determined by the date of a positive culture. Mortality was compared between patients with fungal and bacterial infections in the MILU.

Secondary outcomes of interest included need for cardiopulmonary support, development of acute kidney injury requiring dialysis, transplant evaluation endpoints and length of stay. Three separate lengths of stay were compared: total stay from hospital admission to discharge/death, time from intensive care unit (ICU) admission to ICU discharge and time from hospital admission to ICU discharge. Outcomes were compared between fungal and bacterial cohorts. Comparisons were also conducted on characteristics of acute illness including labs at infection, illness severity scoring and severity of ACLF, if applicable, at the time of culture. Finally, pre-infection predisposing variables were analyzed for differences between bacterial and fungal cohorts, including circulatory failure requiring hemodynamic support, prior antimicrobial use, and admission scores of illness severity.

Variables and definitions

All variables and outcomes were collected through chart extraction. Patients were identified from our longitudinal, prospective registry of all admissions to the MILU, and eligible cases were extracted from the electronic medical record based on culture positivity. ACLF was defined as suggested by the chronic liver failure consortium (CLIF-C OFs), graded by the number and severity of organ failures after an initial insult[30,31]. Infections were considered to have precipitated ACLF if the date of culture was prior to or on the day of syndrome development. Furthermore, grading of ACLF was done at the time of positive culture. Labs of interest at time points of infection were taken within 3 d prior to or after the date of culture, if unavailable at the date of culture. Stress dose steroid use preceding infection was defined as steroid dosing equivalent to 50 mg of hydrocortisone every 8 h, used for at least 3 d in the preceding 3 months from date of positive culture. MDROs were defined using pre-established criteria by an international expert proposal for interim standard definitions for acquired resistance[23,24,26,27]. Elucidation of epidemiology of fungal infection and colonization

within our unit to inform antimicrobial protocols was done using individual culture data.

Statistical analysis

Measures of central tendency (means and standard deviations for normally distributed continuous variables, medians and quartiles for non-normally distributed continuous variables) and frequency distributions were used to characterize the sample. Comparisons between fungal and bacterial cohorts were done using Wilcoxon rank sum and Welch's two-sample *t*-tests for continuous variables. Pearson's chi-square and Fischer's exact tests were used for comparison of categorical variables. A Kaplan-Meier curve was constructed to compare survival from ICU admission. All statistical analyses were conducted using R 4.0.5. Core Team (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2018. URL http://www.R-project.org/). *P* values < 0.05 were considered statistically significant. All statistical analyses were conducted in partnership with biostatisticians from our institution's department of quantitative health sciences.

RESULTS

Study sample and population characteristics

From 2018-2022, 1136 individual patients were treated in the MILU, accounting for 1698 admissions. Of these, we isolated 214 unique patients with positive microbial cultures. Of this population, we further excluded 3 cases with positive cultures as these were clinically treated as contaminants (Figure 1).

Twenty-seven patients with positive fungal cultures, and 183 with bacterial infections were included in our analysis. Ten patients in the fungal cohort had bacterial co-infections. Of the bacterial cohort, 15 patients had 2 instances of separate infections within the same MILU stay. The last infection prior to discharge or death was utilized for analysis in these cases.

There were no differences in baseline demographics of age, race, or sex between the 2 cohorts (Table 1). Both cohorts also had similar Charlson Comorbidity Scores. The fungal and bacterial cohorts had similar proportions of patients admitted with cirrhosis, alcohol associated hepatitis, acute liver failure and severe acute liver injury. Viral hepatitis due to hepatitis B and C infection was more commonly the etiology of liver disease among patients with fungal infections, but other etiologies were similar between cohorts. Patients with fungal infections had higher rates of hepatorenal syndrome. One case of alcohol-associated hepatitis occurred without underlying cirrhosis in the bacterial cohort, while all other cases occurred with comorbid cirrhosis.

Among the fungal cohort, 33% of patients also suffered surgical illnesses including small bowel obstruction, cholecystitis, colitis and abdominal fistula, during their ICU stay. Of those with isolated fungal infection, 71% received 5 d of antibiotic therapy prior to initiation of antifungal treatment.

Infection types and epidemiology

All isolated fungal infections were *Candida* infections (Table 2). *Candida glabrata* was the most common isolated fungus, followed by *Candida albicans*. Isolates were most frequently from blood, followed by ascites and urine. We isolated one case of secondary peritonitis and one case of tracheitis.

Among 183 patients with bacterial infections, 45 (24.5%) had co-infections with multiple bacterial isolates and 15 (8.1%) patients had 2 separate instances of bacterial infection during their MILU stay. Blood was the most frequently isolated source (Appendix). Spontaneous bacterial peritonitis, and respiratory and urinary tract infections were the most common sources of gram-positive infections following bacteremia. There were 117 g-positive cultures, of which the most common organism was *Enterococcus faecium*, followed by methicillin-resistant *Staphylococcus aureus*. There were 126 g-negative isolates, and the majority were caused by *Escherichia coli*, followed by *Klebsiella* species.

Mortality, intensive care resource utilization, and transplant outcomes

The mortality rate among patients with fungal infections was significantly higher than those with bacterial infections (93% *vs* 52%, *P* < 0.001, Figure 2). Median survival among the fungal cohort was 12 d relative to 31 d in the bacterial cohort (Figure 2). The majority of patients with fungal infections had severe ACLF, defined as ACLF grade 2 or higher (90% *vs* 64%, *P* = 0.02, Table 3), and either died or transitioned to hospice during their MILU stay (93% *vs* 52%, *P* < 0.0001). One patient with fungal infection had decompensated cirrhosis without ACLF, while 34 patients in the bacterial cohort had decompensated cirrhosis alone. Significantly higher proportions of those in the fungal cohort required vasopressor support (96% *vs* 70%, *P* = 0.04), mechanical ventilation (96% *vs* 65%, *P* < 0.001), and dialysis initiation due to acute kidney injury (78% *vs* 52%, *P* = 0.014). There were no differences in indication for intubation, MILU length of stay (LOS) or overall hospital LOS. However, those in the fungal cohort had longer hospital LOS prior to MILU admission (8 d *vs* 0 d, *P* = 0.046).

There were no differences between fungal and bacterial cohorts in rate of transplant evaluation initiation (48% *vs* 58%, *P* = 0.3) or rate of listing (31% *vs* 51%, *P* = 0.13). Of those patients who were listed, all patients with fungal infection were subsequently placed on hold, and no patients with fungal infections received a transplant. Patients with fungal infection had higher rate of hold placement (100% *vs* 57%, *P* = 0.14), and lower rates of transplant compared to bacterial counterparts (0% *vs* 50%, 0 = 0.056); however, these differences did not achieve statistical significance.

Table 1 Baseline cohort characteristics between liver intensive care unit patients with fungal and bacterial infections

Characteristic	Bacteria, *n* = 183[1]	Fungal, *n* = 27[1]	*P* value[2]
Age	60 (50, 66)	58 (46, 66)	0.3
Sex			
Female	73 (40)	12 (44)	
Male	110 (60)	15 (56)	
Race			
American Indian/Alaska Native	1 (0.5)	0 (0)	
Asian	1 (0.5)	0 (0)	
Black	27 (15)	6 (22)	
Declined	1 (0.5)	2 (7.4)	
Multiracial/cultural	7 (3.8)	1 (3.7)	
Unavailable	7 (3.8)	1 (3.7)	
White	139 (76)	17 (63)	
Charlson Comorbidity Score	6.00 (5.00, 7.00)	6.00 (4.00, 7.00)	0.3
Hepatocellular carcinoma	18 (9.8)	1 (3.7)	0.5
Principal liver diagnosis			
Acute liver failure	10 (5)	2 (7.4)	> 0.9
Cirrhosis	141 (78)	21 (78)	> 0.9
Alcohol-associated hepatitis (comorbid[3])	16 (9.3)	6 (22)	0.093
Acute severe liver injury/other[4]	34 (17)	4 (14.6)	> 0.9
Etiology of viral disease			
Viral hepatitis	24 (13)	9 (33)	0.016
Alcohol-associated	75 (41)	13 (48)	0.6
Autoimmune	8 (4.4)	1 (3.7)	> 0.9
NASH	44 (24)	5 (19)	0.7
Primary biliary cholangitis	4 (2.2)	1 (3.7)	> 0.9
Primary sclerosing cholangitis	17 (9.3)	1 (3.7)	0.5
Other	34 (19)	6 (22)	0.9
Toxins	4 (2.2)	0 (0)	> 0.9
Ischemic injury	5 (2.7)	1 (3.7)	> 0.9
Cryptogenic	16 (8.7)	1 (3.7)	0.6
Decompensation defining events			
Ascites	144 (79)	23 (85)	0.6
Hepatic encephalopathy	138 (75)	25 (93)	0.08
Hepatorenal syndrome	63 (34)	16 (59)	0.023
EV history/variceal bleeding	106 (58)	16 (59)	> 0.9
HPS	2 (1.1)	0 (0)	> 0.9
PoPHTN	6 (3.3)	0 (0)	0.7
Hepatic hydrothorax	27 (15)	3 (11)	0.8
SBP	45 (25)	12 (44)	0.053
Coagulopathy	122 (67)	21 (78)	0.3
Thrombocytopenia	107 (58)	20 (74)	0.2

[1]Median (IQR); *n* (%).
[2]Welch Two Sample *t*-test; Standardized Mean Difference; Two sample test for equality of proportions.
[3]Only 1 case of alcohol-associated hepatitis occurred without comorbid cirrhosis in bacterial cohort, not included in proportion shown.
[4]Other etiologies include chronic post-transplant patients with liver injury/recurrent portal hypertension, portal vein thrombosis, non-hepatitis viral infection and Caroli disease in conjunction with severe liver injury.
HPS: Hepatopulmonary syndrome; PoPTHN: Portopulmonary hypertension; SBP: Spontaneous bacterial peritonitis.

Table 2 Epidemiology of *Candida* isolates among patients with fungal patients in the intensive care unit

Organism	Urinary source	Bacteremia	Spontaneous peritonitis	Secondary peritonitis	Tracheitis	Total per organism
Candida glabrata	-	6	2	1	-	9
Candida albicans	1	4	2	-	1	8
Candida krusei	-	2	-	-	-	2
Candida dubliniensis	1	3	2	-	-	6
Candida (other)	2	2	-	-	-	4
Total per source	4	17	6	1	1	

Table 3 Transplant and intensive care outcomes comparison between fungal and bacterial cohorts

Characteristic	Bacteria, *n* = 183[1]	Fungal, *n* = 27[1]	*P* value[2]
Intensive care outcomes			
ACLF grade			0.017
< 2	50 (36)	2 (9.5)	
≥ 2	90 (64)	19 (90)	
Death during admission or hospice	95 (52)	25 (93)	< 0.001
Vasopressor requirement	129 (70)	26 (96)	0.004
Mechanical ventilation	118 (65)	26 (96)	< 0.001
Indication for intubation			
Airway protection	93 (79)	22 (85)	0.6
Respiratory failure	25 (21)	4 (15)	
Dialysis due to acute kidney injury	95 (52)	21 (78)	0.014
ICU LOS (d)	5 (2, 10)	6 (4, 16)	0.063
Hospital LOS (d)	16 (7, 28)	17 (12, 30)	0.3
Hosp admit to ICU (d)	0 (0, 6)	8 (0, 13)	0.046
Transplant-related outcomes			
Evaluated for transplant	107 (58)	13 (48)	0.3
Listed	57 (53)	4 (31)	0.13
Organ listed			0.3
Liver	44 (79)	3 (60)	
Liver and kidney	12 (21)	2 (40)	
Hold placed	32 (57)	4 (100)	0.14
Transplant occurred	28 (50)	0 (0)	0.056

[1]*n* (%); Range; Median (IQR).
[2]Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test.
ACLF: Acute on chronic liver failure; ICU: Intensive care unit; LOS: Length of stay.

Characteristics of acute infection and predisposing variables

At the time of positive culture, fungal and bacterial cohorts had similar rates of infection with MDROs (37% *vs* 50%, P = 0.2) and Child-Pugh scores (11 *vs* 11, P = 0.064) (Table 4). Patients in the fungal cohort had higher Model for End-Stage Liver Disease-Sodium (MELD-Na) (33 *vs* 28, P = 0.017) and CLIF scores (13 *vs* 11, P < 0.001). Albumin, lactate, leukocyte count, and C-reactive protein were not significantly different between cohorts.

At the time of MILU admission, patients with fungal infection had significantly higher Acute Physiology and Chronic Health Evaluation (108 *vs* 91, P = 0.003), Acute Physiology Score (86 *vs* 65, P = 0.003), and MELD-Na scores (86 *vs* 65, P = 0.041) (Table 5). There was no significant difference in the rate of central line use in 48 h preceding positive culture in fungal patients (52% *vs* 40%, P = 0.2). Prior infection or colonization with MDRO was more common in the fungal cohort (41% *vs* 21%, P = 0.027). Foley catheter use within 48 h preceding infection was less common among the fungal cohort relative to the bacterial cohort (19% *vs* 44%, P = 0.013). There were no significant differences in preceding stress dose steroid use, screening MRSA nasal swab, regular large volume paracentesis requirement prior to admission (defined as at least monthly paracenteses within the preceding six months of admission), outpatient immunosuppression use, prior antibiotic exposure, prior antifungal exposure, or SARS-CoV-2 infection within preceding 30 d (Table 5).

DISCUSSION

While bacterial infections have been recognized as a major cause of mortality among patients with advanced liver disease, especially as the most common trigger for ACLF, outcomes of fungal infections have not been as well studied. Our study is among the few to examine survival in this population and is among the first to compare outcomes of fungal and bacterial infections in the intensive care setting. Our findings demonstrate survival reductions are associated with fungal infections among patients with advanced liver disease who are receiving care in intensive care units such as the MILU. Further, our findings suggest the need for future work, such as exploration of predictors of poor outcomes to elucidate indications for palliative care, and implications for transplant.

The stark difference in mortality among fungal and bacterial cohorts is the most notable finding of our study. As bacterial infections are common and confer a 4-fold increase in mortality, several studies have examined factors associated with infection acquisition, outcomes, and prevention strategies[13,32-35]. Our findings highlight, comparative to bacterial infections that fungal infections are associated with worse survival, as 93% of patients in our fungal cohort died or transitioned to hospice care. This may be attributable in part to development of ACLF, as the majority of patients with fungal infections had severe ACLF relative to bacterial counterparts. It is clear from our results that fungal infection is likely associated with ACLF severity; however, we were unable to run predictive models given the respective aspect of our study design. Our findings affirm the need for future work to further elucidate associations, and the potential benefits of empiric or prophylactic fungal coverage.

Furthermore, patients with fungal infection had severely reduced rates of transplant. Half of listed patients with bacterial infections received liver transplantation, whereas no patients with fungal infections received liver transplantation. Other studies have shown that in ACLF grades 2-3, non-transplant 90-d mortality ranged from 52.3-79.1% [30]. Transplant is the only ultimate standard therapy for severe ACLF that does not rely on liver regeneration for clinical improvement; 1-year post-transplant survival has been shown to be over 80% regardless of ACLF grade, and is better among transplant recipients compared to non-recipients[36,37]. However, patient selection is crucial given the narrow window for transplant[38,39]. Several studies have examined pre-transplant predictors to prognosticate post-transplant survival in ACLF[39,40]. Certain factors associated with poor post-transplant prognosis, including age > 53 years and mechanical ventilation for respiratory failure, were seen among the majority of our fungal cohort. Serum INR has also been shown to be predictive of short-term post-transplant mortality, and was significantly elevated among the fungal cohort relative to bacterial counterparts[41]. Post-transplant, fungal infection has been found to be the second most common cause of mortality and significantly more common among patients with pre-transplant ACLF[42]. The role of fungal infection as a peri-transplant prognostic factor and whether positive fungal culture is a true contraindication to transplant remains to be seen.

While prior studies have reported relatively lower rates of fungal infection, our study found prevalence of fungal infection among all culture-positive patients in the ICU to be 12.9%, or 10.9% when including only sterile source isolates. This is higher than previously postulated estimates ranging between 2%-7% among hospitalized patients with cirrhosis [15,43], suggesting that fungal infection may be more common specifically in the intensive care setting. The incidence of invasive candidiasis in non-selected patients in the ICU has been reported to be between 1%-2%, and on the rise[44]. Our estimation of prevalence may be subject to bias, however, due to the limited size of our fungal cohort. Nevertheless, underestimation of prevalence of invasive fungal infections has been suggested in the past due to dependence of prior estimates on performance of specific fungal cultures. To enable early recognition, interest in non-culture based diagnostic tools is growing, though current clinical use remains limited[45]. Additionally, similar to our findings, *Candida* infection has been associated with prolonged antibiotic administration prior to diagnosis of *Candidemia*[46], potentially leading to under-diagnosis. Further epidemiologic characterizations of patients with advanced liver disease is thus crucial, as inadequate antimicrobial coverage is associated with increased mortality[15,34]. Our findings demonstrate this, as *Candida glabrata* was the most commonly isolated species, in keeping with recent trends towards the rising prevalence of non-*albicans* species[44]. While echinocandins have been recommended by the Infectious Disease Society of America for empiric antifungal therapy[47], recommendations differ for *C. glabrata* depending on susceptibility due to resistance.

Table 4 Comparison of infection characteristics among liver patients in intensive care unit between fungal and bacterial cohorts

Characteristic	Bacteria, *n* = 183[1]	Fungal, *n* = 27[1]	*P* value[2]
MDRO	90 (50)	10 (37)	0.2
MELD-Na (time of positive culture)	28 (22, 33)	33 (25, 38)	0.017
Child-Pugh score (time of positive culture)	11.00 (9.00, 12.00)	11.00 (10.00, 13.00)	0.064
CLIF-C score (time of positive culture)	11.00 (9.00, 13.00)	13.00 (12.00, 14.50)	< 0.001
Lab values of interest at time of culture			
Leukocyte count	13 (7, 19)	16 (11, 18)	> 0.9
C-reactive protein	6 (3, 12)	5 (4, 8)	0.057
Albumin	2.70 (2.20, 3.30)	3.10 (2.70, 3.40)	0.086
Bilirubin	6 (2, 13)	13 (4, 23)	0.035
Lactate	2.9 (1.9, 5.2)	4.4 (2.2, 7.3)	0.3
International normalized ratio	1.80 (1.40, 2.10)	2.05 (1.78, 3.00)	0.046

[1]*n* (%); Median (IQR).
[2]Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test; Welch Two Sample test.
MDRO: Multi-drug resistant organism; MELD-Na: Model for End Stage Liver Disease-Sodium; CLIF: Chronic liver failure consortium organ failure score.

Table 5 Comparison of pre-infection variables between liver patients in intensive care unit with fungal and bacterial cohorts

Characteristic	Bacteria, *n* = 183[1]	Fungal, *n* = 27[1]	*P* value[2]
APACHE III Score	91 (71, 112)	108 (96, 121)	0.003
Acute Physiology Score	65 (50, 90)	86 (75, 108)	0.003
MELD-Na (admission)	29 (23, 35)	32 (28, 38)	0.041
Stress dose steroid use in past 3 months	29 (16)	6 (22)	0.4
Foley in past 48 h	80 (44)	5 (19)	0.013
Central line in past 48 h	73 (40)	14 (52)	0.2
Positive MRSA nasal swab	15 (8.2)	5 (19)	0.15
Prior MDRO infection/colonization	39 (21)	11 (41)	0.027
Regular LVP	72 (39)	14 (52)	0.2
Immunosuppressive medications (at time of admission)	40 (22)	6 (22)	> 0.9
Charlson Comorbidity Score	6.00 (5.00, 7.00)	6.00 (4.00, 7.00)	0.5
Prior antibiotic classes exposed	4.00 (3.00, 5.00)	4.00 (3.00, 5.00)	0.9
Prior antifungal classes exposed			0.11
0	104 (57)	10 (37)	
1	58 (32)	14 (52)	
2	16 (8.8)	2 (7.4)	
3	3 (1.7)	1 (3.7)	
COVID within 30 d prior	9 (4.9)	2 (7.4)	0.6

[1]*n* (%); Median (IQR).
[2]Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test.
MRSA: Methicillin-resistant *Staphylococcus aureus;* MDRO: Multi-drug resistant organism; LVP: Large-volume paracentesis; MELD-Na: Model for End Stage Liver Disease-Sodium; APACHE: Acute Physiology and Chronic Health Evaluation.

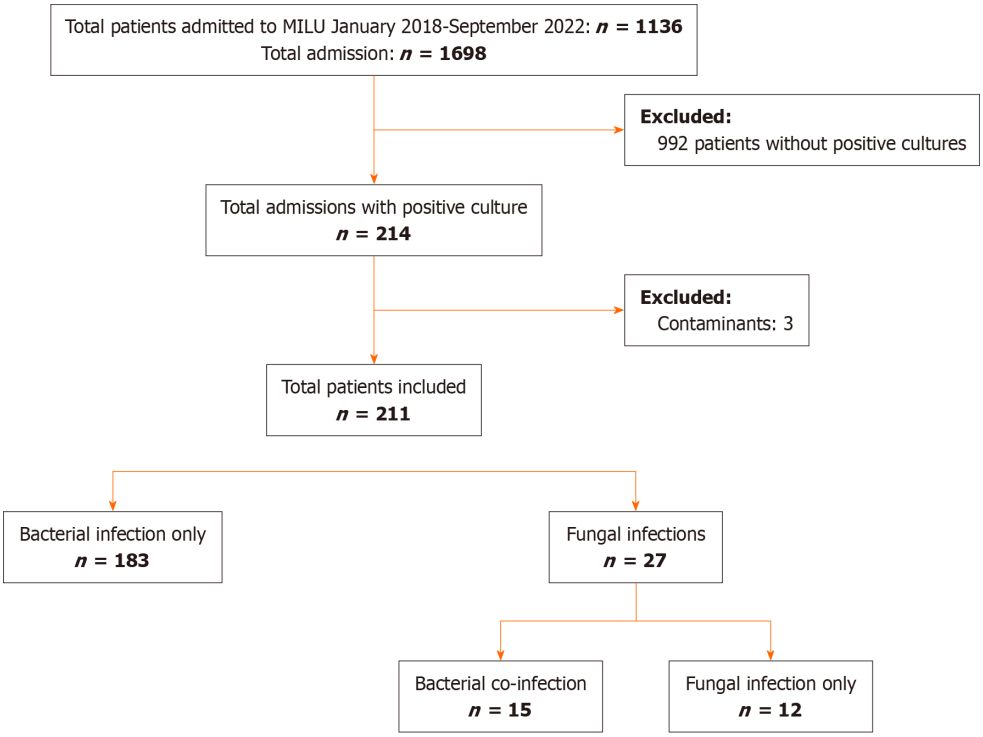

Figure 1 Study population and inclusion criteria. MILU: Medical intensive liver unit.

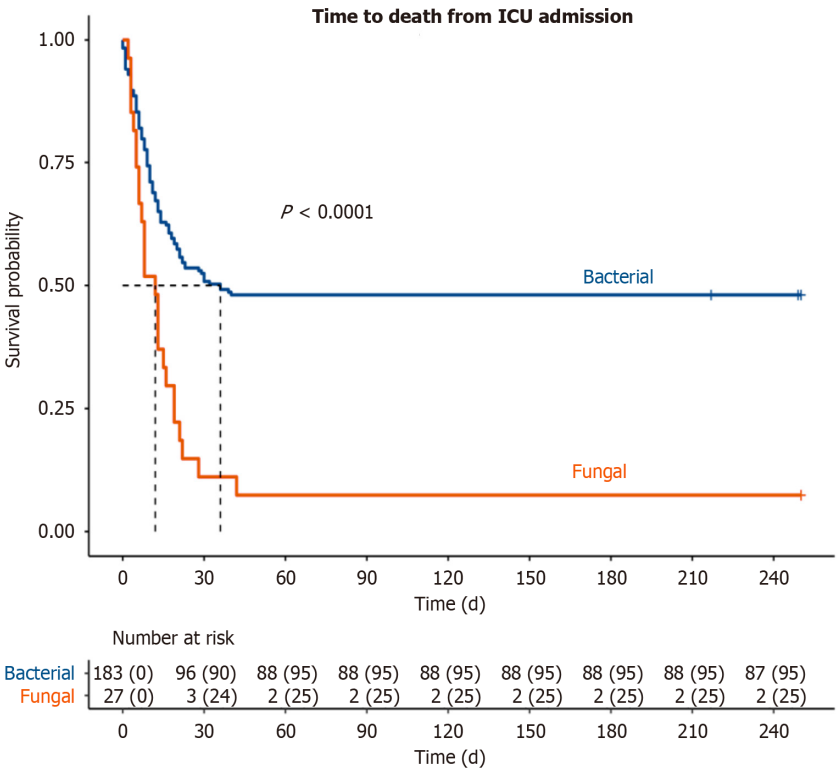

Figure 2 Comparison of survival from intensive care unit admission between fungal and bacterial cohorts. ICU: Intensive care unit.

In addition to empiric therapy, the severe mortality associated with fungal infections raises the importance of early risk stratification for potential prophylactic therapy. Markers of utility may be MELD-Na or ACLF grade cutoffs; MELD-Na has previously been shown to be predictive of fungal infection development[48]. In our study, MELD-Na was higher both at MILU admission and at time of infection among the fungal cohort. A prior study has also raised the possibility of prophylaxis in waitlisted patients with severe ACLF[49]. Our findings on pre-infection variables of interest may represent useful targets of future work to identify appropriate indications for prophylactic antifungals. In our cohort, fungal infections were associated with a higher rate of prior multi-drug resistant colonization and infection. Prior bacterial

infection has been established as a risk factor for subsequent fungal infection development[19,43,50], and may represent an important indication to explore for prophylactic antifungal therapy in critically-ill liver patients. Additionally, bilirubin and INR were significantly higher among patients with fungal infections, though this may represent collinearity with the MELD-Na score. Further studies investigating these markers for independent inclusion in predictive models would be valuable. A prolonged hospital stay prior to ICU admission in the fungal cohort compared to the bacterial cohort may also suggest an increased rate of nosocomial infections in this population. Invasive fungal infections have been reported as important causes of healthcare-acquired infections, and may warrant further study in this setting[46].

Parallel to the need for early aggressive treatment among patients with fungal infections is also the need to develop prognostication tools to guide goals of care. The concepts of futility and palliative care in severe ACLF are rising due to the associated reductions in quality of life beyond that associated with decompensated cirrhosis alone. In our study, despite their poor survival, the fungal cohort had similar lengths of stay in the intensive care unit compared to bacterial counterparts, with higher rates of vasopressor support, mechanical intubation, and dialysis initiation. Furthermore, prior work has shown that survival with *Candida* infection despite timely administration of antifungals is poor[51]. Our findings suggest the importance of exploring the potential prognostic role of positive fungal culture in severe ACLF, to better inform advanced care planning, improve end of life quality, and reduce psychosocial patient and family burden.

Several factors set our study apart from others. We provide data from a large population of unique MILU patients and used data from a prospectively maintained database over the course of four years. Within the unit, all patients are daily co-managed by hepatologists and intensivists, ensuring multi-disciplinary comprehensive care. We report on a critically ill, unique population of patients with complex pathologies seeking care at a quaternary center. We are also one of few studies to provide granular data on fungal infections in the critical care setting, and to comment on interplay with ACLF. An important limitation of some prior studies has been the use of population-based databases[37].

With these strengths, our study had some notable limitations. Despite data extracted from a prospective registry, our population had a limited sample size, and for this reason our study was limited in its ability to construct predictive models. Though being a quaternary referral center provides complexity and allows study of a critically ill population, data on patients' pre-care from prior hospital admissions is at times unavailable; this may have impacted our comparison of pre-infection variables. Finally, due to our requirement for culture positivity, our study did not include patients who may otherwise meet criteria for infection despite lack of microbiological isolation. Selection by culture may allow bias towards selection of a population with higher illness severity; however, our study aimed to investigate infections in this cohort of critically ill patients, and culture positivity is crucial for differentiating infection from other acute states of decompensation/inflammation.

CONCLUSION

Our findings demonstrate that fungal infection is associated with severe ACLF and marked increase in mortality among critically ill patients with advanced liver disease. We highlight the poor outcomes in this population despite aggressive supportive care and efforts towards stabilization for transplant evaluation. Future multi-center prospective studies are necessary to predict infection and prognosticate trajectory of care.

ARTICLE HIGHLIGHTS

Research background

Advanced liver disease predisposes critically ill patients to the development of fungal infections. While bacterial infections have been well-studied as the most common cause of acute-on-chronic liver failure and associated mortality, fungal infections have been relatively under-studied in the intensive care setting.

Research motivation

Infections increase mortality four-fold among critically ill liver patients, but few studies have compared predictors and outcomes of fungal infections to bacterial infections in this population.

Research objectives

We compared outcomes of fungal and bacterial infections among critically ill patients who were admitted to our unique medical intensive liver unit (MILU) from 2018-2022. We also conducted a comprehensive comparison of predictors and illness severity scores between these cohorts. Finally, we characterized microbiologic epidemiology of infections within our unit.

Research methods

Patients were identified for inclusion from a prospectively-curated database of all admissions to our MILU during the study period. Infections were defined based on culture positivity and clinical presentation. Data on outcomes and predictors of interest were collected manually through chart review.

Research results

We found that fungal infections among our patients were all caused by *Candida* species and were most frequently blood isolates. Mortality was significantly worse among the fungal cohort relative to patients with bacterial infections, as the majority of these patients died or transitioned to hospice during the intensive care unit (ICU) stay. The majority of patients in the fungal cohort developed severe acute on chronic liver failure, and they had higher need for vasopressors, mechanical ventilation and acute kidney injury. Further, patients who developed fungal infections were sicker on admission to the unit. Patients with fungal infection had higher rate of transplant hold placement, and lower rates of transplant; however, differences did not achieve statistical significance.

Research conclusions

Fungal infection is a poor prognostic marker for patients with advanced liver disease in the critical care setting, and it is associated with significantly worse mortality than bacterial infection. This may be in large part due to development of severe acute on chronic liver failure. Patients who developed fungal infections had higher Model for End-Stage Liver Disease-Sodium, Acute Physiology and Chronic Health Evaluation, and Acute Physiology Score scores on admission to the ICU.

Research perspectives

We believe our work highlights the importance of a need for future studies to investigate associations between fungal infections and acute on chronic liver failure. Furthermore, research efforts examining prognostic markers, potential indications for prophylactic/empiric antifungal use, and transplant outcomes would be equally important and informative for clinical practice.

FOOTNOTES

Author contributions: All authors were involved in study design; Khan S and Hong H collected data and designed the data collection tool; Khan S, Wang X, Wang Y, Sims O and Lindenmeyer CC were responsible for statistical analysis; Khan S, Bass S, Sims O, Koval C, Kapoor A and Lindenmeyer CC were involved in analysis and interpretation of results of statistical testing; Khan S, Sims O and Lindenmeyer CC were involved in writing the manuscript; all authors were involved in manuscript appraisal and approval.

Institutional review board statement: The study was reviewed and approved by the Cleveland Clinic Foundation Institutional Review Board [IRB# 22-721].

Informed consent statement: Our Institutional Review Board allowed our study to proceed without the need for informed consent.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Lean body mass index is a marker of advanced tumor features in patients with hepatocellular carcinoma

Andrew Scott deLemos, Jing Zhao, Milin Patel, Banks Kooken, Karan Mathur, Hieu Minh Nguyen, Areej Mazhar, Maggie McCarter, Heather Burney, Carla Kettler, Naga Chalasani, Samer Gawrieh

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Abstract

BACKGROUND

Obesity is an independent risk factor for the development of hepatocellular carcinoma (HCC) and may influence its outcomes. However, after diagnosis of HCC, like other malignancies, the obesity paradox may exist where higher body mass index (BMI) may in fact confer a survival benefit. This is frequently observed in patients with advanced HCC and cirrhosis, who often present late with advanced tumor features and cancer related weight loss.

AIM

To explore the relationship between BMI and survival in patients with cirrhosis and HCC.

METHODS
This is a retrospective cohort study of over 2500 patients diagnosed with HCC between 2009-2019 at two United States academic medical centers. Patient and tumor characteristics were extracted manually from medical records of each institutions' cancer registries. Patients were stratified according to BMI classes: < 25 kg/m^2 (lean), 25-29.9 kg/m^2 (overweight), and > 30 kg/m^2 (obese). Patient and tumor characteristics were compared according to BMI classification. We performed an overall survival analysis using Kaplan Meier by the three BMI classes and after adjusting for Milan criteria. A multivariable Cox regression model was then used to assess known risk factors for survival in patients with cirrhosis and HCC.

RESULTS
A total of 2548 patients with HCC were included in the analysis of which 11.2% (n = 286) were classified as non-cirrhotic. The three main BMI categories: Lean (n = 754), overweight (n = 861), and obese (n = 933) represented 29.6%, 33.8%, and 36.6% of the total population overall. Within each BMI class, the non-cirrhotic patients accounted for 15% (n = 100), 12% (n = 94), and 11% (n = 92), respectively. Underweight patients with a BMI < 18.5 kg/m^2 (n = 52) were included in the lean cohort. Of the obese cohort, 42% (n = 396) had a BMI ≥ 35 kg/m^2. Out of 2262 patients with cirrhosis and HCC, 654 (29%) were lean, 767 (34%) were overweight, and 841 (37%) were obese. The three BMI classes did not differ by age, MELD, or Child-Pugh class. Chronic hepatitis C was the dominant etiology in lean compared to the overweight and obese patients (71%, 62%, 49%, P < 0.001). Lean patients had significantly larger tumors compared to the other two BMI classes (5.1 *vs* 4.2 *vs* 4.2 cm, P < 0.001), were more likely outside Milan (56% *vs* 48% *vs* 47%, P < 0.001), and less likely to undergo transplantation (9% *vs* 18% *vs* 18%, P < 0.001). While both tumor size (P < 0.0001) and elevated alpha fetoprotein (P < 0.0001) were associated with worse survival by regression analysis, lean BMI was not (P = 0.36).

CONCLUSION
Lean patients with cirrhosis and HCC present with larger tumors and are more often outside Milan criteria, reflecting cancer related cachexia from delayed diagnosis. Access to care for hepatitis C virus therapy and liver transplantation confer a survival benefit, but not overweight or obese BMI classifications.

Key Words: Hepatocellular carcinoma; Cirrhosis; Obesity; Body mass index class; Sarcopenia; Chronic hepatitis C

Core Tip: This study explores the impact of different body mass index (BMI) strata on patient survival following the diagnosis of hepatocellular carcinoma (HCC). We stratified patients with cirrhosis by lean (BMI < 25 kg/m^2), overweight BMI (25-29.9 kg/m^2), and obese (BMI ≥ 30 kg/m^2) categories, and analyzed patient and tumor characteristics. Lean patients with HCC presented with significantly larger tumors as well as more advanced tumors. Survival was significantly reduced in lean HCC patients in the overall cohort but was restricted to those patients outside Milan criteria following sub-group analysis. We included a survival analysis by BMI class according to the three most common chronic liver diseases: Chronic hepatitis C, alcoholic liver disease, and nonalcoholic fatty liver disease. Lastly, we found no significant difference in survival comparing the three BMI classes from our sub-group of 286 patients with HCC but without cirrhosis.

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INTRODUCTION

The epidemiology of cirrhosis and hepatocellular carcinoma (HCC) is evolving as the burden of disease shifts toward a future predominated by alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD). A recent study from Canada projects that 92% of incident cases of cirrhosis will be due to either NAFLD or ALD in 2040[1]. The incidence of NAFLD-related HCC in the United States is predicted to increase by 137% to 12240 cases by 2030[2]. These alarming estimates underscore the present mandate to identify patients at risk for cirrhosis and HCC, presently the third leading cause of cancer death worldwide[3].

While the risk of HCC development varies depending on the underlying etiology of liver disease, ample data now supports a higher risk among chronic liver disease (CLD) patients with superimposed metabolic syndrome[4]. In a retrospective cohort of NAFLD patients, the presence of diabetes, hypertension, and dyslipidemia was shown to confer the highest risk for progression to HCC relative to patients with obesity alone[5]. A report from the International Agency

for Research on Cancer, however, clearly establishes a higher body mass index (BMI) as a risk factor for HCC with a relative risk of 1.8 compared to a normal reference BMI[6]. A recent meta-analysis of 22 prospective studies encompassing over 6 million patients followed for liver cancer occurrence found that a higher BMI was associated with an increased risk of HCC, with hazard ratios (HR) that increased from 1.36 to 1.77 to 3.08 in overweight, obese class I, and obese class II/III patients respectively[7].

Although obesity is a recognized risk factor for incident HCC, whether a high BMI translates into poorer survival following the diagnosis of HCC remains unclear. In fact, a survival analysis of a nationwide cancer registry of 10578 patients with HCC from South Korea found that overweight men with a BMI of 25-29.9 kg/m^2 had a better prognosis than normal weight men[8]. This "obesity paradox", or a survival benefit in overweight or mildly obese patients with cancer may in fact be apparent in patients with HCC such as has been shown in other types of cancer[9,10]. The "obesity paradox" may also be applicable in the context of cirrhosis. The presence of obesity was found by multivariate analysis to be associated with a lower risk of inpatient mortality in 32000 cirrhotic patients from the Nationwide Inpatient Sample [11]. Additionally, a BMI ≥ 30 kg/m^2 was recently identified as a variable associated with improved survival in cirrhotic patients undergoing surgery[12].

This study aims to investigate the relationship between BMI at diagnosis of HCC, tumor characteristics and patient survival. We contrasted patient and tumor characteristics, as well as overall survival across 3 BMIs: BMI < 25 kg/m^2 (lean), BMI 25-29.9 kg/m^2 (overweight), and BMI ≥ 30 kg/m^2 (obese) in over 2500 patients diagnosed with HCC over the last decade. To our knowledge, this is the first United States-based study comprised of individually collected patient data to address the "obesity paradox" in patients with HCC.

MATERIALS AND METHODS

Study design

This retrospective study included patient data from 2 academic medical centers (Atrium Health in Charlotte, North Carolina and Indiana University School of Medicine in Indianapolis, Indiana). HCC cases diagnosed from January of 2009 through June of 2019 were identified from each institutions' cancer registries. A detailed explanation of the cohort composition was described previously[13]. A confirmation of the HCC diagnosis based upon histological and/or radiographic evidence consistent with American Association for Study of Liver Disease guidelines was made by direct review of the individual electronic health record (EHR)[14]. Following verification of the HCC diagnosis, patient and tumor characteristics were then manually extracted from the EHR into a shared REDCap database. Tumor variables collected included alpha fetoprotein (AFP), largest tumor diameter, tumor-node-metastasis stage, and whether the HCC was within Milan criteria[15,16]. The method of HCC diagnosis was ascertained whenever possible and categorized as by routine screening, symptom work-up, and/or incidentally. All HCC treatment modalities were recorded from the medical record for analysis as well.

Patients were classified according to 3 BMI classes: BMI < 25 kg/m^2 (lean), BMI 25-29.9 kg/m^2 (overweight), and BMI ≥ 30 kg/m^2 (obese). BMI was individually recorded from each EHR at the nearest timepoint from initial date of HCC diagnosis. Provider documentation, again through manual chart review was used in concert with confirmatory laboratory testing to assess for the presence of co-morbid metabolic risk factors including diabetes, dyslipidemia, coronary artery disease and hypertension. Patients were classified as either cirrhotic or non-cirrhotic according to criteria published previously by Mittal *et al*[17] and externally validated by our group[17,18]. The underlying etiology of CLD was determined by review of hepatology provider notes and supportive clinical testing. A patient with combined chronic hepatitis C (CHC) and alcohol abuse was categorized as CHC and we captured whether a sustained virologic response (SVR) was known to have occurred. Laboratory testing for a model for end-stage liver disease (MELD) calculation closest to the time of HCC diagnosis was recorded. The presence or absence of liver-related complications (ascites, hepatic encephalopathy, varices, and spontaneous bacterial peritonitis was collected through the last documentation in the EHR.

Patient survival was established from cancer registries and medical records. For patients who are still alive or died with an unknown date of death, the date of last contact available in the medical record was used to define the time of censoring for the survival analysis. Each participating site had local Institutional Review Board approval to conduct the study.

Statistical analyses

BMI group differences of patient and tumor characteristics were compared using analysis of variance, Kruskal-Wallis test, chi-square test, and Fisher's Exact test, as appropriate. Cirrhotic and non-cirrhotic cases were analyzed separately. Survival curves among BMI classes was estimated through the Kaplan-Meier method. Subgroup analyses by Milan criteria as well as etiology of CLD were conducted and included a subgroup of patients with BMI ≥ 35 kg/m^2. To better evaluate potential survival differences, multivariable Cox regression models were conducted to assess risk factors for survival with HR and 95%CI presented. Risk factors included gender, race, diabetes, alcohol use, etiology of CLD, AFP, Milan criteria, screening within 2 years before HCC diagnosis, liver transplantation, and BMI categories. Statistical analyses were performed using SAS statistical software (version 9.4; SAS Institute, Cary, NC, United States).

RESULTS

Study population

A total of 2548 patients with HCC were included in the analysis of which 11.2% (n = 286) were classified as non-cirrhotic (Figure 1). The three main BMI categories: Lean (n = 754), overweight (n = 861), and obese (n = 933) represented 29.6%, 33.8%, and 36.6% of the total population overall. Within each BMI class, the non-cirrhotic patients accounted for 15% (n = 100), 12% (n = 94), and 11% (n = 92), respectively. Underweight patients with a BMI < 18.5 kg/m^2 (n = 52) were included in the lean cohort. Of the obese cohort, 42% (n = 396) had a BMI ≥ 35 kg/m^2.

Clinical features of cirrhotic HCC patients

Out of 2262 patients with cirrhosis and HCC, 654 (29%) were lean, 767 (34%) were overweight, and 841 (37%) were obese (Table 1). The mean age at HCC diagnosis for cirrhotic patients was 62 years and did not differ among the three BMI classes (P = 0.43). Although women represented a minority of HCC cases overall (21%), they were overrepresented in the obese cohort accounting for 26% of cases. By comparison, men accounted for a higher percentage of cases in the lean (80%) and overweight (85%) groups (P < 0.001). Lean patients with HCC were less frequently white or Hispanic and more frequently Black or Asian. As expected, the rate of diabetes, dyslipidemia, hypertension, and coronary artery disease was highest in the obese cohort compared to the overweight and lean groups (P < 0.001 for each risk factor; Table 1). There were no significant or clinical differences in laboratory tests or MELD-Na score across the three groups.

Lean patients with HCC had the highest frequency of alcohol abuse (53%), followed by overweight (50%) and obese patients (38%, P < 0.001). As anticipated, NAFLD was the etiology of cirrhosis in 27% of obese patients with HCC and accounted for 14% and 5% in the overweight and lean groups, (P < 0.001). Correspondingly, CHC accounted for 49%, 62%, and 71% of cases across the three BMI classes (P < 0.001). SVR rates were similar across the 3 CHC BMI groups, ranging from 34% to 41% (P = 0.07). The prevalence of ALD as the only etiology of liver disease was also similar across the 3 BMI classes (13%-15%, P = 0.51).

There were no differences in the distribution of Child-Pugh classes, presence of ascites or portal vein thrombosis across the 3 groups. In contrast, the presence of encephalopathy (36% *vs* 33% *vs* 26%, P < 0.001) and varices (51% *vs* 48% *vs* 40%, P < 0.001) were significantly higher in the obese relative to the overweight and lean groups.

Tumor characteristics among BMI classes in cirrhotic population

The lean HCC cohort presented with significantly larger tumors than the overweight and lean cohorts (mean 5.1 *vs* 4.2 *vs* 4.2 cm, P < 0.001). An AFP level > 200 ng/mL was also more frequently encountered in the lean HCC group in comparison to the other two groups (36% *vs* 23% *vs* 26%, P < 0.001). The lean cohort presented with more aggressive tumors as evidenced by the lowest rate of single tumors (35% *vs* 43% *vs* 46%) and highest rate of vascular invasion or extrahepatic spread (30% *vs* 22% *vs* 22%, P < 0.001 for overall clinical tumor stage). Predictably, the lean cohort was least likely to fall within Milan criteria (44% *vs* 52% *vs* 53%, P = 0.003) and to undergo liver transplantation (9% *vs* 18% *vs* 18%, P < 0.001). Lastly, the lean HCC group was most likely to be diagnosed as part of a symptom workup (48% *vs* 42% *vs* 38%, P = 0.003) and least likely by screening (46% *vs* 49% *vs* 55%, P = 0.007), compared to the overweight and obese groups (Table 2).

Patient survival by BMI classification

Median survival in the lean HCC cohort was 1.28 years (P < 0.0001, 95%CI 1.03-1.44) and was significantly lower compared to the overweight; 2.13 years (95%CI 1.79-2.59) and obese; 2.14 years (95%CI 1.83-2.51) cohorts (Figure 2A). The reduction in overall survival for lean cirrhotic HCC patients did not persist upon multivariate analysis (P = 0.36; Table 3). Although there was no difference in survival by BMI class when adjusting for patients with HCCs within Milan criteria (P = 0.35), there was a significantly increased mortality for lean cohort patients with HCCs outside Milan criteria compared to the other 2 BMI groups (P < 0.0001; Figure 3). This observation remained significant on multivariate analysis as patients with tumors within Milan criteria had a significant survival benefit (HR = 0.59, 95%CI 0.48-0.72, P < 0.0001) as did those patients undergoing liver transplantation (HR = 0.10, 95%CI 0.06-0.17, P < 0.0001). A SVR from CHC infection was also associated with a survival benefit (HR = 0.27, 95%CI 0.21-0.35, P < 0.0001) while an AFP > 200 ng/mL (HR = 1.93, 95%CI 1.61-2.32, P < 0.0001) and tumor size (cm) (HR = 1.08, 95%CI 1.05-1.12) were associated with worse survival (Table 3).

A final survival analysis was performed after stratifying by the three most common etiologies of CLD: Hepatitis C virus (HCV), alcohol, and NAFLD (Figure 2B-D). For each etiology, the obese HCC cohort was further subdivided into Class I obese (BMI: 30-34.9 kg/m^2) and Class II & III (BMI ≥ 35 kg/m^2). Median survival was significantly lower for the lean HCC cohort with underlying HCV (1.42 years, P = 0.01, 95%CI 1.2-1.79) and alcohol (0.7 years, P = 0.0007, 95%CI 0.21-1.15) compared to the three other BMI groups. The lean NAFLD-related HCC cohort patients were predictably low in number (n = 23) and their median survival, while lower at 1.44 years (95%CI: 0.39-) did not reach statistical significance compared to the overweight (2.95 years, 95%CI 1.5-4.14), obese class I (1.99 years 1.42-) and obese class II (2.23 years, 95%CI 1.54-2.87) (P overall = 0.84 among 4 BMI classes).

Patient and tumor characteristics among BMI classes in non-cirrhotic population

The 286 patients with HCC but without cirrhosis were evaluated according to BMI classification (Supplementary Table 1). Patients with non-cirrhotic HCC presented at a mean age of 66, 69, and 67 years old in the lean, overweight, and obese cohorts respectively (P = 0.22). Interestingly, women accounted for 30% of the non-cirrhotic HCC cohort (compared to 21% in the cirrhotic HCC cohort) though there was no significant difference in gender distribution across the three BMI

Table 1 Patient characteristics for cirrhotic hepatocellular carcinoma, *n* (%)

Variable	BMI < 25 kg/m² (*n* = 654)	BMI 25.0-29.9 kg/m² (*n* = 767)	BMI ≥ 30 kg/m² (*n* = 841)	*P* value
Age (yr)	62.0 ± 8.9	62.6 ± 8.7	62.5 ± 8.4	0.43
Male	522 (79.8)	650 (84.7)	620 (73.7)	< 0.001
Race				
White	437 (66.8)	599 (78.6)	697 (83.1)	< 0.001
Black	166 (25.4)	110 (14.4)	98 (11.7)	
Hispanic	14 (2.1)	27 (3.5)	31 (3.7)	
Asian	24 (3.7)	14 (1.8)	9 (1.1)	
Other	13 (2.0)	12 (1.6)	4 (0.48)	
Diabetes	144 (22.1)	256 (33.5)	440 (52.3)	< 0.001
Hypertension	325 (49.8)	450 (58.8)	579 (68.8)	< 0.001
Dyslipidemia	127 (19.4)	192 (25.1)	252 (30.0)	< 0.001
Coronary artery disease	105 (16.1)	110 (14.4)	181 (21.5)	< 0.001
Peripheral vascular disease	74 (11.3)	76 (9.9)	83 (9.9)	0.6
ALT (Units/L)	63.0 ± 67.9	64.3 ± 68.8	56.8 ± 112.1	0.19
AST (Units/L)	103.5 ± 147.4	94.3 ± 99.0	90.6 ± 183.2	0.25
Total bilirubin (mg/dL)	2.1 ± 3.0	2.3 ± 3.7	2.2 ± 3.2	0.62
Alkaline phosphatase (Units/L)	161.3 ± 142.8	144.7 ± 108.2	146.6 ± 104.6	0.017
Albumin (g/dL)	3.15 ± 0.67	3.24 ± 0.69	3.16 ± 0.63	0.015
Platelets (k/cumm)	146.9 ± 96.8	122.4 ± 74.5	123.5 ± 76.1	< 0.001
Creatinine (md/dL)	1.03 ± 0.84	1.02 ± 0.63	1.07 ± 0.71	0.37
Sodium (mEq/L)	135.7 ± 4.1	136.6 ± 3.9	136.8 ± 3.8	< 0.001
INR	1.3 ± 0.31	1.3 ± 0.41	1.3 ± 0.39	0.1
MELD-Na score	14.3 ± 5.6	14.0 ± 5.5	14.3 ± 5.4	0.72
Alcohol abuse	342 (53.4)	380 (50.1)	312 (37.8)	< 0.001
Etiology of chronic liver disease				
All HCV	465 (71.2)	474 (61.8)	409 (48.6)	< 0.001
HCV with known SVR	158 (34)	183 (38.6)	167 (40.8)	0.07
Alcohol alone	82 (12.5)	106 (13.8)	123 (14.6)	0.51
NAFLD	34 (5.2)	108 (14.1)	230 (27.3)	< 0.001
AIH/PBC/PSC	7 (1.1)	12 (1.6)	14 (1.7)	0.61
HBV	32 (4.9)	35 (4.6)	19 (2.3)	0.01
HC/A1AT	4 (0.61)	7 (0.91)	15 (1.8)	0.08
Child-Pugh classification				
Child A	277 (43.0)	328 (43.5)	336 (40.8)	0.58
Child A-B	15 (2.3)	26 (3.4)	25 (3.0)	
Child B	263 (40.8)	284 (37.7)	345 (41.9)	
Child C	89 (13.8)	116 (15.4)	118 (14.3)	
Complications				
Ascites	300 (54.1)	383 (49.9)	404 (48.0)	0.31
Encephalopathy	170 (26.0)	252 (32.9)	302 (35.9)	< 0.001

Varices	259 (39.6)	368 (48.0)	427 (50.8)	< 0.001
Portal vein thrombus	148 (22.6)	164 (21.4)	181 (21.5)	0.83

BMI: Body mass index; MELD: Model for end-stage liver disease; HCV: Hepatitis C virus; NAFLD: Non-alcoholic fatty liver disease; AIH: Autoimmune hepatitis; PBC: Primary biliary cholangitis; PSC: Primary sclerosing cholangitis; HBV: Hepatitis B virus; HC: Hemochromatosis; A1AT: Alpha 1 antitrypsin deficiency; SVR: Sustained virologic response; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; INR: International normalized ratio.

Table 2 Tumor, diagnosis, and treatment characteristics for cirrhotic hepatocellular carcinoma, *n* (%)

Variable	BMI < 25 kg/m², (*n* = 654)	BMI 25.0-29.9 kg/m², (*n* = 767)	BMI ≥ 30 kg/m², (*n* = 841)	*P* value
Tumor size (cm)	5.1 ± 4.2	4.2 ± 3.0	4.2 ± 3.2	< 0.001
AFP (ng/mL) category				
< 20	269 (43.7)	399 (54.8)	417 (51.9)	< 0.001
20-200	125 (20.3)	164 (22.5)	178 (22.1)	
> 200	221 (35.9)	165 (22.7)	209 (26.0)	
Tumor stage				
Single	230 (35.2	331 (43.2)	386 (46.0)	< 0.001
3 tumors < 3 cm	88 (13.5)	97 (12.7)	116 (13.8)	
Large multinodular	142 (21.7)	173 (22.6)	153 (18.2)	
Vascular invasion or extrahepatic spread	193 (29.6)	165 (21.5)	184 (21.9)	
Anatomic stage				
Stage I	173 (32.8)	239 (41.0)	293 (43.3)	< 0.001
Stage II	147 (27.9)	169 (29.0)	179 (26.5)	
Stage IIIA	36 (6.8)	38 (6.5)	49 (7.2)	
Stage IIIB	55 (10.4)	38 (6.5)	65 (9.6)	
Stage IIIC + IVA + IVB	116 (22.0)	99 (17.0)	90 (13.3)	
Tumor outside of Milan criteria	363 (55.6)	370 (48.3)	397 (47.3)	0.003
Tumor differentiation				
Well	70 (27.1)	109 (31.5)	116 (32.6)	0.15
Moderate	128 (49.6)	175 (50.6)	184 (51.7)	
Poor	53 (20.6)	59 (17.0)	54 (15.2)	
Undifferentiated/anaplastic	7 (2.7)	3 (0.9)	2 (0.5)	
How was HCC diagnosed?				
Part of screening	236 (45.8)	285 (48.6)	350 (54.8)	0.007
Incidental	120 (23.3)	120 (20.5)	116 (18.2)	0.098
Symptoms work up	246 (47.8)	244 (41.6)	241 (37.7)	0.003
Evidence of screening within 2 years	190 (36.8)	259 (44.0)	334 (52.1)	< 0.001
Liver transplantation	61 (9.3)	134 (17.5)	150 (17.8)	< 0.001
Palliative care/hospice	268 (41.0)	271 (35.3)	265 (31.5)	< 0.001

BMI: Body mass index; AFP: Alpha fetoprotein; HCC: Hepatocellular carcinoma.

strata. As observed in cirrhotic HCC (Table 1), the non-cirrhotic obese group was more often white than in the overweight or lean groups (88% *vs* 79% *vs* 72%, respectively) and less often Black (9% *vs* 10% *vs* 21%, respectively, $P = 0.015$). As expected, 78% of the cases had Aspartate aminotransferase to platelet ratio index scores < 1.0% and 76% had no record of undergoing HCC screening. No CLD etiology could be ascertained in 48% (137/286) of the cases, while the remaining were either NAFLD or viral hepatitis. HCC tumor size on presentation tended to be larger in the lean cohort (9.2 cm)

Table 3 Multivariable cox regression model of cirrhotic patient and tumor risk factors associated with survival after hepatocellular carcinoma diagnosis

Characteristic	Adjusted HR (95%CI)	*P* value
Age	1.0 (0.99-1.01)	0.58
Female (Ref.: Male)	0.94 (0.76-1.16)	0.57
Race (Ref.: White)		0.72
Asian	0.98 (0.55-1.76)	
Black	0.87 (0.70-1.08)	
Hispanic	0.94 (0.57-1.55)	
Other	0.81 (0.45-1.45)	
BMI classification (kg/m²) (Ref.: BMI < 25)		0.36
BMI 25.0-29.9	0.87 (0.72-1.06)	
BMI ≥ 30	0.90 (0.73-1.11)	
Diabetes	0.96 (0.81-1.14)	0.64
Alcohol abuse	1.10 (0.90-1.34)	0.35
HCV SVR	0.27 (0.21-0.35)	< 0.0001
Tumor size (cm)	1.08 (1.05-1.12)	< 0.0001
AFP (ng/mL) (Ref.: < 20)		< 0.0001
20-200	1.51 (1.23-1.85)	
> 200	1.93 (1.61-2.32)	
Tumor within Milan criteria	0.59 (0.48-0.72)	< 0.0001
Liver transplantation	0.10 (0.06-0.16)	< 0.0001
Any method of screening within 2 years before HCC diagnosis (Ref.: No)		0.0634
Yes	1.01 (0.85-1.21)	
Unknown	0.79 (0.63-0.99)	

BMI: Body mass index; AFP: Alpha fetoprotein; HCC: Hepatocellular carcinoma; HR: Hazard ratios; HCV: Hepatitis C virus; SVR: Sustained virologic response.

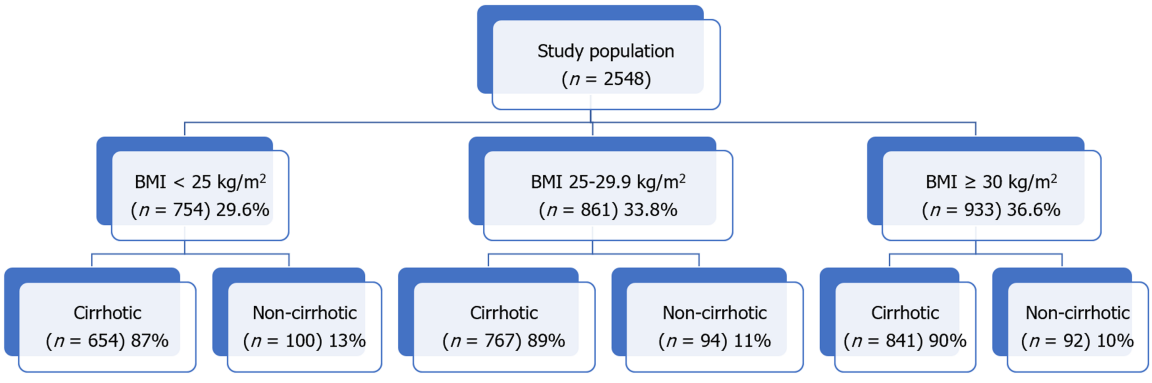
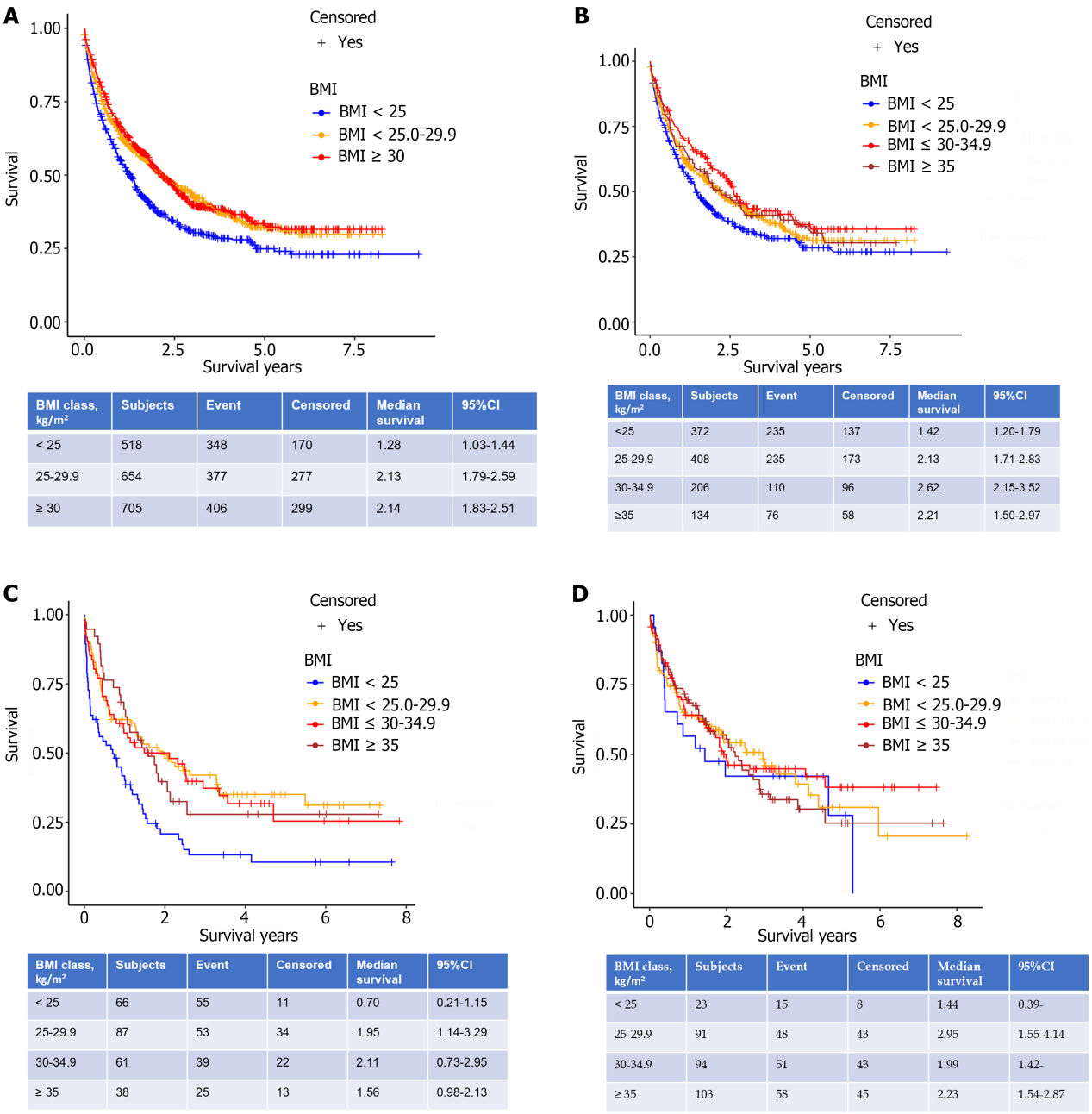


Figure 1 Study cohort by body mass index class. BMI: Body mass index class.

compared to the overweight (7.6 cm) and obese (7.7 cm) cohorts respectively (P = 0.06) though there was no difference among the BMI classes in clinical tumor stage with 51% of the tumors presenting as single lesions and 23% presenting with vascular invasion or extrahepatic spread. Median survival in non-cirrhotic HCC patients in the lean (2.95 years, 95%CI 1.12-6.52), overweight (2.14 years 95%CI 0.96-2.96), and obese (2.77 years 95%CI 1.33-3.17) cohorts was not significantly different.



BMI class, kg/m²	Subjects	Event	Censored	Median survival	95%CI
< 25	518	348	170	1.28	1.03-1.44
25-29.9	654	377	277	2.13	1.79-2.59
≥ 30	705	406	299	2.14	1.83-2.51

BMI class, kg/m²	Subjects	Event	Censored	Median survival	95%CI
<25	372	235	137	1.42	1.20-1.79
25-29.9	408	235	173	2.13	1.71-2.83
30-34.9	206	110	96	2.62	2.15-3.52
≥35	134	76	58	2.21	1.50-2.97

BMI class, kg/m²	Subjects	Event	Censored	Median survival	95%CI
< 25	66	55	11	0.70	0.21-1.15
25-29.9	87	53	34	1.95	1.14-3.29
30-34.9	61	39	22	2.11	0.73-2.95
≥ 35	38	25	13	1.56	0.98-2.13

BMI class, kg/m²	Subjects	Event	Censored	Median survival	95%CI
< 25	23	15	8	1.44	0.39-
25-29.9	91	48	43	2.95	1.55-4.14
30-34.9	94	51	43	1.99	1.42-
≥ 35	103	58	45	2.23	1.54-2.87

Figure 2 Patient survival. A: Patient survival with hepatocellular carcinoma according to body mass index class (BMI); B: Overall survival (OS) across four BMI groups by liver disease etiology: Hepatitis C virus; C: OS across four BMI groups by liver disease etiology: Alcohol; D: OS across four BMI groups by liver disease etiology: Nonalcoholic fatty liver disease. BMI: Body mass index class.

DISCUSSION

Our analysis of over 2500 patients diagnosed with HCC over the last decade focused on the differences between overweight and obese BMI classifications relative to a cohort of lean patients. The lean group, at the time of HCC diagnosis was enriched with hepatitis C and alcohol abuse and presented with significantly larger tumors as well as more aggressive tumors which resulted in lower frequency of liver transplantation compared to the overweight and obese groups. By multivariate analysis, however, the impact of BMI classification on patient survival was eclipsed by established survival outcomes such as presenting within Milan criteria and achieving a cure of CHC infection.

Our results should be interpreted within the context of emerging evidence demonstrating the significance of sarcopenia in survival following the diagnosis of HCC. In a Japanese study of over 1200 patients with HCC who underwent computed tomography for body composition assessment; sarcopenia, intramuscular fat deposition, and high visceral adiposity, but not BMI were significant predictors of survival by multivariate analysis[19]. Progressive skeletal muscle volume loss as measured by the psoas muscle index in patients undergoing locoregional therapy for HCC has also recently been associated with poor prognosis[20,21]. We acknowledge that the use of BMI as a variable to evaluate survival in patients with HCC has its limitations. However, our findings reinforce what one would anticipate contrasting BMI classes. The lean cohort, presenting more often without prior HCC screening and with more advanced tumors as we

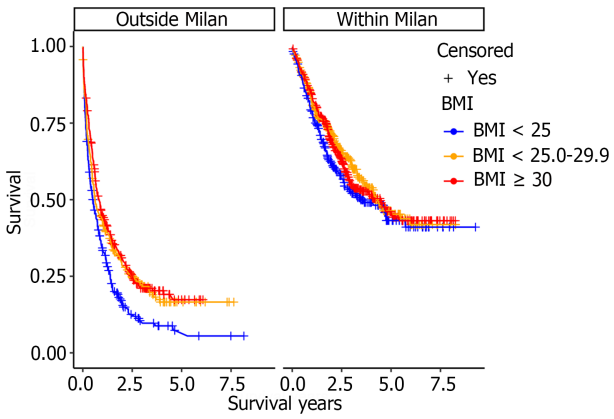


Figure 3 Patient survival after hepatocellular carcinoma diagnosis by body mass index class adjusted for Milan criteria. BMI: Body mass index class.

found in our analysis, likely comprises patients with cancer related cachexia. Rich *et al*[22] investigated the impact of cachexia defined as > 5% weight loss in the six months prior to HCC diagnosis compared to pre-cachexia (2%-5% weight loss) and stable/increased weight patients[22]. Approximately 25% of 600 patients met criteria for cachexia. Notably, BMI in the cachexia cohort was significantly lower than in the pre-cachexia and stable weight groups (25.4 *vs* 28.3 *vs* 28.5, $P <$ 001). The authors found that cachexia was independently associated with increased mortality with a median overall survival of 11.3 months which is comparable to the 15.4 months we found in our lean cohort. Thus BMI, while not an ideal surrogate of cachexia, is still of consequence particularly when evaluated in a considerably larger cohort such as ours.

A strength of our study was including a sub-group survival analysis of BMI classes according to HCV, ALD, and NAFLD etiologies. Since 53% of the patients from the lean HCC cohort were classified as having a history of alcohol abuse, the interaction between alcohol use and HCV could have led to more aggressive tumors in the lean cohort which was comprised of 71% HCV-related HCCs. The differences in screening rates preceding HCC diagnosis among the three BMI cohorts is a natural limitation from a retrospective study and highlights the fundamental challenge in routine cirrhosis management, namely access to screening and the diagnostic accuracy of our screening methodology. A recent detailed investigation of the limitations of screening found that just over a third of patients diagnosed with HCC had regular outpatient care in the year before presenting with HCC[23]. Furthermore, the adequacy of ultrasound visualization for HCC screening was reported to be sub-optimal in nearly 20% of cirrhosis patients, particularly in obese patients with NAFLD and ALD[24]. While newer blood-based biomarkers hold promise and may improve upon ultrasound for surveillance[25,26], issues surrounding access to testing will undoubtedly persist.

CONCLUSION

Reconsidering the use of the term "obesity paradox" in patients with advanced HCC outside the Milan criteria is a salient conclusion to draw from our study. In fact, our results reinforce the larger impact of cancer related weight loss which is at least in part a result of delayed diagnoses. The present focus on creating a robust screening apparatus for our liver disease patients at risk for HCC is of critical importance to prevent the past from repeating itself[27].

ARTICLE HIGHLIGHTS

Research background

This study examines a large cohort of patients diagnosed with hepatocellular carcinoma (HCC) at two academic medical centers where liver transplantation is offered. Extensive data collection on patient and tumor variables were obtained to investigate the relationship between body mass index (BMI) classification and outcomes of patients with HCC.

Research motivation

The motivation for our research study is to explore how different BMI strata impact survival in patients with HCC.

Research objectives

It is apparent that a lean BMI in patients at the time of HCC diagnosis reflects advanced tumor burden but is not independently associated with worse survival.

Research methods

Patient and tumor characteristics were compared according to BMI < 25 kg/m^2 (lean), BMI 25-29.9 kg/m^2 (overweight), and BMI ≥ 30 kg/m^2. The Kaplan-Meier method was used to estimate survival by BMI categories. A multivariable model was performed to investigate risk factors (including the three BMI strata) associated with survival following HCC diagnosis.

Research results

Our research demonstrates interesting differences when comparing patients across BMI categories. For example, women with HCC were more likely to be in a higher BMI classification than men. Chronic hepatitis C infection was by far the most common reason for chronic liver disease in our cohort, and achieving sustained virologic response, not unexpectedly was associated with improved survival. We did not see significant differences in the Child-Pugh class or model for end stage liver disease scores according to the three different BMI. We did not see a survival difference by BMI class in our large cohort of 286 non-cirrhotic HCC cases patients.

Research conclusions

The relevant conclusion that one can draw from this study is the importance of identifying patients early in their presentation as our results confirm well established risk factors for reduced survival in patients with HCC trump the perceived protection of the "obesity paradox".

Research perspectives

The future research in this field needs to focus on improving patient access to screening for HCC to prevent a delay in diagnosis.

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FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Prediction model for hepatitis B e antigen seroconversion in chronic hepatitis B with peginterferon-alfa treated based on a response-guided therapy strategy

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Abstract

BACKGROUND

Models for predicting hepatitis B e antigen (HBeAg) seroconversion in patients with HBeAg-positive chronic hepatitis B (CHB) after nucleos(t)ide analog treatment are rare.

AIM

To establish a simple scoring model based on a response-guided therapy (RGT) strategy for predicting HBeAg seroconversion and hepatitis B surface antigen (HBsAg) clearance.

METHODS

In this study, 75 previously treated patients with HBeAg-positive CHB underwent a 52-week peginterferon-alfa (PEG-IFNα) treatment and a 24-wk follow-up. Logistic regression analysis was used to assess parameters at baseline, week 12, and week 24 to predict HBeAg seroconversion at 24 wk post-treatment. The two best predictors at each time point were used to establish a prediction model for PEG-IFNα therapy efficacy. Parameters at each time point that met the corresponding optimal cutoff thresholds were scored as 1 or 0.

RESULTS

The two most meaningful predictors were HBsAg ≤ 1000 IU/mL and HBeAg ≤ 3 S/CO at baseline, HBsAg ≤ 600 IU/mL and HBeAg ≤ 3 S/CO at week 12, and HBsAg ≤ 300 IU/mL and HBeAg ≤ 2 S/CO at week 24. With a total score of 0 *vs* 2 at baseline, week 12, and week 24, the response rates were 23.8%, 15.2%, and 11.1% *vs* 81.8%, 80.0%, and 82.4%, respectively, and the HBsAg clearance rates were 2.4%, 3.0%, and 0.0%, *vs* 54.5%, 40.0%, and 41.2%, respectively.

CONCLUSION

We successfully established a predictive model and diagnosis-treatment process using the RGT strategy to predict HBeAg and HBsAg seroconversion in patients with HBeAg-positive CHB undergoing PEG-IFNα therapy.

Key Words: Chronic hepatitis B; Hepatitis B e antigen-positive; Peginterferon-alfa; Prediction model; Response-guided therapy strategy

Core Tip: This study identified the optimal independent predictors of treatment response in previously treated patients with hepatitis B e antigen (HBeAg)-positive chronic hepatitis B who received peginterferon alpha therapy. Using single-factor and multi-factor logistic regression analyses, scoring prediction models and response-guided therapy strategies were established. These tools offer guidance for physicians to adjust treatment plans for patients who have not achieved HBeAg seroconversion after long-term nucleos(t)ide analog therapy, carrying significant practical implications for alleviating social and medical burdens.

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INTRODUCTION

Hepatitis B virus (HBV) infection poses a major public health threat worldwide. The World Health Organization estimated that approximately 296 million people worldwide were infected with chronic hepatitis B (CHB) in 2019, with approximately 820000 deaths from cirrhosis, liver failure, and hepatocellular carcinoma (HCC) caused by CHB[1]. The goal of antiviral therapy is to effectively suppress HBV DNA replication, with sustained hepatitis B surface antigen (HBsAg) clearance as the ideal endpoint[2], which significantly improves overall survival and reduces the risk of HCC and HBV-related mortality[3].

Currently, recommended antiviral treatment options include long-term nucleos(t)ide analogs (NAs) and a limited course of peginterferon alpha (PEG-IFNα) therapy. Most patients with CHB choose NAs because of their availability, affordability, ability to inhibit viral replication, and minimal side effects. The APASL Guideline[2] suggests the possibility of discontinuing antiviral treatment after 1-3 years of NA consolidation therapy following hepatitis B e antigen (HBeAg) seroconversion. However, numerous studies have demonstrated a high clinical relapse rate after discontinuing NAs in both HBeAg-positive and HBeAg-negative patients[4-6]. Maintaining good treatment compliance becomes challenging with long-term or lifelong oral medications, resulting in spontaneous or irregular drug withdrawal. Interferon has direct antiviral and immunomodulatory effects and can significantly reduce the incidence of liver cirrhosis and liver cancer in HBeAg-positive patients after HBeAg seroconversion[7]. Therefore, interferon is appropriate for young patients with CHB seeking permanent treatment cessation. However, the low HBeAg seroconversion rate, multiple contraindications and side effects, high price, and frequent follow-up times significantly limit the use of interferon[8,9].

In clinical practice, many patients with CHB choose NAs for various reasons. However, HBeAg seroconversion remains elusive after years of treatment, and discontinuing the drug is unsafe. Further investigation is needed to determine whether these patients should choose interferon for HBeAg seroconversion or HBsAg clearance. Therefore, optimal treatment strategies are urgently needed for patients pretreated with NAs who have not achieved HBeAg seroconversion.

PEG-IFNα has demonstrated a significantly greater effect in reducing HBsAg levels compared to NAs[10]. The large SWITCH study revealed that switching to PEG-IFNα in HBeAg-negative patients with CHB on long-term NAs could result in high rates of HBsAg loss[11]. Moreover, add-on or switching to PEG-IFNα therapy can optimize therapeutic response[12,13]. However, current studies on the efficacy of PEG-IFNα in previously treated HBeAg-positive patients with CHB are scarce. Several studies have demonstrated that lower baseline HBsAg levels and the extent of HBsAg decline during early treatment are strong predictors of HBeAg seroconversion and clearance in HBeAg-positive patients with CHB previously on NAs after PEG-IFNα therapy[14-16]. However, these studies mainly focus on the performance of a single parameter or predictors either at baseline or early treatment[14,16,17], resulting in limited predictive power.

Developing accurate prediction models for monitoring response to PEG-IFNα therapy and viable response-guided therapy (RGT) strategy in HBeAg-positive patients with CHB is necessary. Therefore, this study aimed to establish a simple and practical scoring model based on the RGT strategy for predicting HBeAg seroconversion and clearance.

MATERIALS AND METHODS

Study population

In this open, polycentric, retrospective study conducted from January 2010 to May 2023, 101 NAs-treated HBeAg-positive patients with CHB previously on NAs who received PEG-IFNα-2a/2b treatment were enrolled and followed up at the Second Affiliated Hospital, Anhui Provincial Hospital and the Fuyang Second People's Hospital of Anhui Medical University. The inclusion criteria were HBsAg positivity for at least 6 months, previous anti-HBV therapy (NAs treatment for at least 6 months), HBeAg-positive status before the current PEG-IFNα treatment, and patients who received at least one PEG-IFNα therapy. The exclusion criteria included co-infection with hepatitis C virus, hepatitis delta virus, or human immunodeficiency virus; resistance to lamivudine, adefovir dipivoxil, or telbivudine; neutrophil count < 1.0×10^9/L; platelet count < 50×10^9/L; de-compensated liver disease; immunologically-mediated disease; incomplete primary data; non-treatment in our hospital for the whole course; alcohol or drug abuse; and pregnancy or lactation. Following the Helsinki Declaration of 1975, the Ethics Committee of Anhui Medical University approved the study, and written informed consent was obtained from all patients.

Study medications

Patients were treated weekly with 180 μg PEG-IFNα-2a/2b (Pegasys; Roche, Shanghai, China or Peginterferonα-2b; Amoytop Biotech, Xiamen, China) by subcutaneous injection for 52 wk, followed by 24 wk off-treatment. Those with PEG-IFNα intolerance received a reduced dose depending on the situation. Patients who completed at least one round of PEG-IFNα therapy were included in this analysis according to the principles of intention-to-treat analysis.

Follow-up and measurements

Clinical assessments were performed from the initial treatment stage, baseline, on-treatment (weeks 12, 24, and 52), and the end of follow-up (EOF) of PEG-IFNα therapy. Commercially available enzyme immunoassays (Abbott, Chicago, IL, United States) were used to measure HBV serological markers (HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc; the lower limit of quantification of HBsAg was 0.05 IU/mL). TaqMan-based real-time polymerase chain reaction) assay (Shanghai ZJ BioTech, Shanghai, China) was used to quantify serum HBV DNA with a lower quantification limit of 500 IU/mL. Serum alanine aminotransferase (ALT) levels, expressed as multiples of the upper limit of normal (40 U/L), were assessed using an automatic biochemical analyzer (Roche, Basel, Switzerland). Blood cells were sorted and counted using an automatic blood cell analyzer (Aptio, Sysmex, Shanghai, China).

Study endpoints

The responses at the end of treatment (EOT) and EOF were defined as HBeAg seroconversion at the end of 52 wk of PEG-IFNα therapy and 24 wk off-treatment, respectively. For a few patients who changed their treatment regimen midway, data at 52 or 76 wk of PEG-IFNα therapy were analyzed for EOT or EOF evaluation. Patients with HBeAg seroconversion were defined as responders; otherwise, they were defined as non-responders. The primary endpoint was the HBeAg seroconversion rate at EOF, and the secondary endpoint was HBsAg clearance at EOF.

Statistical analysis and model establishment

Statistical analyses were conducted using the SPSS software version 26.0 (SPSS Inc., Chicago, IL, United States). Graphic production was performed using GraphPad Prism version 9 (GraphPad Prism 9.3.1, Santiago, United States). Descriptive statistics were expressed as mean ± SD or median (interquartile range) for parametric or non-parametric continuous data and were compared using Student's *t*-test or Mann-Whitney *U* test when necessary. Categorical parameters were expressed as counts (percentage) and compared using the χ^2 test or Fisher's exact test as required.

The best cut-off values of parameters were determined based on the areas under the receiver operating characteristic curve (AUROC). In addition, the values adjacent to the best cutoff values (integer, if possible) were used as the best predictive cutoff values (hereafter referred to as the best predictors) for clinical practicability. Univariate and multivariate logistic regression analyses were conducted to identify the best predictors of treatment outcomes. All statistical tests were two-sided, and $P < 0.05$ was considered significant.

The most significant independent predictors associated with the response at EOF were selected through logistic regression analysis at baseline, week 12, and week 24 using stepwise regression or entry methods. The two best predictors were selected at each time point to establish the prediction models. If the parameters met the optimal threshold, the score was 1. Otherwise, the score was 0, and the sum was the total score.

RESULTS

Baseline characteristics

Out of the 101 patients treated and followed up, 75 (74.3%) were included in the final analysis, with 26 patients excluded (Supplementary Figure 1). At EOF, HBeAg seroconversion occurred in 27 patients (36.0%), eight (10.7%) experienced HBsAg loss, and seven (9.3%) developed anti-HBs.

At the initial treatment stage, ALT levels in responders were higher than those in non-responders, while HBV DNA, HBsAg, and HBeAg levels were lower in responders. After a period of NAs treatment (median of 2 years), the above indexes decreased significantly, with ALT decreasing to normal levels, HBV DNA below the detection limit, and HBeAg to an extremely low level, and the decline was more pronounced in responders (Figure 1D). The initial therapy and pretreatment duration were comparable.

Compared with non-responders, responders had longer PEG-IFNα treatment duration (13 *vs* 9 months), lower baseline HBsAg levels (3.26 *vs* 3.51 Lg IU/mL), lower HBeAg levels (0.43 *vs* 1.01 Lg S/CO), and lower initial HBsAg and HBeAg levels. The two groups did not differ significantly with respect to sex, age, baseline ALT, HBV DNA, duration of pretreatment, type of NAs, or current treatment strategies (Table 1).

Treatment and follow-up

ALT levels fluctuated during the treatment, and no differences were observed between the two groups at each time point. After a period of NAs treatment, HBV DNA was undetectable at the beginning of PEG-IFNα therapy for most patients (63/75, 84.0%), and no rebound occurred during the entire period (Figure 1A and B).

Throughout the process, the HBsAg and HBeAg levels of the responders decreased gradually, while that of the non-responders fluctuated at week 52 because the PEG-IFNα treatment course was less than 52 wk. Furthermore, the decline in HBsAg level was more pronounced and persistent. HBeAg showed the most significant decrease at week 12 and gradually decreased continuously thereafter (Figure 1C and D).

Performance of traditional single parameters in predicting response at the EOF

HBsAg and/or HBeAg levels are reliable predictors of response to PEG-IFNα in naïve patients with CHB. HBsAg levels were sub-grouped according to the following criteria[18]: HBsAg < 1500 IU/mL, 1500 ≤ HBsAg ≤ 20000 IU/mL, and HBsAg > 20000 IU/mL. When efficacy was evaluated based on EOF response, no obvious differences were observed between the HBsAg subgroups at baseline and week 12, but significant differences were observed at week 24 ($P < 0.001$). However, only 34 patients (with HBsAg ≥ 1500 IU/mL at week 24) with a poor response (expected response rate ≤ 15.0%) were considered for PEG-IFNα discontinuation. Similarly, when HBsAg clearance at EOF was assessed, the predictive values at baseline, week 12, and week 24 were extremely limited ($P = 0.024$), and the highest predictive HBsAg loss rates (with HBsAg level < 1500 IU/mL) were all poor (21.4%, 18.2%, and 19.5%, respectively) (Supplementary Figure 2A-C).

Similarly, HBeAg levels were classified at each time point[18]: HBeAg < 20 S/CO, 20 ≤ HBeAg ≤ 500 S/CO, and HBeAg > 500 S/CO. When efficacy was evaluated based on the response at EOF, no significant differences were observed between the HBeAg subgroups at baseline, week 12, and week 24. Only 6 (with HBeAg > 500 S/CO at week 12) and 13 (with HBeAg ≥ 20 S/CO at week 24) patients with a poor response (expected response rate ≤ 15.0%) were advised to discontinue PEG-IFNα. After evaluating the HBsAg loss rate at EOF, no significant differences were observed among the HBeAg subgroups at each time point (Supplementary Figure 2D-F).

Performance of single parameters in predicting response at the EOF

Univariate/multivariate analyses of relevant parameters at each time point were performed. Furthermore, the optimal cutoff values at each time point were determined using AUROC and adjusted for clinical practicality (preferably using integers). The two best predictors of response at EOF were HBsAg ≤ 1000 IU/mL and HBeAg ≤ 3 S/CO at baseline, HBsAg ≤ 600 IU/mL and HBeAg ≤ 3 S/CO at week 12, and HBsAg ≤ 300 IU/mL and HBeAg ≤ 2 S/CO at week 24 (Table 2, Supplementary Tables 1-3).

When predicting efficacy at EOF using a single parameter at each time point, patients were divided into high-response and low-response groups based on the proportion of HBeAg seroconversion. Only the HBsAg ≤ 300 IU/mL group at week 24 showed no predictive value for HBsAg loss, whereas the predictive power of a single factor was better at other time points ($P < 0.05$). However, when HBeAg seroconversion at EOF was used as the evaluation criterion, the predictive value of univariate grouping was not satisfactory in most cases (except for the HBeAg subgroup at weeks 12 and 24). The response rate in the low response group ranged between 15.2% and 28.3%; however, the proportion was as high as 57.3% to 70.7% (Figure 2A-C). Among 27 patients who achieved response at EOF, only 44.4%-63.0% (HBsAg subgroup) and 51.9%-74.1% (HBeAg subgroup) were from the high-response group, indicating the limited effectiveness of using a single parameter to predict response (Figure 2D-F).

The HBsAg and HBeAg values at each time point were used to create scatter diagrams. Scatter plots of HBsAg and HBeAg are shown in Supplementary Figure 3A-C. HBsAg plots of responders below the cutoff values (1000 IU/mL at baseline, 600 IU/mL at week 12, and 300 IU/mL at week 24) were 44.4%, 55.6%, and 63.0%, respectively, while non-responders below the cutoff values were all 20.8% (Supplementary Figure 3A-C). For the HBeAg subgroup, plots of responders below the cutoff values (3 S/CO at baseline, 3 S/CO at week 12, and 2 S/CO at week 24) fluctuated between 51.9%-74.1%, while that of non-responders was between 16.7%-25.0% (Supplementary Figure 3D-F).

Table 1 Baseline characteristics of patients stratified by the follow-up endpoint

Characteristics	RS (*n* = 27)	NRS (*n* = 48)	*P* value
Sex			0.057
Male	18 (66.7%)	41 (85.4%)	
Female	9 (33.3%)	7 (14.6%)	
Age (yr)	32.26 ± 6.96	34.33 ± 7.38	0.237
Initial data			
ALT (ULN)	3.01 (0.69-4.88)	2.00 (0.98-6.20)	0.773
HBV DNA (lg IU/mL)	4.57 (2.70-7.62)	7.15 (5.35-7.85)	0.057
HBsAg (lg IU/mL)	3.90 (2.84-4.38)	4.30 (3.68-4.70)	0.022
HBeAg (lg S/CO)	2.36 (0.89-2.93)	2.96 (2.05-3.17)	0.014
Prior antiviral therapy			
ETV	13 (48.2%)	28 (58.3%)	0.653
TDF	9 (33.3%)	14 (29.2%)	
LAM/ADV/LdT	5 (18.5%)	6 (12.5%)	
Pre-treatment duration (yr)	2 (1.0-3.5)	2 (1.0-4.0)	0.399
Baseline			
ALT (ULN)	0.90 (0.45-1.88)	0.99 (0.65-1.56)	0.446
HBV DNA (lg IU/mL)	2.70 (2.70-2.70)	2.70 (2.70-2.70)	0.375
HBsAg (lg IU/mL)	3.26 (2.61-3.65)	3.51 (3.05-3.82)	0.089
HBeAg (lg S/CO)	0.43 (0.18-1.70)	1.07 (0.63-1.89)	0.035
WBC (× 10^9/L)	5.89 ± 1.85	5.60 ± 1.71	0.517
N (× 10^9/L)	3.34 ± 1.37	3.06 ± 1.21	0.385
RBC (× 10^9/L)	4.99 ± 0.53	5.00 ± 0. 47	0.901
Hb (× 10^9/L)	146.75 ± 13.64	150.57 ± 15.72	0.323
PLT (× 10^9/L)	193.63 ± 56.65	192.19 ± 66.45	0.929
Current therapy			
PEG-IFNα monotherapy	7 (25.9%)	6 (12.5%)	0.326
PEG-IFNα + ETV	6 (22.2%)	14 (29.2%)	
PEG-IFNα + TDF	14 (51.9%)	28 (58.3%)	
PEG-IFNα duration (month)	13 (12-18)	9 (6-12)	< 0.001

Values are presented as number (percentage) or mean ± SD or median (Q1-Q3). RS: Responders; NRS: Non-responders; ALT: Alanine aminotransferase; ULN: Upper limit of normal; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; ETV: Entecavir; TDF: Tenofovir disoproxil; LAM: Lamivudine; ADV: Adefovir dipivoxil; LdT: Telbivudine; PEG-IFNα: Peginterferon-alfa; WBC: White blood cell; RBC: Red blood cell; N: Nitride; Hb: Hemoglobin; PLT: Platelet count.

Performance of multiple parameters in predicting response at the EOF

At baseline, two independent predictors of response at EOF, HBsAg ≤ 1000 IU/mL and HBeAg ≤ 3 S/CO, were used to construct the prediction model. At baseline, 10 patients (23.8%) scored 0 attained a response, and one (2.4%) achieved HBsAg clearance. Nine patients with a score of 2 (81.8%) experienced a response, and HBsAg loss was achieved in six patients (54.5%) (Figure 3A).

At week 12, two meaningful parameters were HBsAg level ≤ 600 IU/mL and HBeAg level ≤ 3 S/CO. After using the above predictors to establish the model, 15 patients scored 2, with response and HBsAg clearance rates of 80.0% and 40.0%, respectively. Out of 33 patients with a score of 0, HBeAg seroconversion occurred in only five (15.2%) (Figure 3B).

At week 24, the most significant predictive parameters were HBsAg level ≤ 300 IU/mL and HBeAg ≤ 2 S/CO. Using these variables to construct a prediction model, 17 patients scored 2, of whom 14 (82.4%) attained a response, and seven (41.2%) achieved HBsAg seroclearance. Thirty-six patients scored 0, and only four (11.1%) had HBeAg seroconversion (Figure 3C).

Table 2 Selected variables by multivariate analysis at baseline, week 12, and week 24 to construct predictive models for response at 24 wk post-treatment

	Selected predictive variables	Multivariate analysis OR (95%CI)	*P* value
Baseline	HBsAg ≤ 1000 IU/mL	0.466 (0.153-1.421)	0.180
	HBeAg ≤ 3 S/CO	0.222 (0.074-0.671)	0.008
12 wk	HBsAg ≤ 600 IU/mL	0.271 (0.091-0.810)	0.019
	HBeAg ≤ 3 S/CO	0.230 (0.079-0.668)	0.007
24 wk	HBsAg ≤ 300 IU/mL	0.225 (0.067-0.759)	0.016
	HBeAg ≤ 2 S/CO	0.089 (0.027-0.297)	< 0.001

HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; OR: Odds ratio; CI: Confidence interval.

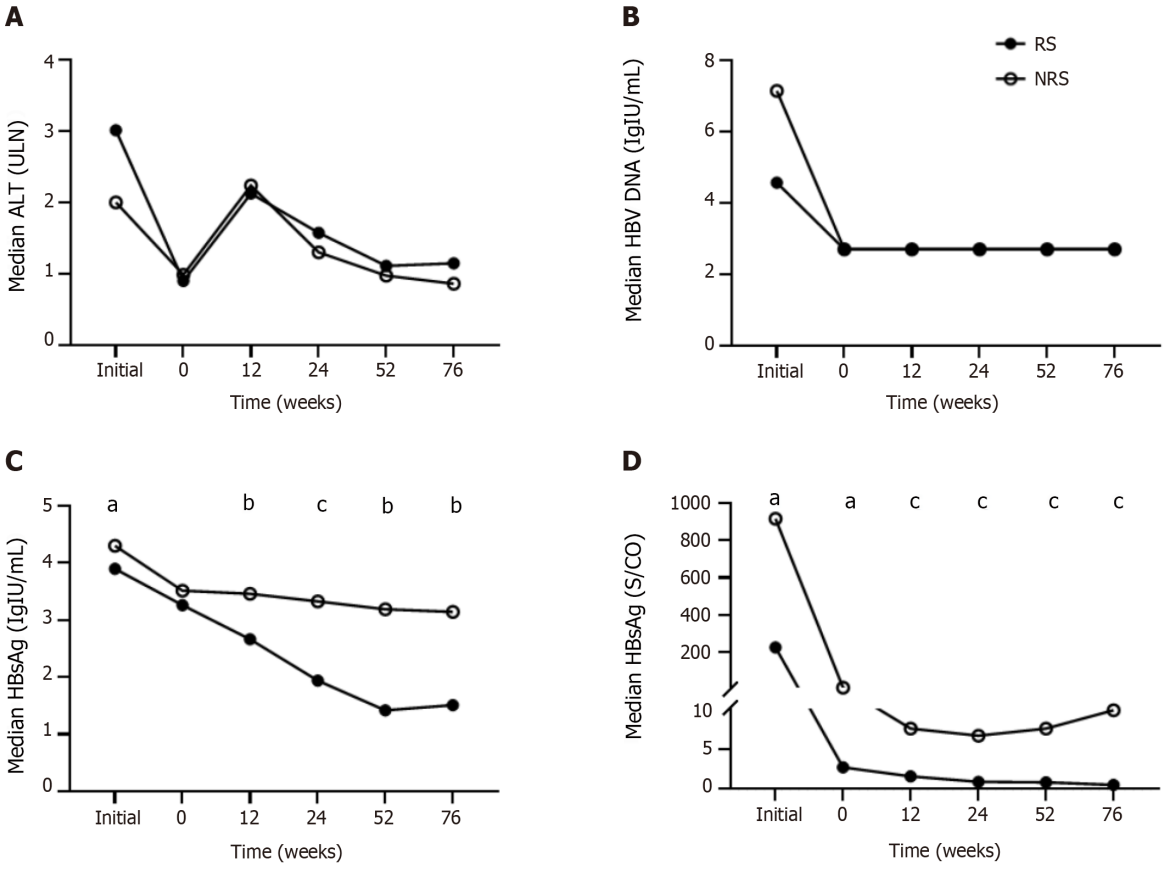

Figure 1 Kinetics of serum markers in patients with chronic hepatitis B during peginterferon alpha treatment and follow-up between responders and non-responders. A: Alanine aminotransferase; B: Hepatitis B virus DNA; C: Hepatitis B surface antigen; D: Hepatitis B e antigen. [a]*P* < 0.05, [b]*P* < 0.01, [c]*P* < 0.001. RS: Responders; NRS: Non-responders; ALT: Alanine aminotransferase; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen.

RGT strategies

At each time point, a higher score indicated a better curative effect. However, the Kappa consistency analysis of patients scores at each time point revealed a Kappa coefficient between 0.542 and 0.677 after pairwise comparison, suggesting the scores of the same patient at different time points were moderately consistent (Supplementary Tables 4-7).

According to a comprehensive analysis of scores at each time point, the possibility of obtaining a response was very low for patients who scored 0 at any time point, and the possibility of attaining a response decreased with an increase in the number of patients scoring 0. In contrast, among patients who scored 2, the more they frequently scored 2, the higher the response and HBsAg clearance rates. Compared with patients who scored 0 at all three-time points, patients who scored 2 at two or three-time points were significantly more likely to experience HBsAg clearance (Supplementary Figure 4A-D).

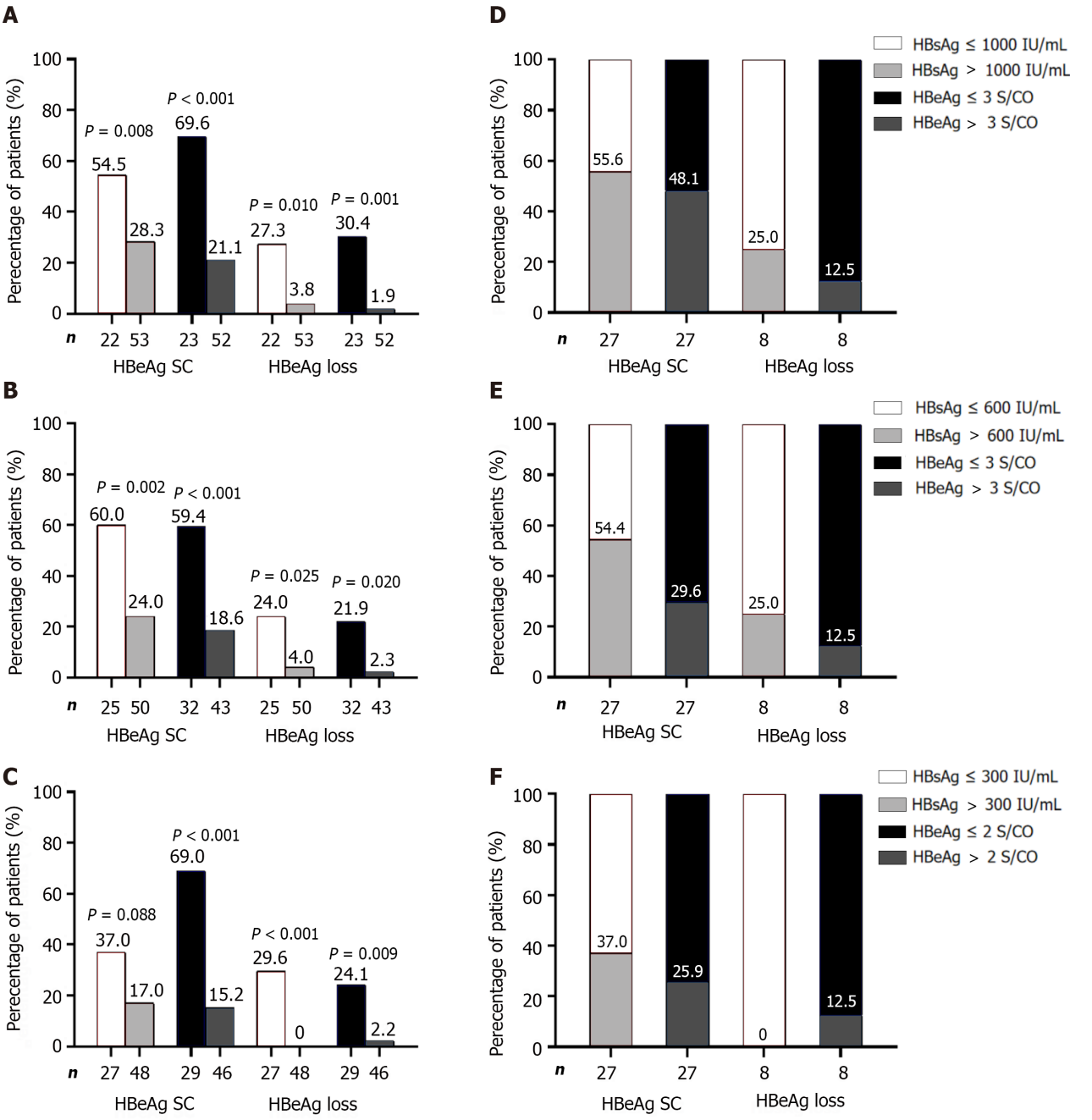

Figure 2 Response and hepatitis B surface antigen loss rates at 24 wk post-treatment based on patients who met single hepatitis B surface antigen or hepatitis B e antigen cutoffs at each time point. A-F: Moreover, the proportion of patients who met the single hepatitis B surface antigen (HBsAg) or hepatitis B e antigen (HBeAg) cutoffs at each time point was determined based on patients that achieved response and HBsAg loss at 24 wk post-treatment. Baseline (A and D), week 12 (B and E), week 24 (C and F). HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen.

Conversely, among patients who scored 0 at baseline, the percentage of attaining a response at EOF was 23.8%, which was obviously higher than that of patients at weeks 12 and 24. The percentage of patients who experienced a response at EOF among patients who scored 0 continued to decrease (15.2%-11.1%) as treatment progressed (Figure 3A-C). Owing to the limited predictive efficacy at baseline, it is crucial to make real-time treatment decisions based on timely clinical indicators.

Based on the optimal cutoff values for HBsAg and HBeAg levels at each time point, an RGT strategy was proposed. At baseline, if the patient's indicators meet both cutoff values (HBsAg ≤ 1000 IU/mL and HBeAg ≤ 3 S/CO), undergoing PEG-IFNα treatment is highly recommended because the chances of achieving a favorable response are very high. If only one of the criteria is met, PEG-IFNα therapy is recommended. If neither of the above conditions is met, PEG-IFNα treatment can still be considered, as approximately one-fifth of patients may achieve a response at the end of therapy. At week 12, continuing PEG-IFNα treatment is advisable if patients' parameters meet either one or both cutoffs (HBsAg ≤ 600 IU/mL and HBeAg ≤ 3 S/CO). However, if none of the criteria are met, discontinuing treatment is advisable because the likelihood of achieving a response is low, thereby helping to avoid treatment-related side effects and reducing the financial burden on patients. After 24 wk of therapy, if the patient's indicators meet either one or both criteria (HBsAg ≤

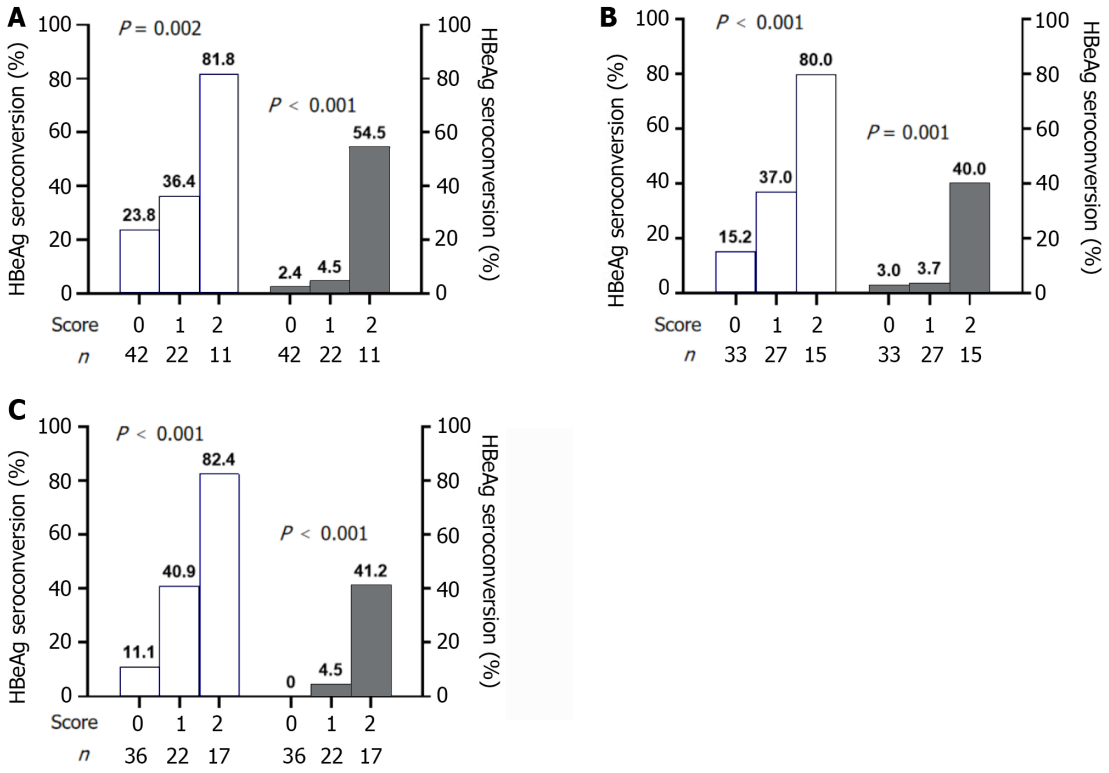

Figure 3 Performance of predictive models at baseline, week 12, and week 24 for evaluating hepatitis B e antigen seroconversion (blue bars) and hepatitis B surface antigen loss (pink bars) at 24 wk post-treatment in patients with chronic hepatitis B treated with peginterferon alpha. A: Baseline; B: Week 12; C: Week 24. HBeAg: Hepatitis B e antigen.

300 IU/mL and HBeAg ≤ 2 S/CO), it is recommended to continue and complete the PEG-IFNα treatment. If neither criterion is met, discontinuing PEG-IFNα treatment is advised (Figure 4).

DISCUSSION

Achieving a clinical cure for HBeAg-positive patients with CHB previously treated with NAs is unlikely. HBeAg seroconversion can be achieved with PEG-IFNα therapy, thus allowing drug withdrawal. Several large randomized controlled studies aimed at treatment-naïve HBeAg-positive patients with CHB reported HBeAg seroconversion and HBsAg clearance rates of 29.0%-36.7% and 3.0%-7.0%, respectively[9,19,20]. In our study, the HBeAg seroconversion and HBsAg loss rates were 36.0% and 10.7%, respectively. Similar to previous studies, no significant differences were observed in the HBeAg seroconversion and HBsAg loss rates between treatment-naïve and PEG-IFNα-treated patients. Increasing evidence suggests that long-term NA therapy could enhance and promote the immunomodulatory effects of interferon therapy in patients with CHB. Chi *et al*[21] showed that PEG-IFNα therapy increased the likelihood of HBeAg seroconversion (30% *vs* 7%) in HBeAg-positive patients treated with entecavir (ETV)/tenofovir disoproxil (TDF) for at least 1 year, compared to continuing NAs treatment. A meta-analysis reported that the PEG-IFNα combination strategy in NAs-treated patients resulted in higher HBeAg seroconversion (59% *vs* 31%) and HBsAg clearance (9% *vs* 6%) rates than the "*de novo*" strategy[22]. These findings indicate that the PEG-IFNα treatment strategy remains effective for treated HBeAg-positive patients.

Numerous studies have shown that treatment-naïve HBeAg-positive CHB patients have high levels of ALT, HBV DNA, HBsAg, HBeAg, and anti-HBc at baseline[16,17,23]. When the patients in our study initially chose NAs for antiviral therapy, the above parameters were similarly high. However, after approximately 2 years of antiviral treatment, ALT and HBV DNA reduced to normal levels in most patients. Additionally, HBsAg and HBeAg levels also decreased significantly, although HBeAg remained positive. This aligns with the baseline results of treated patients, as reported in various studies[17,24-26]. In naïve HBeAg-positive patients treated with PEG-IFNα, age, sex, baseline ALT, HBV DNA load, HBsAg, HBeAg, and anti-HBC levels may be closely related to HBeAg seroconversion and/or HBsAg loss at EOF, which can be used as predictors of clinical efficacy[20,23,27]. This study included only 75 patients in the final analysis. The reason for the small sample size was that the number of patients who did not undergo serological conversion after NAs and subsequently switched to PEG-IFN therapy was small in the literature and clinical practice. Since HBeAg seroconversion did not occur after long-term treatment, drug withdrawal was not advisable. To ensure that early drug discontinuation is safe, efforts have been made to switch to or complement PEG-IFNα therapy. For such patients, it is important to identify meaningful factors affecting the response and establish early prediction models for better efficacy at EOF.

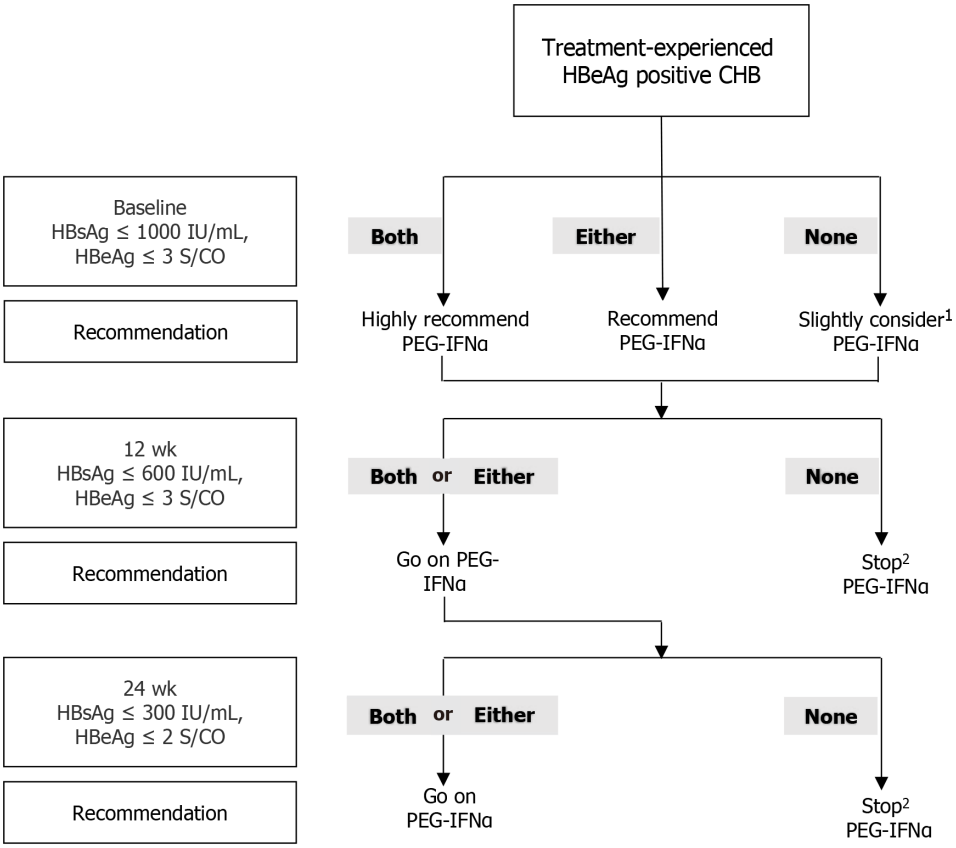

Figure 4 Response-guided therapy strategy for predicting response at 24 wk post-treatment based on hepatitis B surface antigen and hepatitis B e antigen levels at baseline, week 12, and week 24 in the management of previously treated patients with hepatitis B e antigen-positive chronic hepatitis B. [1]According to the prediction models, patients who didn't meet the two cutoffs at baseline had a response of 23.8% at end of follow-up. So we gave a recommendation of slightly considering peginterferon-alfa. [2]For patients who didn't meet corresponding two cutoffs at week 12 or week 24 but met cutoffs at baseline, peginterferon-alfa could be considered because there was a possibility of attaining a response at end of follow-up. CHB: Chronic hepatitis; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; PEG-IFNα: Peginterferon-alfa.

Several parameters, including ALT, HBV DNA, HBeAg, HBsAg levels, and the early decline in HBsAg during treatment, have been associated with HBeAg seroconversion after PEG-IFNα treatment in previously treated HBeAg-positive patients[16,17,28]. Li *et al*[16] demonstrated that HBeAg-positive patients who started PEG-IFNα combination therapy after 2 years of ETV treatment had a higher HBeAg seroconversion rate (64.2%) if their baseline HBeAg was < 200 S/CO. Patients with baseline HBsAg levels < 1000 IU/mL had a higher HBsAg loss rate (31.8%). Liem *et al*[17] also reported that the response rate was the highest, reaching up to 70%, in HBeAg-positive patients who started combination therapy with PEG-IFNα, with baseline HBsAg levels < 4000 IU/mL and HBV DNA levels < 50 IU/mL. Moreover, the response rate of patients meeting only one of the above criteria was only 44%. Some other factors, such as PEG-IFNα monotherapy or combination therapy with NAs, seem unrelated to treatment efficacy. Our study indicated that the occurrence of response at EOF was not significantly correlated with the treatment regimen, whether it was PEG-IFNα monotherapy, PEG-IFNα + ETV, or PEG-IFNα + TDF. However, a recent meta-analysis indicated that compared to IFN monotherapy, IFN + NAs combination therapy had a higher e-antigen serological response at EOT[29]. These influencing factors may include whether the patient has received prior treatment, viral load, HBsAg levels, HBeAg status, and the degree of liver fibrosis[9,30,31]. These studies suggested that baseline HBsAg or HBeAg levels and on-treatment dynamics could be valuable in predicting response to PEG-IFNα. However, most of these studies employed univariate analyses or only analyzed parameters at baseline.

In this study, HBsAg and/or HBeAg levels and their decline at baseline, week 12, and week 24 were valuable for predicting HBeAg seroconversion and HBsAg clearance at EOF. However, the predictive power of single parameters is extremely limited, with unsatisfactory sensitivity, specificity, as well as positive and negative predictive values. The HBeAg seroconversion rate in the low-response group remained between 20% and 30% at baseline, posing significant challenges for physicians' and patients' decision-making. Combining two predictors to establish a prediction model can greatly improve the efficiency and accuracy of the prediction power. Patients who scored 0 at week 12 and week 24 had a response below 15%, while most patients achieved satisfactory outcomes, and HBsAg clearance occurred in patients who scored 2 at each time point.

Sonneveld *et al*[28] developed a preliminary RGT strategy for PEG-IFN treatment to guide HBeAg-positive patients with CHB according to the different genotypes and HBsAg levels. Patients with the B or C genotype and HBsAg > 20000 IU/mL at week 12 were advised to stop treatment. Similarly, those with HBsAg > 20000 IU/mL at week 24, irrespective of genotype, should stop treatment. Therefore, the decision to continue the original antiviral therapy regimen should be

based on the on-treatment response. In this study, the multivariate prediction models based on responses at baseline, week 12, and week 24 had good predictive values. However, the effect of the baseline prediction model alone was limited. The HBeAg seroconversion rates at EOF for patients who scored 0, 1, and 2 at baseline were 23.8%, 36.4%, and 81.8%, respectively. Excluding patients who scored 0 from the PEG-IFNα therapy was difficult. Therefore, adjusting the treatment strategy according to the on-treatment response is necessary.

To facilitate clinical practice, we evaluated the possibility of response at EOF based on HBsAg and HBeAg levels at different time points. Thereafter, we created a strategy map for the RGT approach, providing recommendations on whether to continue or stop PEG-IFNα therapy at each time point (Figure 4). Patients who did not achieve HBeAg seroconversion after NAs therapy and met both HBsAg and HBeAg thresholds at baseline were highly likely to experience HBeAg seroconversion at EOF. Therefore, PEG-IFNα therapy was recommended. However, the likelihood of a response is not high when either of the above thresholds is satisfied. It is recommended that NAs should be continued until appropriate, and PEG-IFNα therapy should not be initiated without the patient's desire for it. After 12 wk of treatment, PEG-IFNα therapy was recommended to be continued in patients with scores of 1 or 2 and should be stopped in patients that scored 0 unless their baseline score was 2. At week 24, if the patient scored 1 or 2, continuing PEG-IFNα treatment for 52 wk is highly recommended; otherwise, PEG-IFNα treatment should be stopped unless the total score at baseline and week 12 was 2. This RGT strategy can be used to effectively select patients with good outcomes, allowing both doctors and patients to make reasonable decisions.

CONCLUSION

In summary, our study successfully established predictive models for the response to PEG-IFNα in treatment-experienced patients with HBeAg-positive CHB. The prediction models are simplistic and practical, and the RGT strategy can help optimize the use of PEG-IFNα. However, this study was a single-center exploratory study with a limited sample size, and no genotypes were tested. These results need to be further confirmed by multicenter, large-scale prospective studies.

ARTICLE HIGHLIGHTS

Research background

Hepatitis B virus (HBV) infection poses a major public health threat worldwide. Recently, many studies on the efficacy of peginterferon-alfa (PEG-IFNα) in treatment-experienced hepatitis B e antigen (HBeAg)-positive chronic hepatitis B (CHB) patients are scarce. Models for predicting HBeAg seroconversion in patients with HBeAg-positive CHB after nucleos(t)ide analog (NAs) treatment are necessary.

Research motivation

In clinical practice, many NAs-treated patients with HBeAg-positive CHB did not attain HBeAg seroconversion, and drug withdrawal is unsafe. Currently, IFN is appropriate for young patients with CHB who desire to end treatment permanently. It is necessary to explore accurate prediction models for the response to PEG-IFNα therapy and viable response-guided therapy (RGT) strategy in patients with HBeAg-positive CHB.

Research objectives

The key significance of this study is to establish a simple scoring model based on a RGT strategy for predicting HBeAg seroconversion and hepatitis B surface antigen (HBsAg) clearance for treatment-experienced patients with HBeAg-positive CHB.

Research methods

In this study, seventy-five treatment-experienced patients with HBeAg-positive CHB underwent a 52-wk PEG-IFNα treatment and a 24-wk follow-up. Logistic regression analysis was used to assess parameters at baseline, week 12, and week 24 to predict HBeAg seroconversion at 24 wk off-treatment. The two best predictors at each time point were applied to establish a prediction model for PEG-IFNα therapy efficacy. Parameters at each time point meeting the corresponding optimal cut-off thresholds were scored as 1 or 0.

Research results

We found that the two most meaningful predictors were HBsAg ≤ 1000 IU/mL and HBeAg ≤ 3 S/CO at baseline, HBsAg ≤ 600 IU/mL and HBeAg ≤ 3 S/CO at week 12, and HBsAg ≤ 300 IU/mL and HBeAg ≤ 2 S/CO at week 24. For a total score of 0 *vs* 2 at baseline, week 12, and week 24, the response rates were 23.8%, 15.2%, and 11.1% *vs* 81.8%, 80.0%, and 82.4%, respectively, and the HBsAg clearance rates were 2.4%, 3.0%, and 0.0%, *vs* 54.5%, 40.0%, and 41.2%, respectively.

Research conclusions

We successfully established a predictive model and diagnosis-treatment process based on the RGT strategy to predict HBeAg and HBsAg seroconversion to PEG-IFNα therapy in patients with HBeAg-positive CHB.

Research perspectives

The prediction models established for treatment-experienced patients with HBeAg-positive CHB are simplistic and practical, and the RGT strategy can help to optimize the use of PEG-IFNα. These results need to be further confirmed by multicenter, large-scale prospective studies.

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FOOTNOTES

Author contributions: Zhang PX and Zheng XW contributed to the data analysis and drafting of the manuscript; Zhang PX, Zheng XW, Zhang YF, Ye J, Li W, Tang QQ, and Zou GZ contributed to the data acquisition; Zhang YF, Zhu J, Zou GZ, and Zhang ZH contributed in the process assessment; Zhang ZH contributed in the study conception and design; and all authors have read and approved the final version of the manuscript.

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Informed consent statement: Written informed consent was obtained from all patients.

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ORIGINAL ARTICLE

Retrospective Study

Retrospective study of the incidence, risk factors, treatment outcomes of bacterial infections at uncommon sites in cirrhotic patients

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Abstract

BACKGROUND

Bacterial infections (BI) negatively affect the natural course of cirrhosis. The most frequent BI are urinary tract infections (UTI), pneumonia, and spontaneous-bacterial peritonitis (SBP).

AIM

To assess the relevance of bacterial infections beyond the commonly recognized types in patients with cirrhosis and to investigate their relationship with other clinical variables.

METHODS

We retrospectively analyzed patients with cirrhosis and BI treated between 2015 and 2018 at our tertiary care center. BIs were classified as typical and atypical, and clinical as well as laboratory parameters were compared between the two groups.

RESULTS

In a cohort of 488 patients with cirrhosis, we identified 225 typical BI (95 UTI, 73

SBP, 72 pulmonary infections) and 74 atypical BIs, predominantly cholangitis and soft tissue infections (21 each), followed by intra-abdominal BIs (n = 9), cholecystitis (n = 6), head/throat BIs (n = 6), osteoarticular BIs (n = 5), and endocarditis (n = 3). We did not observe differences concerning age, sex, or etiology of cirrhosis in patients with typical *vs* atypical BI. Atypical BIs were more common in patients with more advanced cirrhosis, as evidenced by Model of End Stage Liver Disease (15.1 ± 7.4 *vs* 12.9 ± 5.1; P = 0.005) and Child-Pugh scores (8.6 ± 2.5 *vs* 8.0 ± 2; P = 0.05).

CONCLUSION

Atypical BIs in cirrhosis patients exhibit a distinct spectrum and are associated with more advanced stages of the disease. Hence, the work-up of cirrhosis patients with suspected BI requires detailed work-up to elucidate whether typical BI can be identified.

Key Words: Bacterial infection; Empirical antibiotic therapy; End-stage liver disease; Escherichia coli; Multi-resistant pathogens

Core Tip: Bacterial infections (BI) affect the natural course of liver cirrhosis and can trigger decompensation or death. The most frequent BI in cirrhosis (urinary tract infections, pneumonia or spontaneous-bacterial peritonitis) were retrospectively compared to infections at other body sites, which are thought to be less frequently affected (so-called "atypical BI"). When comparing typical/atypical BI, no differences in age, sex, or etiology of cirrhosis were found. Notably, for atypical BI, the stage of cirrhosis was less advanced, as expressed by laboratory parameters and clinical scores (*e.g.* Model of End Stage Liver Disease - and Child-Pugh-Score).

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INTRODUCTION

Bacterial infections (BI) significantly affect the natural history of cirrhosis and may lead to a dramatic increase in mortality of infected patients[1-3]. Furthermore, BI are the most common event causing hepatic decompensation[4]. The more severe course of BI is attributed to the acquired immunodeficiency of patients with cirrhosis, the increased bacterial translocation from the intestinal tract, and the consequences of portal hypertension. The most common BI in cirrhosis include urinary tract infection (UTI), pneumonia, and spontaneous-bacterial peritonitis (SBP)[3]. Whereas infections at other body sites also occur relatively frequently in patients with cirrhosis (herein further called "atypical BI"), these have been investigated far less in-depth, in particular due to the lack of sufficiently large cohorts of patients with these specific BI in the setting of cirrhosis.

Accurate microbiological diagnostics are essential for targeted antibiotic therapy. This is often challenging in patients with cirrhosis, as invasive collecting of samples (*e.g.* ascites, or sputum) is not always feasible. Commonly, empirical antibiotic therapy is insufficient. Indeed, Lameirão Gomes *et al*[5] showed in a retrospective analysis that in only 60% of cases, empirical therapy was adequate against the infection-causing pathogens.

Here, we aimed to specifically compare the clinical and microbiological characteristics of patients with cirrhosis and typical BI (pneumonia, UTI and SBP) as compared to atypical BI, by exploiting a large database[6] (INCA database) of patients with BI and cirrhosis.

MATERIALS AND METHODS

Study population

This analysis was carried out as sub-study of the INCA trial, the study protocol of which has been published[6]. The study analyzed data from inpatients with cirrhosis and BI who received treatment at Saarland University Medical Center in Homburg, Southwest Germany, between January 1, 2015, and December 31, 2018. All hospitalized patients with cirrhosis were considered for inclusion. Patients with severe comorbidities such as end-stage heart failure, HIV infection and non-resectable cancer (except hepatocellular carcinoma Barcelona Lever Clinic Classification stages A-C), as well as patients in whom a BI could not be confirmed were excluded. Cirrhosis was defined by (1) biopsy; (2) a combination of clinical, laboratory, ultrasound and endoscopic findings; or (3) transient elastography > 13.0 kPa[7]. In patients with

transient elastography < 19.7 kPa, diagnosis of cirrhosis was additionally confirmed by (1) or (2). Results pertaining to different disease aspects of this cohort have been reported previously[7]. Overall, 488 patients with cirrhosis and BI requiring antibiotic therapy were finally included. BI were categorized applying stringent criteria (Supplementary Table 1). The electronic medical records were reviewed for clinical data, and further information regarding medication use (such as antibiotic therapy, beta-blocker, lactulose, statins) and laboratory parameters at the time of inclusion were recorded. The use of long-term antibiotics (prescribed for prophylaxis of SBP or for recurrent hepatic encephalopathy) was also documented.

Bacterial infections and antibiotic therapy

All atypical BI cases were analyzed using the microbiological databases HyBASE® (epiNET AG, Germany) and M/Lab (Dorner, Germany) at Saarland University Medical Center. The diagnostics carried out during the event period, the main detected pathogens, and the related antibiotic therapy were recorded. Of note, all microbiological diagnostic procedures such as Gram staining, culture techniques and identification methods were performed using standard operating procedures. Species identification of culture-grown bacterial colonies was carried out using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker, Germany). Subsequently, the pathogens were grouped into Gram-positive and Gram-negative pathogens. In addition, the available antibiograms were interpreted with respect to resistance behavior using the multi-drug resistance (MDR) classification by Magiorakos *et al* [8]. The antibiotic therapy was categorized into the following antibiotic classes: Penicillins, cephalosporins, carbapenems, quinolones, macrolides, glycopeptides, linezolid, metronidazole, and others. In addition, the assessment included the administration of monotherapy and combination therapies, the length of therapy given, and the effectiveness of empirical therapy.

Statistical analyses

All variables are described as proportions, means with standard deviations, or medians with interquartile ranges (IQR). The univariate analysis was performed with chi^2-square test, *t*-test, or Mann-Whitney *U* test, according to the distribution of the test variable. The statistical analyses were performed with SPSS 22.0 (SPSS, Munich, Germany). Two-sided *P* values < 0.05 were regarded as significant.

RESULTS

Overall, the retrospective search of the electronic data records of hospitalized patients with cirrhosis yielded 1128 patients with cirrhosis. Among them, 488 (43.3%) patients were treated with antibiotics due to BI. Figure 1 illustrates the workflow for the inclusion of patients into the study cohort. Tables 1 and 2 summarizes the detailed baseline and specific characteristics of these patients.

The patients were predominantly men (*n* = 322, 66.1%). The median age was 61 [Range 26-92, (IQR 54-68)], and the predominant etiology of cirrhosis was alcohol-associated (*n* = 259, 53.1%). Most patients were in Child-Pugh stage (CPS) B. Figure 1 shows the distribution of the BI. In general, patients with BI were in an advanced stage of cirrhosis, as reflected by lower serum sodium and albumin concentrations as well as hemoglobin levels and higher creatinine, bilirubin and international normalized ratio, as compared to patients with cirrhosis and no BI. No differences were found concerning the presence of age, sex, or diabetes.

Concerning the common BI, 95 urinary tract infections, 73 SBP, 72 pulmonary infections, and 11 *Clostridioides difficile* infections were recorded. The most frequently atypical BI were soft-tissue infections (*n* = 21), bacterial cholangitis (*n* = 21), and intra-abdominal BI (*n* = 9) (Figure 1). Regardless of Gram classification, cholangitis (*n* = 21, 28.4% each) and soft tissue infections (*n* = 21, 28.4%) were the most common atypical BI presentations. These were followed by intra-abdominal infections, including cholecystitis (*n* = 15, 19%). Among neck and head infections, peritonsillar abscesses and parotitis were equally common (2 each).

The most frequent bacterial detections for atypical BI were detected in the Gram negative (*n* = 20; most frequently *Escherichia coli* (*E. coli*), *Pseudomonas* spp.) spectrum, *e.g.* being responsible for 8 out of 20 cholangitis cases and 6 out of 20 soft tissue infections. Most MDR detections were Gram-negative (8/20), and *Escherichia coli* (*E. coli*) (6/8) was the most frequently detected pathogen (Table 3).

A total of 70 cases (94.6%) were treated with empirical antibiotic therapy, with penicillin predominating (Table 4), followed equally by cephalosporins and metronidazole (19.2% each). Metronidazole was always used as a combination partner, with cephalosporin being the most frequently used combination (11.0%). The administered antibiotic therapy was most common targeted against Gram-positive pathogens (35.6%) and frequently administered over a period of up to two weeks (38.4%). Looking at the efficiency of empirical antibiotic therapy in terms of microbiological detection, the most common problem was that sufficient microbiological tests were not performed, and hence no microbiological analysis was performed (32.9%) (Table 4).

When comparing patients with common *vs* atypical BI, the stage of cirrhosis in patients with atypical BI was less advanced, as reflected by lower creatinine levels (1.14 ± 0.60 *vs* 1.38 ± 1.17; *P* = 0.018) as well as CPS (7.99 ± 2.15 *vs* 8.61 ± 2.50; *P* = 0.05) and Model of End Stage Liver Disease (MELD) scores (12.9 ± 5.1 *vs* 15.1 ± 7.44; *P* = 0.005). No differences were found with respect to sex or diabetes. Long-term antibiotics (*P* = 0.002), lactulose (*P* = 0.03) and proton pump inhibitors (*P* = 0.013) were prescribed more frequently for patients with common BI.

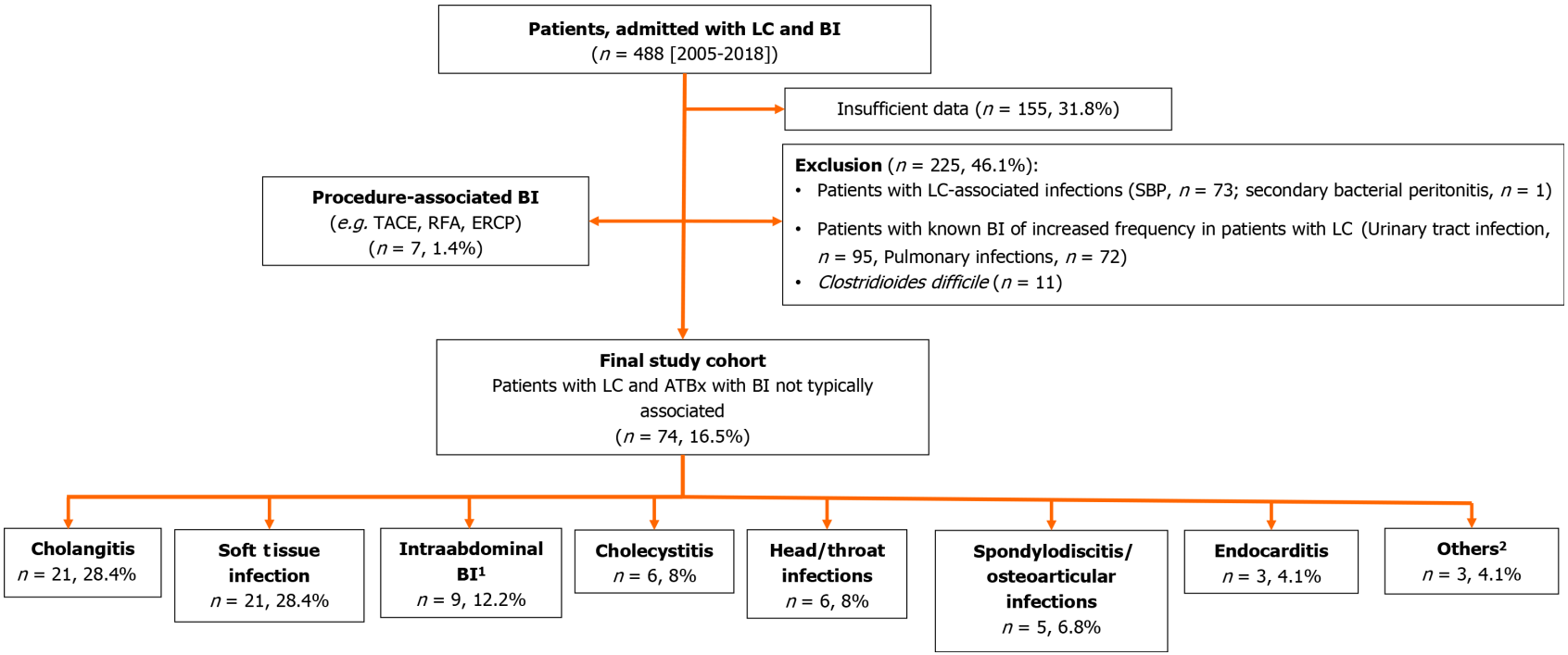

Figure 1 illustrates the workflow for the inclusion of patients into the study cohort. [1]6× enteritis, 1× liver abscess, 1× appendicitis with peritonitis, 1× diverticulitis. [2]1× Epididymitis, 1× SBP equivalent of pleura, 1× vascular catheter associated infection; ATBx: Antibiotic therapy; BI: Bacterial infection; LC: Liver cirrhosis; TACE: Transarterial Chemoembolisation; RFA: Radiofrequency ablation; ERCP: Endoscopic retrograde cholangiopancreatography; SBP: Spontaneous bacterial Infection.

DISCUSSION

Bacterial Infections remain a major contributor to morbidity in patients with liver cirrhosis, but data on less frequently occurring infections are scarce. In this retrospective analysis we compared less frequent BI (termed "atypical BI"), such as soft tissue infections, and found them to be present in a relevant proportion of BI in patients with cirrhosis. Our cohort of patients resembled a typical cohort of patients with cirrhosis in Western countries with respect to age, etiology of cirrhosis (predominantly alcoholic), and sex (predominantly male patients). Notably, the stage of cirrhosis in patients with atypical BI was less advanced. The typical BI frequently observed in cirrhosis were associated with liver function. We also confirmed previous observations that BI occurred more commonly in patients with advanced stage of cirrhosis, as expressed by higher MELD score and CPS[9,10].

Of note, the definition of atypical BI is not consistent in the literature. Even though pneumonia, UTI and SBP are consistently reported as common BI, discrepancies exist for other infections, in particular cellulitis. For example, in their recent analysis, Fricker *et al*[11] subsumed cellulitis as atypical BI. Other study groups *e.g.* Jalan *et al*[12] included cellulitis among the more frequent BI. Additionally, the localization of skin- and soft tissue BI is usually not further specified.

Table 1 Comparing common and non-common bacterial infections

	Atypical BI (*n* = 74)	Common BI (*n* = 225)	No BI (*n* = 640)	*P* value[1]	*P* value[2]	*P* value[3]
Sex (female)	20 (27.0)	80 (35.6)	218 (34.1)	0.20	0.243	0.88
Age (yr)	61.14 ± 12.61	61.34 ± 11.95	60.45 ± 10.76	0.901	**0.61**	
Diabetes (yes)	28 (37.8)	69 (30.7)	199 (31.1)	0.26	0.238	0.706
Etiology of cirrhosis						
Alcoholic	35 (47.3)	147 (65.3)	317 (49.5)			
Hepatitis C	2 (2.7)	29 (12.9)	101 (15.8)			
Hepatitis B	1 (1.4)	3 (1.3)	21 (3.3)			
NASH	5 (6.8)	7 (3.1)	60 (9.4)			
Cryptogenic	6 (8.1)	19 (8.4)	72 (11.3)			
PSC	7 (9.5)	2 (0.9)	6 (0.9)			
Others	11 (14.9)	10 (4.4)	48 (7.5)			
Hemochromatosis	3 (4.1)	1 (0.4)	10 (1.6)			
PBC	4 (5.4)	2 (0.9)	5 (0.8)			
Medication						
Beta blocker	33 (45.8)	115 (51.8)	297 (47.1)	0.42	0.901	0.359
Long term ATBx	13 (18.1)	74 (33.1)	73 (11.6)	**0.017**	0.128	**< 0.001**
Lactulose	24 (33.3)	108 (48.6)	196 (31.1)	**0.029**	0.689	**< 0.001**
PPI	49 (68.1)	183 (82.4)	419 (66.3)	**0.013**	0.794	**< 0.001**
Laboratory parameters						
Serum sodium (mmol/L)	137.62 ± 4.04	136.56 ± 5.03	138.10 ± 4.61	0.10	0.346	**< 0.001**
Creatinine (mg/dL)	1.14 ± 0.60	1.38 ± 1.17	1.029 ± 0.52	**0.018**	0.106	**< 0.001**
Total bilirubin (mg/dL)	2.83 ± 4.00	3.73 ± 5.88	2.21 ± 3.96	0.14	0.205	**< 0.001**
Albumin (g/dL)	33.24 ± 6.73	32.95 ± 6.91	36.64 ± 7.14	0.75	**< 0.001**	**< 0.001**
Hemoglobin (g/dL)	11.98 ± 2.21	11.19 ± 2.34	12.66 ± 2.48	**0.009**	**0.025**	**< 0.001**
INR	1.27 ± 0.34	1.37 ± 58	1.25 ± 0.32	0.15	0.639	**0.001**
ASAT	108.69 ± 257.76	80.55 ± 104.89	84.75 ± 185.15	0.31	0.475	0.930
ALAT	71.46 ± 219.92	53.63 ± 121.43	69.08 ± 164.11	0.39	0.911	0.327
Platelets	164.93 ± 110.00	150.74 ± 88.46	150.27 ± 79.27	0.26	0.151	0.527
MELD	12.86 ± 5.13	15.10 ± 7.44	11.60 ± 5.13	**0.005**	**0.049**	**< 0.001**
CPS	7.99 ± 2.15	8.61 ± 2.50	7.19 ± 5.44	**0.05**	**0.003**	**< 0.001**
Fibroscan (kPa)	41.96 ± 21.94	46 ± 21.90	37.06 ± 21.44	0.22	0.106	**< 0.001**

[1]Uncommon bacterial infections (BI) versus common BI.
[2]Uncommon BI versus no BI.
[3]Any BI versus no BI.
Data is presented as frequency and percentage or median and standard deviation. Significant *P* values are marked in bold. ALAT: Alanine aminotransferase; ASAT: Aspartate aminotransferase; ATBx: Antibiotic therapy; BI: Bacterial infection; CPS: Child-Pugh-Score; CRP: C-reactive-protein; INR: International normalized ratio; MELD: Model of End Stage Liver Disease; WBC: White blood cells; UTI: Urinary tract infections; SBP: Spontaneous-bacterial peritonitis. Long term ATBx = minimum 28 d, in the case of hepatic encephalopathy or prophylaxis of SBP.

Compared to typical BI, cellulitis is often a purely clinical diagnosis without a confirmatory laboratory method, making it much more difficult to classify and this may be one of the reasons why the definition and classification in the literature varies. Due to the clinically frequent presence of peripheral edema with dysfunction of the skin barrier, skin and soft tissue infections of the lower limb are more likely to occur in cirrhotics and should therefore be given more attention as a potential typical focus of infection.

Table 2 Characteristics of patients with atypical bacterial infection (at the time of bacterial infection diagnosis)

	Atypical BI (*n* = 74)
Outcome	
Dead within 30 d	7 (9.5)
Sepsis	9 (12.7)
Laboratory parameters (at BI)	
Serum sodium (mmol/L)	137 ± 5.5
Creatinine (mg/dL)	1.13 ± 13.5
Total bilirubin (mg/dL)	1.9 ± 6.67
Albumin (g/dL)	30.0 ± 6.25
Hemoglobin (g/dL)	11.8 ± 3.00
INR	1.20 ± 0.48
ASAT (U/l)	67.0 ± 42.47
ALAT (U/l)	44 ± 30.92
Platelets	154 ± 103
MELD	14.5 ± 6.23
CPS	8 ± 1.86
WBC ($\times 10^9$)	8.2 ± 5.05
CRP (mg/dL)	43.1 ± 61.77

Data are presented as frequency and percentage or median and standard deviation. Significant *P* values are marked in bold. ALAT: Alanine aminotransferase; ASAT: Aspartate aminotransferase; BI: Bacterial infection; CPS: Child-Pugh-Score; CRP: C-reactive-protein; INR: International normalized ratio; MELD: Model of End Stage Liver Disease; WBC: White blood cells.

Multidrug resistance is an increasingly important issue[13]. The range here is wide, from 29% Extended Spectrum Beta Lactamase-producing Enterobacterales in Korea to rather Gram-positive problems, with 9% vancomycin-resistant enterococci in the United States[14,15]. Fricker *et al*[11] reported an antibiotic resistance in 38% of cases, but did not specify how resistance was defined and which antibiotic classes were considered. Jalan *et al*[12] also discuss that depending on the geographical region, multidrug-resistant bacterial infections have become more frequent. In our analysis, we were able to show that when a pathogen was detected, resistance tended to occur in the Gram-negative range and one major pathogen was *E. coli*. In our study, not many multi-resistant pathogens were detected. However, it must be considered that only the cases with microbiological pathogen identification were considered. Internationally, gram-negative pathogens predominate in infections of liver cirrhotic patients, whereby no distinction is made between typical and atypical infections. Our data showed an empirically more frequent antibiotic coverage in the gram-positive spectrum with, however, more frequent detection of a gram-negative infection. Hillert *et al*[16] found, that a gram-positive pathogen was detected in 54% of cases, with the most common single pathogen detection being *E. coli*. Hillert *et al*[16] inclusion criterion was the presence of ascites.

Our data indicate that the general recommendations for antibiotic therapy can also be followed for atypical BI in cirrhotics and that empirical antibiotic therapy should be based on the localization of the clinical infection focus. Despite immunosuppression and multiple contacts in the health care system, broader antibiotic coverage is not empirically necessary, especially not for multidrug-resistant pathogens. In addition to the clinical localization, the presence of a long-term antibiotic therapy must also be included in the consideration of antibiotics therapy in cirrhotics and need further studies.

To our knowledge, there is no study evaluating how microbiological diagnostics and long-term use of antibiotics in liver cirrhosis patients influence infections and whether previous long-term antibiotics should be included in empirical treatment decisions.

A limiting factor in this data collection is the retrospective method, which makes it difficult to objectively assess appropriate microbiological diagnostics and the resulting decisions. Furthermore, the inclusion of many centers to collect sufficient case numbers and other experiences would certainly be useful to avoid monocentric aspects.

CONCLUSION

Cirrhosis is expected to further increase worldwide in the coming years, e.g. due to the increase in non-alcoholic steato-

Table 3 Microbiological characteristics of selected infections

Pathogen	Organs frequently affected (*n*)	% MDR[1]
Gram positive (*n* = 17)		
Staphylococcus aureus (*n* = 10)	Soft tissue infection[3], abscess[3], discitis/osteomyelitis[2], endocarditis[2]	1/10
Streptococcus spp. (*n* = 4)	Cholangitis/cholecystitis[1], endocarditis[1], meningitis[1], epididymitis[1]	NU
Enterococcus faecium (*n* = 3)	Cholangitis/cholecystitis[3]	NA
Gram negative (*n* = 20)		
Escherichia coli (*n* = 7)	Cholangitis[5], soft tissue infection[2]	6/7
Klebsiella spp. (*n* = 3)	Cholangitis[1], soft tissue infection[1], appendicitis[1]	1/3
Enterobacter spp. (*n* = 2)	Cholangitis[1], periprothetic infection of hip joint[1]	1/2
Pseudomonas spp. (*n* = 4)	Soft tissue infection[2], cholangitis[1], abscess[1]	0/4
Campylobacter spp. (*n* = 3)	Colitis[3]	0/3
Acinetobacter baumanii (*n* = 1)	Soft tissue infection[1]	0/1

[1]Multi-drug classification used by[8]. NU: No MDR classification; NA: Not available.

Table 4 Characteristics of antibiotic therapy

Variable	Number (*n* = 73)[1]
Empirical antibiotic treatment	
Monotherapy	40 (54.8)
Combination therapy with > 2 antibiotics (*n*)	24 (32.9)
Combination therapy with > 3 antibiotics (*n*)	6 (8.2)
Unspecific antibiotic information	3 (4,1)
Antibiotic classes[1]	
Penicillins	25 (34.2)
Cephalosporins	14 (19.2)
Metronidazole	14 (19.2)
Carbapenems	13 (17.8)
Other	13 (17.8)
Quinolones	11 (15.1)
Glycopeptides	6 (8,2)
Not assessable	4 (5.5)
Most frequent antibiotic combinations	
Cephalosporins with Metronidazole	8 (11)
Carbapenems with others	4 (5.5)
Quinolones with Metronidazole	3 (4.1)
Coverage	
Gram positive	26 (35.6)
Gram negative	17 (23.3)
Gram positive and negative	12 (16.4)
Gram negative and anaerobic	12 (16.4)
Non-rankable/gram positive. Negative and anaerobic	6 (8.2)

Duration of therapy	
One week	18 (24.7)
Up to two weeks	28 (38.4)
More than two weeks	10 (13.7)
No data	18 (24.7)
Efficacy of empirical antibiotic therapy	
No sufficient data	17 (23.3)
No resistance to antibiotics being used	19 (26)
Change in multi-resistant germ under antibiotic therapy	2 (2.7)
Antibiotic therapy not adequate	7 (9.6)
No germ detection with adequate diagnostics. Effectiveness of antibiotic therapy cannot be assessed	5 (6.8)
No germ detection in the absence of microbiological diagnostics	24 (32.9)

[1]*n* = 73 because of one patient without specific treatment.

hepatitis[17,18]. BI remain a major cause of morbidity and mortality in these patients. The relevance of a correct adequately chosen antibiotic in face of an increasing antimicrobial resistance rate worldwide is paramount[19]. Out data shows that atypical BI in patients with cirrhosis have different characteristics. With an increasing degree of liver failure, the severity and the spectrum of BI change. Prospective multicentric studies are needed to improve our understanding of an optimal diagnostic and therapeutic management of these disease entities in patients with liver cirrhosis. Further research is also warranted to identify whether infections at atypical body sites and more common sites differ depending on the causative bacterial species.

ARTICLE HIGHLIGHTS

Research background

Typical infections in patients with liver cirrhosis have standardized diagnostic algorithms and are therefore recognized and treated quickly. Clinically, however, unusual infections are also more frequent in patients with cirrhosis. These are not included in guidelines and are therefore often not adequately addressed in diagnostic and therapeutic algorithms.

Research motivation

The study aimed to analyze a cirrhosis cohort for typical and atypical infections. The aim is to derive improved diagnostic and therapeutic algorithms from these analyses in the future.

Research objectives

The main aim is to identify the most common pathogens for atypical infections and their resistance patterns in relation to the stage of liver cirrhosis. Algorithms for the improved detection of infections, including atypical situations, can then be developed.

Research methods

For the analysis, data were analyzed in relation to the research question in a cirrhosis cohort.

Research results

The cohort showed that atypical infections are not so rare overall and should be clinically investigated more frequently in order to initiate the correct diagnosis and treatment. It was also shown that the pathogen spectrum recorded did not always correspond correctly with the empirical therapy, and that microbiological diagnostics are therefore particularly relevant in this patient population.

Research conclusions

We were able to show that the stage of cirrhosis is associated with a change in infections and that this needs to be taken into account. The relevance of these findings must be considered in the light of the increasing role of liver disease and its sequelae in the global burden of disease.

Research perspectives

Confirmation of these results in larger multicenter studies and development of corresponding algorithms.

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FOOTNOTES

Author contributions: Reichert MC, Schneitler S, Lammert F, and Becker SL designed the study; Reichert MC, Casper M, Schneider C, and Schneitler S participated in the acquisition of clinical data, drafted the manuscript, and together with Krawczyk M and Becker SL, analyzed the data and finalized the manuscript, which was then revised by all authors; the final draft of the manuscript was approved by all authors.

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ORIGINAL ARTICLE

Observational Study

Palliative long-term abdominal drains *vs* large volume paracenteses for the management of refractory ascites in end-stage liver disease

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Abstract

BACKGROUND

Long-term abdominal drains (LTAD) are a cost-effective palliative measure to manage malignant ascites in the community, but their use in patients with end-stage chronic liver disease and refractory ascites is not routine practice. The safety and cost-effectiveness of LTAD are currently being studied in this setting, with preliminary positive results. We hypothesised that palliative LTAD are as effective and safe as repeat palliative large volume paracentesis (LVP) in patients with cirrhosis and refractory ascites and may offer advantages in patients' quality of life.

AIM

To compare the effectiveness and safety of palliative LTAD and LVP in refractory ascites secondary to end-stage chronic liver disease.

METHODS

A retrospective, observational cohort study comparing the effectiveness and safety outcomes of palliative LTAD and regular palliative LVP as a treatment for refractory ascites in consecutive patients with end-stage chronic liver disease followed-up at our United Kingdom tertiary centre between 2018 and 2022 was conducted. Fisher's exact tests and the Mann-Whitney *U* test were used to compare qualitative and quantitative variables, respectively. Kaplan-Meier survival estimates were generated to stratify time-related outcomes according to the type of drain.

RESULTS

Thirty patients had a total of 35 indwelling abdominal drains and nineteen

patients underwent regular LVP. The baseline characteristics were similar between the groups. Prophylactic antibiotics were more frequently prescribed in patients with LTAD (P = 0.012), while the incidence of peritonitis did not differ between the two groups (P = 0.46). The incidence of acute kidney injury (P = 0.014) and ascites/drain-related hospital admissions (P = 0.004) were significantly higher in the LVP group. The overall survival was similar in the two groups (log-rank P = 0.26), but the endpoint-free survival was significantly shorter in the LVP group (P = 0.003, P < 0.001, P = 0.018 for first ascites/drain-related admission, acute kidney injury and drain-related complications, respectively).

CONCLUSION

The use of LTAD in the management of refractory ascites in palliated end-stage liver disease is effective, safe, and may reduce hospital admissions and utilisation of healthcare resources compared to LVP.

Key Words: Decompensated liver cirrhosis; Indwelling abdominal catheter; Rocket drain; Palliative care; Safety; Quality of life

Core Tip: The standard treatment of refractory ascites in palliated patients with end-stage liver disease is repeated large volume paracentesis (LVP) with albumin infusion. This study focuses on real-world data comparing the effectiveness and safety of long-term abdominal drains (LTAD) in comparison with LVP. The incidence of acute kidney injury, ascites and drain-related hospital admissions was lower in the LTAD group. There was no difference in the overall survival between the two groups, but time to acute kidney injury, first ascites/drain-related hospital admission and drain-related complications were shorter in the LTAD group.

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INTRODUCTION

In Europe, liver-related mortality has risen from 2.3% of all deaths in 1990 to 3% in 2019[1]. Patients with advanced liver disease who are not eligible for transplant frequently need palliative care due to their high risk of death, high burden of symptoms, poor quality of life, and frequent hospitalizations. Early provision of palliative care can lead to improvements in quality of life and a reduction of the physical and psychological symptom burden, with the potential for reduced utilisation of healthcare resources and even improved survival for patients with serious illnesses[2]. Similarly, timely palliative care can improve health-related quality of life and reduce the need for hospitalisation of patients with advanced liver cirrhosis[3-5]. Ascites remains the most common complication in cirrhosis that necessitates hospitalisation, and progresses to refractory ascites (RA) in up to 30% of cases[6]. As many as 20% of patients presenting with ascites die within the first year of diagnosis[7]. RA is classified as either diuretic resistant or diuretic intractable and, following the onset of RA, patients have a median lifespan of 6-12 months in the absence of liver transplantation[8]. The current guidelines for the management of RA recommend large volume paracentesis (LVP)[8] with intravenous albumin infusion to decrease the risk of paracentesis-induced circulatory dysfunction[9]. Although LVP is considered safe, it requires patient-hospital contact as often as weekly and is associated with poor quality of life and malnutrition which, together, increase morbidity and mortality[8,10,11].

In selected patients with RA, transjugular intrahepatic portosystemic shunt (TIPS) and Automated Low-Flow Ascites Pump System [alfapump Ò (AP) system] are therapeutic alternatives to repeated LVP[10,11]. However, TIPS is contraindicated in patients with marked pulmonary arterial hypertension, heart failure, hepatic encephalopathy, coagulopathy, and elevated right or left heart pressures[12], whereas the alfapump® system is contraindicated in patients with obstructive uropathy, advanced sarcopenia, bed confinement and abdominal skin infections[13]. Clinical trials are still being conducted to determine the best candidates for the alfapump® device and its cost effectiveness[14].

Individuals with RA who are not eligible for TIPS or liver transplantation, in particular those with a limited life expectancy, should be considered for palliative care. Repeated LVP is the conventional main treatment in these cases[8].

Long-term abdominal drains (LTAD) are tunnelled drains inserted under local anaesthesia, that enable community nurses or trained caregivers to drain small amounts (1-2 L) of ascitic fluid at home, up to three times a week, thus reducing hospital visits and the use of healthcare resources[15,16]. They represent a reliable and cost-effective strategic option in the palliative management of recurrent malignant ascites and are currently being studied as a palliative measure in RA[16-19].

Absolute contraindications to the insertion of LTAD include loculated or chylous ascites, candidacy for liver transplantation or TIPS, and very short life expectancy, whilst severe renal impairment, previous life-threatening spontaneous bacterial peritonitis and active infection are considered relative contraindications[18].

There are currently two types of LTAD available in the United Kingdom: PleurX™, recently rebranded as PeriX™ (United Kingdom Medical, Basingstoke, United Kingdom) and Rocket® (Rocket Medical plc, Watford, United Kingdom) [20].

In 2022, the British Association for the Study of the Liver/British Society of Gastroenterology End of Life Special Interest Group published a consensus to help standardise the use of long-term abdominal drains in cirrhosis, including patient selection and community management[20]. A recent feasibility trial conducted in the United Kingdom compared palliative LTAD with LVP in refractory ascites secondary to advanced liver disease[18]. The trial yielded favourable results of LTAD in terms of efficacy, safety, acceptability by patients and clinical staff, and decreased healthcare resource utilisation[18]. However, pending the results of a national multicentre randomised controlled trial (REDUCe2, ISRCTN26993825), LTAD are currently not used as standard of care in advanced decompensated cirrhosis.

To contribute real-world data to the available scarce evidence, our study aimed to further investigate this subject by retrospectively evaluating the effectiveness and safety of LTAD in comparison with recurrent LVP, which is the current standard of care, in palliated patients with end-stage liver disease and RA followed-up at a United Kingdom tertiary centre.

MATERIALS AND METHODS

Study design

This is a retrospective, single-centre, observational cohort study aimed at analysing the effectiveness and safety of palliative LTAD in comparison with repeat palliative LVP in patients with end-stage liver disease and RA followed-up at the Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom, between January 2018 and December 2022.

Patient characteristics

All consecutive patients above 18 years of age referred to palliative care owing to end-stage liver disease of any aetiology and RA defined according to the International Ascites Club criteria[21] (but without loculated, chylous, or malignant ascites), who were not eligible for TIPS and liver transplantation and had undergone palliative treatment of ascites at our centre during the 5-year study period with either repeat LVP or LTAD, were included.

Data collection

Data was retrospectively collected from electronic patient records to avoid recollection bias, and included: age at diagnosis of RA, aetiology of liver disease, Child-Pugh score at the time of diagnosis of RA, ascites proteins (as a protein concentration of ≤ 15 g/L in ascitic fluid has been associated with an increased risk of developing spontaneous bacterial peritonitis[8]), use of diuretics, comorbidities, presence of hepatocellular carcinoma, presence of hepatic encephalopathy, date of LTAD insertion, perioperative complications, baseline creatinine, eGFR and sodium, date of referral to palliative care, use of prophylactic antibiotics, occurrence and date of cellulitis, peritonitis, other localised infections, sepsis, bacteria identified in the case of infection, leakage and bleeding on the site of the abdominal drain, drain displacement, blockage, hypotension, acute kidney injury (AKI), date and reason for hospital admissions, total number of hospital admissions, frequency of ascitic drainage per week, litres of ascites drained each time, need for additional LVP, date and cause of death. The presence of shortness of breath, abdominal pain/discomfort, anorexia and poor mobility before and after the insertion of LTAD were also evaluated.

Large volume paracentesis

All paracenteses were undertaken in our dedicated Hepatology Day Case Unit by two Hepatology Advanced Clinical Practitioners (ACPs). In preparation for the paracentesis drain insertion, patients received appropriate advice regarding withholding current anticoagulant treatment, according to the local protocols. Bloods, including full blood count and clotting, were taken within 5 d of the drain insertion. An international normalized ratio (INR) > 2 and/or a platelet count < 50×10^9 were considered contraindications to drain insertion requiring correction.

A safe insertion site was confirmed using bedside ultrasound and usually chosen slightly above the iliac crest, avoiding the inferior epigastric vessels and any visible vessels. Local anaesthetic (5-10 mL of 2% lidocaine) was injected and a Bonanno Safe-T-centesis 18G catheter (Becton, Dickinson and Company, Franklin Lakes, New Jersey, United States) was inserted with aseptic technique following a small incision with a sterile scalpel. Human albumin was administered (8-10 g/L of ascitic fluid removed)[8] to prevent paracentesis-induced circulatory dysfunction.

At the time of the first paracentesis, the ascitic fluid was tested for cell count, bacterial cultures, proteins, amylase, triglycerides and cytology. Ascitic neutrophil count was routinely tested at every subsequent LVP.

The drain was left in situ for up to six hours. Long-term antibiotic use was not routinely administered unless a history of spontaneous bacterial peritonitis (SBP) was present. The frequency of LVP varied depending on clinical need and patients' symptoms.

LTAD

Rocket® (Rocket Medical) LTAD insertion was performed in Interventional Radiology under local anaesthesia using ultrasound guidance, as previously described[22]. Bloods, including full blood count and clotting, were taken within 5 d of the drain insertion. Correction of clotting parameters was considered necessary prior to the procedure if INR > 2 and/

or a platelet count < 50 × 10^9. Active anticoagulation was withheld before drain insertion according to the local protocols. At the time of LTAD insertion, ascitic cell count, bacterial cultures and proteins were assessed. Until 2020, the decision to commence prophylactic antibiotics was made on a case-by-case basis. Thereafter, all patients with a LTAD were prescribed long-term prophylactic ciprofloxacin 250 mg twice a day. The Hepatology ACPs arranged referral to district nurses for ascitic drainage of 1-2 litres twice a week in the community. Further follow-up in the Hepatology Day Case Unit was decided on a case-by-case basis.

Outcomes

The primary endpoint was the difference in overall survival between patients with LTAD and patients undergoing repeat LVP. Secondary endpoints were differences in the incidence of drain-related complications in the two groups and endpoint-free survival for first ascites/drain-related hospitalisation, time to AKI (defined as an absolute increase in serum creatinine of at least 26.5 micromol/L within 48 h or by a > 50% increase in serum creatinine from baseline within 7 d, or a urinary output of less than 0.5 mL/kg/h over > 6 h[23]) and time to drain-related complications between the two groups.

Ethics

This study was conducted in accordance with the Declaration of Helsinki Ethical Principles and Good Clinical Practice and approved by the Clinical Audit Division at Oxford University Hospitals NHS Foundation Trust (REC:8587). No ethical approval and informed consent were required for this study, as the information used was collected as part of the normal clinical care and data were collected retrospectively by the care team involved, and were anonymised.

Statistical analysis

Categorical variables were expressed as number and percentage. In the LTAD group, the percentage of patient-related outcomes was calculated using the total number of patients with LTAD as a denominator, while the percentage of drain-related complications was computed using the total number of drains inserted as a denominator. Time 0 of follow-up was considered the time of LTAD insertion (for the LTAD group) or the time of the first LVP since deemed palliative/referred to palliative care (for the LVP group). A complete-case analysis approach was used.

Kolmogorov-Smirnov and Shapiro-Wilk test of normality were used to assess the distribution of quantitative variables, which were expressed as mean and standard deviation (SD) or median and interquartile range (IQR), as appropriate. Fisher's Exact test and Mann-Whitney *U* test were used to compare qualitative and quantitative variables, respectively.

Kaplan-Meier survival estimate curves were generated to stratify outcomes according to the type of drainage. Patients were censored at death or at the time of last encounter, in case they were alive on 31/12/2022 or lost to follow-up. Statistical analysis was performed using SPSS (v.29.0; IMB® SPSS®, Inc, Chicago, IL, United States). A two-sided *P* value < 0.05 was considered statistically significant.

RESULTS

Overall characteristics of patients

Forty-nine patients met the criteria for this study. Thirty (61%) had LTAD and 19 (39%) were treated with repeated LVP only. The median individual follow-up after the decision to provide palliative care was 165 (IQR 360) d, for the whole cohort. Median follow-up with LTAD in place or undergoing LVP, age, Child-Pugh score, liver disease aetiology, baseline renal function, ascitic protein, and the presence of hepatocellular carcinoma were not significantly different between the two cohorts (Table 1).

LTAD cohort

A total of 35 drains were placed in 30 patients. The amount of ascites drained at each home visit was 1-2 litres. The median time with drain in place was 135 (IQR 226) d. This group had a mean age of 71 ± 11 years; 18 (60%) patients were male. The most common aetiology of liver cirrhosis was alcohol (40%), followed by metabolic dysfunction-associated steatotic liver disease (MASLD, 30%). At the time of insertion of the indwelling drains, 9 (30%) patients were classified as Child-Pugh B8, 10 (33%) patients were classified as B9, and 9 (30%) patients were classified as Child-Pugh C. The insertion of LTAD was successful in all cases, with no procedure-related deaths or perioperative complications.

Among the 30 patients in the LTAD group, shortness of breath, abdominal discomfort, anorexia and poor mobility were present in 11 (37%), 21 (70%), 13 (43%), and 24 (80%), respectively. Following LTAD insertion, symptomatic relief of shortness of breath and abdominal pain was seen in 71% and 69% of cases, respectively, while anorexia and poor mobility resolved in 46% and 37% of cases, respectively.

Data on prophylactic antibiotics was available for 31 out of the 35 cases of LTAD insertion. Prophylactic antibiotics were prescribed in 25 (81%) cases (Table 1). Ciprofloxacin was the most common choice (88% of cases), while trimethoprim/sulfamethoxazole was prescribed in 2 (12%) cases. One (4%) patient was initially on prophylaxis with ciprofloxacin but was switched to trimethoprim/sulfamethoxazole following development of SBP.

Hospital admission due to ascites or drain-related complications occurred in 11 (37%) patients with LTAD. The median time to first admission following insertion of the LTAD was 44 (IQR 93) d.

Drain displacement occurred in 4 (11%) cases and prompted drain removal in 3 patients; catheter blockage occurred in 2 (5%) cases, requiring drain removal in 1. Two patients (5%) had self-limiting bleed at the drain site, which did not require hospitalisation or removal of the indwelling catheter. Four (11%) patients developed abdominal cellulitis, one of

Table 1 Comparison of baseline characteristics of cirrhotic patients with refractory ascites palliated with long-term abdominal drain or repeat large volume paracentesis, *n* (%)

Baseline characteristics	LTAD (*n* = 30)	LVP (*n* = 19)	*P* value
Age, yr (SD)	71 (11)	66 (12)	0.07
Male sex	18 (60)	15 (79)	0.22
Child-Pugh score (IQR)	9 (2)	9 (2)	0.48
Child-Pugh class B/C	24/11 (69/31)	12/7 (63/37)	0.76
Aetiology (MASLD/ArLD/Viral/Other)	9/12/2/7 (30/40/7/23)	3/10/1/5 (16/53/5/26)	0.69
HCC	5 (17)	4 (21)	0.46
Proteins in ascites ≤ 15 g/L	14 (47)	9 (47)	0.76
Prophylactic antibiotics	25/31[1] (81)	8/19 (42)	**0.012**
Previous peritonitis	2 (7)	5 (26)	0.86
T2DM	12 (40)	8 (42)	1.00
Use of metformin	3 (10)	3 (16)	0.66
Use of diuretics	18 (60)	12 (63)	1.00
Use of NSBBs	13 (43)	4 (21)	0.13
Use of antihypertensive	2 (7)	2 (10)	0.66
Use of lactulose	13 (43)	13 (68)	0.14
Baseline creatinine (IQR)	104 (68)	84 (143)	0.44

[1]Data on prophylactic antibiotics were available for 31/35 long-term abdominal drains.

LTAD: Long-term abdominal drain; LVP: Large volume paracentesis; SD: Standard deviation; IQR: Interquartile range; MASLD: Metabolic dysfunction-associated steatotic liver disease; ArLD: Alcohol-related liver disease; HCC: Hepatocellular carcinoma; T2DM: Type 2 diabetes mellitus; NSBBs: Non-selective beta-blockers; AKI: Acute kidney injury.

which was also diagnosed with concurrent bacterial peritonitis. Blood and ascitic cultures yielded multisensitive Gram-positive *S. aureus* for this patient. These infections were treated successfully with antibiotics and resolved without removal of the catheter. Five out of 30 (17%) patients developed bacterial peritonitis (total number of peritonitis episodes 10; 3 patients had a single episode, one patient had 3 episodes and one patient had 4 episodes), despite 2 of them receiving prophylaxis with ciprofloxacin and 1 with trimethoprim/sulfamethoxazole. Among these 5 patients, ascitic fluid cultures detected multisensitive *E. coli*, multisensitive *S. aureus*, multi-resistant coagulase negative staphylococci, *E. cloacae* and *Pseudoglutamicibacter cumminsii*. None of these cases resulted in death.

LVP cohort

The 19 patients in the LVP group had a mean age of 66 ± 12 years, and 15 (79%) were male. Alcohol-related liver disease (53%) and MASLD (16%) were again the most common causes of chronic liver disease. Five (26%) patients were classified as Child-Pugh B8 and 4 as B7 (21%), while 7 (37%) patients were in Child-Pugh class C. The median drain frequency was 21 (IQR 7) d. The median follow-up time for these patients was 80 d (IQR 239).

Twelve (63%) of the 19 patients in this group were on diuretic treatment, and 8 (42%) were prescribed prophylactic antibiotics (Table 1). In particular, 4 (21%) patients were prescribed ciprofloxacin and 3 (16%) trimethoprim/sulfamethoxazole. One (5%) patient developed peritonitis whilst on ciprofloxacin and was then switched to trimethoprim/sulfamethoxazole.

Hospital admission due to ascites or drain-related complications occurred in 13 (68%) patients undergoing LVP, with a median time to first admission of 7.5 (IQR 35) d. Two (11%) patients had drain-related cellulitis, 1 of which required hospitalisation for concurrent confusion. One (5%) LVP was complicated by abdominal wall hematoma requiring interventional radiology-guided embolisation of the bleeding vessel. Five (28%) patients developed bacterial peritonitis despite receiving antibiotic prophylaxis, *i.e.*, 4 patients with ciprofloxacin and 1 with trimethoprim/sulfamethoxazole. In 2 cases, these infections resulted in death. Ascitic cultures identified *E. coli* in one case, while in another case there was no growth despite elevated white cell count on the ascitic fluid and the presence of symptoms compatible with peritonitis. Streptococcus species (*S. orallis*, *S. gordonii* and *S. anginosus*) were isolated in the remaining 3 cases.

Comparison of outcomes

The comparison of the outcomes of interest in the two cohorts is reported in Table 2. Long-term prophylactic antibiotics were more frequently prescribed in the LTAD group compared to the LVP group (81% *vs* 42%; $P = 0.012$). The incidence of peritonitis did not differ between the two groups ($P = 0.46$).

Table 2 Comparative outcomes and safety events by type of ascitic drainage, *n* (%)

Outcomes	LTAD (*n* = 30)	LVP (*n* = 19)	*P* value
Median survival, d	124 (330)	297 (438)	0.06
Median follow-up (with drain in place/undergoing LVP), d	135 (226)	80 (239)	0.98
Ascites/drain related admissions	11 (37)	17 (89)	**0.004**
Time to first hospitalisation, (IQR), d	44 (93)	10 (35)	**0.002**
AKI	8 (27)	11 (58)	**0.014**
Drain-related complications	14 (47)	11 (58)	0.06
Patients with peritonitis	5 (17)	5 (26)	0.46
Total No. of peritonitis episodes	10 (33)	5 (26)	0.98
Cellulitis	4 (13)	2 (10)	1.00
Site leakage	12 (40)	2 (10)	0.10
Bleeding of drain site	2 (7)	1 (5)	1.00
Hypotension	6 (20)	4 (21)	0.71

LTAD: Long-term abdominal drain; LVP: Large volume paracentesis; AKI: Acute kidney injury.

Despite a similar use of diuretics, non-selective beta-blockers, antihypertensive, metformin and laxatives in the two groups (Table 1; concomitant pharmacological treatments for individual patients are listed in Supplementary Table 1), the incidence of AKI was significantly lower in patients with LTAD (P = 0.014). Furthermore, ascites/drain-related hospital admissions occurred less frequently in the LTAD cohort (P = 0.004) (Table 2). Median time to first hospitalisation was also significantly longer in these patients, compared to the LVP cohort (44 *vs* 10 d, respectively; P = 0.002).

Other clinical endpoints, such as cellulitis, peritonitis, site leakage, bleeding at drain site and hypotension were not significantly different between the groups (Table 2).

The overall survival (since palliation) was not significantly different between the two groups (log-rank P = 0.26), Figure 1. Nevertheless, endpoint-free survival was significantly shorter in the LVP group for time to first ascites/drain-related hospitalisation (P = 0.003), time to AKI (P < 0.001) and time to the development of drain-related complications (P = 0.018) (Figure 2).

A "safety" composite endpoint including (1) Death secondary to drain-related complications; (2) Bleeding at the insertion site; (3) Bacterial peritonitis; and (4) Cellulitis was also compared between the two cohorts. Again, this was significantly shorter for the LVP group (log-rank P = 0.018) (data not shown).

DISCUSSION

In our single-centre retrospective evaluation of the use of palliative LTAD in comparison with repeat palliative LVP for the management of RA in patients with end-stage liver disease, LTAD was associated with a reduced incidence of AKI, as well as a reduced number of ascites- or drain-related hospital admissions and time to first hospitalisation. Time to the development of AKI and of drain-related complications was also significantly shorter in patients with LTAD.

The scarcity of real-world data on indwelling abdominal drains precludes international societies from making strong recommendations on their use. In its guidelines on the outpatient therapy of cirrhosis, the British Society of Gastroenterology (BSG) has mentioned long-term abdominal drains as an experimental approach that may be considered for patients with advanced liver disease in palliative care[24].

Following promising results from the REDUCe trial[18], a 12-wk feasibility randomised controlled trial comparing the use of LVP (19 patients) *vs* LTAD (17 patients) in RA due to end-stage liver disease, which showed preliminary evidence that LTAD are acceptable and safe in end-stage liver disease and lead to a reduction in healthcare resource utilisation, the use of LTAD is currently being evaluated in the REDUCe2 study, a United Kingdom multicentre randomised controlled clinical trial. To our knowledge, our study represents the largest set of real-world data comparing the use of LVP *vs* Rocket® indwelling peritoneal catheters in a cohort of palliated cirrhotic patients with RA.

We found no significant difference in the incidence of peritonitis between the 2 groups. All the microorganisms identified were typical for SBP. This is likely the consequence of the more frequent administration of prophylactic antibiotics in patients with indwelling catheters compared to those undergoing LVP (83% *vs* 42%, P = 0.012). In a systematic review from 2019 assessing the use of LTAD in end-stage liver disease[25], the rates of bacterial peritonitis (BP) varied from 0% to 42% across individual studies, with an overall combined rate of 17%, similarly to our study findings. However, it is unclear whether all reported cases of BP in this systemic review were true BP or there were cases of positive bacterial cultures secondary to colonisation. The more regular follow-up schedule in the setting of a clinical trial and the universal treatment with prophylactic antibiotics in both groups are likely accountable for the lower rates of

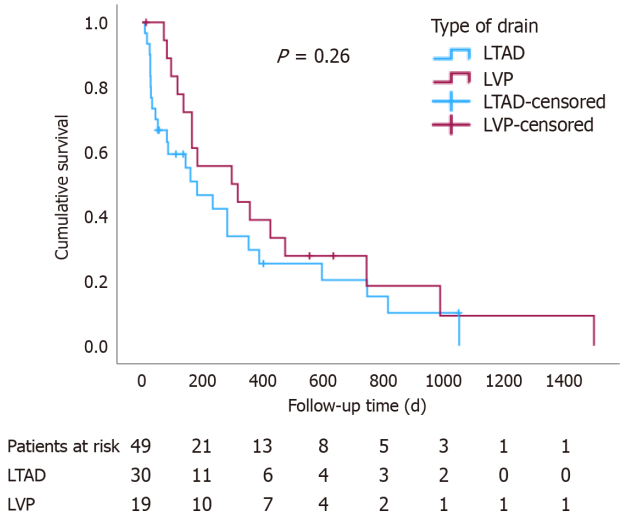

Figure 1 Comparison of overall survival between cirrhotic patients with refractory ascites palliated with long-term abdominal drain or repeat large volume paracentesis. LTAD: Long-term abdominal drain; LVP: Large volume paracentesis.

peritonitis recorded in the REDUCe study (6% *vs* 11% in the LTAD *vs* LVP group, respectively)[18], compared to real-world data. In the trial, the LTAD group did not show an increased rate of peritonitis compared to the LVP group. The incidence of peritonitis reported in our study may further decrease in the future, as since 2020, antibiotic prophylaxis is prescribed to all palliated patients with RA undergoing LTAD insertion at our centre, as per BSG recommendation[20].

When comparing the occurrence of complications between the two treatment modalities, there was a significantly lower rate of AKI in the LTAD group (P = 0.014) despite similar use of diuretics between the two cohorts. Previous studies have focused on changes in creatinine over time, which hinders a direct comparison between our findings and other published reports[25]. Contributing factors to the higher incidence of AKI in the LVP group are likely a higher rate of circulatory dysfunction following drainage of larger quantities of ascites (despite regular administration of intravenous albumin), as well as the higher rate of ascites and drain-related admissions seen in this group, underlining the multi-factorial cause of AKI in these patients.

Episodes of leakage and cellulitis were comparable in both groups. These were typically managed with minimal medical intervention and did not require LTAD removal in any of the cases. Though higher rates of site leakage and cellulitis were noted in the LTAD group in our study (34% and 11%, respectively) compared to the aforementioned systematic review (8% and 6%, respectively)[25], a comparable incidence of cellulitis/leakage (41% collectively) was observed in the REDUCe study[18].

There was no significant difference in the overall survival between the LVP and LTAD groups. However, the endpoint-free survival for all other time-related events (time to first ascites/drain-related hospitalisation, time to AKI, and time to drain-related complications) was significantly longer for patients with LTAD.

Symptomatic relief of shortness of breath and abdominal discomfort was seen in 70% of cases following LTAD placement, while anorexia resolved in 50% of patients. These findings corroborate the results of the REDUCe trial, showing that LTAD improves quality of life for patients with RA. Furthermore, the trial has shown that indwelling drains are also cost-effective, as they reduce healthcare resource utilisation and inpatient burden. In fact, median fortnightly total costs were about 15% lower in the LTAD group, as the overall hospital costs were higher in the LVP group[18]. We did not undertake a cost analysis, as our hospital and community databases are not merged and tariffs for community support workers and community costs were not available. As the REDUCe trial was also undertaken in the United Kingdom setting, we would not expect significant differences with regards to costs, in our study.

A consensus on the palliative management of patients with decompensated cirrhosis and RA was published only in 2023[24]. Until then, the treatment of these patients exclusively relied upon local standard operating protocols and the discretion of the individual specialist teams. Accordingly, despite our cohort coming from a single centre, the lack of a unified approach may have resulted in differences in antibiotic prophylaxis, time of referral for LTAD and/or specialist palliative treatment, and management of complications associated with RA. Timing and duration of follow-up might have also led to differences in patients' management, as new technologies and evidence arose between 2018 and 2022. Moreover, the type and dose of diuretics might have changed over time for each individual patient (according to symptoms, creatinine and electrolyte levels), and this may represent a confounding factor. The variable frequency of LVP and amount of ascites removed on each occasion, as well as the concomitant use of other medications (such as non-selective beta-blockers, metformin, antihypertensive and laxatives, although these were not significantly different between the two groups), or possible episodes of hepatic encephalopathy, all of which can favour the occurrence of AKI, are further potential confounding factors. Given the limited sample size, multivariate regression analysis was deemed unsuitable.

The single-centre observational design and the relatively small sample size are limitations of our study that should be taken into consideration in interpreting the results. Larger, more heterogeneous cohorts and randomised controlled trials are needed to validate our findings.

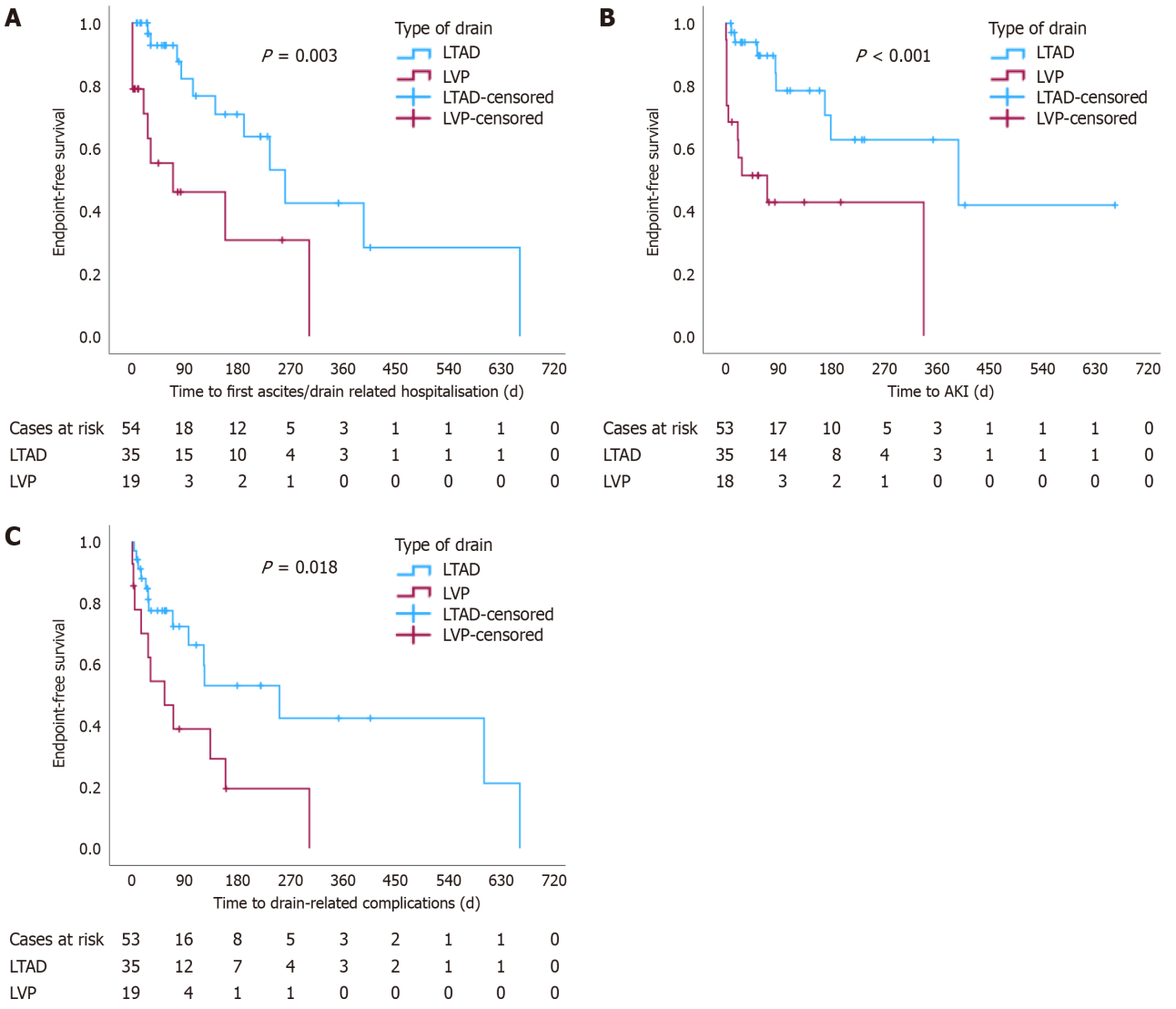

Figure 2 Kaplan-Meier curves illustrating prediction of endpoints according to drain type. A: Comparison of time to first ascites/drain-related hospitalisation between patients with long-term abdominal drains (LTAD) and patients undergoing large volume paracentesis (LVP); B: Comparison of time to acute kidney injury between patients with LTAD and patients undergoing LVP; C: Comparison of time to drain-related complications between patients with LTAD and patients undergoing LVP. LTAD: Long-term abdominal drain; LVP: Large volume paracentesis; AKI: Acute kidney injury.

CONCLUSION

In conclusion, our study demonstrates that the use of palliative LTAD is effective and overall safe for the management of RA in patients with end-stage liver disease. Compared to LVP, LTAD may reduce the incidence of renal dysfunction, hospital admissions and healthcare resource utilisation. Results are eagerly awaited from a randomised controlled trial currently recruiting in the United Kingdom, comparing LVP and LTAD.

ARTICLE HIGHLIGHTS

Research background

Repeat large volume paracentesis (LVP) with albumin infusion is currently the standard treatment for the management of refractory ascites (RA) in patients with end-stage liver disease who are not eligible for transjugular intrahepatic portosystemic shunt or liver transplant, including those on a palliative care pathway. This treatment requires frequent patient-hospital contact and is associated with poor quality of life. Long-term abdominal drains (LTAD) are a reliable and cost-effective strategic option in the palliative management of recurrent malignant ascites, but are currently not routine practice in patients with end-stage liver disease and RA. The safety and cost-effectiveness of LTAD are currently being studied in this setting, with preliminary encouraging results.

Research motivation
As the use of LTAD may improve the quality of life of palliated patients with end-stage liver disease and RA, it is important to assess their utility and safety in this setting. We aimed to provide real-world data from our own experience to the available scarce evidence.

Research objectives
The objective of this study was to retrospectively assess the effectiveness and safety of LTAD in comparison with recurrent LVP for the management of ascites in palliated patients with end-stage liver disease and RA.

Research methods
This observational study included 49 consecutive patients with end-stage liver disease and RA requiring palliative drainage of ascites. Overall survival, the incidence of drain-related complications and endpoint-free survival for first ascites/drain-related hospitalisation, time to acute kidney injury and time to drain-related complications were compared between 30 patients who were managed with LTAD and 19 patients who underwent LVP.

Research results
The study found similar incidence of peritonitis between the two groups, although prophylactic antibiotics were more frequently prescribed in patients with LTAD. However, the incidence of acute kidney injury, ascites- and drain-related hospital admissions was lower in the LTAD group. There was no difference in the overall survival between the two groups, but time to acute kidney injury, first ascites/drain-related hospital admission and drain-related complications were shorter in the LTAD group.

Research conclusions
The use of palliative LTAD for the management of RA in patients with end-stage liver disease appears to be effective and overall safe. Compared to LVP, the use of LTAD in this setting may reduce the incidence of renal dysfunction, hospital admissions and healthcare resource utilisation.

Research perspectives
Larger, more heterogeneous cohorts and randomised controlled trials are needed to validate the findings of this study.

FOOTNOTES

Co-first authors: Senamjit Kaur and Rodrigo V Motta.

Author contributions: Saffioti F conceptualised, designed and supervised the study, performed statistical analysis, created the artwork and made critical revisions; Kaur S and Motta RV conducted the literature review, collected the data, did the analysis, interpreted the data and drafted the original manuscript; Chapman B and Wharton V collected the data and contributed to writing the manuscript; Collier JD contributed to the conception and design of the study and made critical revisions for important intellectual content; All authors have read and approved the final manuscript.

Institutional review board statement: According to the Integrated Research Application System (https://www.myresearchproject.org.uk), in the United Kingdom, research undertaken by staff within a care team using data previously collected in the course of care for their own patients or clients does not require Research Ethics Committee review provided that data is anonymised in conducting the research.

Informed consent statement: No informed consent was required for this study, as the information used was collected as part of the normal clinical care and data were collected retrospectively by the care team involved and were anonymised.

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ORIGINAL ARTICLE

Basic Study

Comprehensive prognostic and immune analysis of sterol O-acyltransferase 1 in patients with hepatocellular carcinoma

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Abstract

BACKGROUND

Sterol O-acyltransferase 1 (SOAT1) is an important target in the diagnosis and treatment of liver cancer. However, the prognostic value of SOAT1 in patients with hepatocellular carcinoma (HCC) is still not clear.

AIM

To investigate the correlation of SOAT1 expression with HCC, using RNA-seq and gene expression data of The Cancer Genome Atlas (TCGA)-liver hepatocellular carcinoma (LIHC) and pan-cancer.

METHODS

The correlation between SOAT1 expression and HCC was analyzed. Cox hazard regression models were conducted to investigate the prognostic value of SOAT1 in HCC. Overall survival and disease-specific survival were explored based on TCGA-LIHC data. Biological processes and functional pathways mediated by SOAT1 were characterized by gene ontology (GO) analysis and the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of differentially expressed genes. In addition, the protein-protein interaction network and co-expression analyses of SOAT1 in HCC were performed to better understand the regulatory mechanisms of SOAT1 in this malignancy.

RESULTS
SOAT1 and SOAT2 were highly expressed in unpaired samples, while only SOAT1 was highly expressed in paired samples. The area under the receiver operating characteristic curve of SOAT1 expression in tumor samples from LIHC patients compared with para-carcinoma tissues was 0.748, while the area under the curve of SOAT1 expression in tumor samples from LIHC patients compared with GTEx was 0.676. Patients with higher SOAT1 expression had lower survival rates. Results from GO/KEGG and gene set enrichment analyses suggested that the PI3K/AKT signaling pathway, the IL-18 signaling pathway, the calcium signaling pathway, secreted factors, the Wnt signaling pathway, the Jak/STAT signaling pathway, the MAPK family signaling pathway, and cell–cell communication were involved in such association. SOAT1 expression was positively associated with the abundance of macrophages, Th2 cells, T helper cells, CD56^{bright} natural killer cells, and Th1 cells, and negatively linked to the abundance of Th17 cells, dendritic cells, and cytotoxic cells.

CONCLUSION
Our findings demonstrate that SOAT1 may serve as a novel target for HCC treatment, which is helpful for the development of new strategies for immunotherapy and metabolic therapy.

Key Words: Sterol O-acyltransferase 1; Hepatocellular carcinoma; Prognostic; Immune

Core Tip: As patients would greatly benefit from early detection of hepatocellular carcinoma, the complementary study of hepatocellular carcinoma-associated proteins in serum samples using state-of-the-art proteomics would be a very attractive direction for future exploration.

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INTRODUCTION

Liver cancer is one of the leading causes of death worldwide. Hepatocellular carcinoma (HCC) is the most devastating type of liver cancer[1], commonly diagnosed at an advanced stage, with a high rate of mortality and aggressive clinical course. The well-known risk factors for HCC include age, sex, alcohol consumption/abuse, environmental toxins, aflatoxin exposure, chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, and non-alcoholic fatty liver disease[2].

Liver transplantation, radical surgical resection, and radiofrequency ablation are commonly used in early-stage HCC. However, the majority of patients do not meet the criteria for radical treatment and are treated with systemic or local treatment instead[3]. Advanced HCC always presents a poor prognosis, although several new treatment modalities, such as immunotherapy and trans-arterial chemoembolization plus systemic treatments, have been proposed[4-6]. Therefore, exploring effective therapeutic targets for HCC is of great importance to both individuals and society.

Sterol O-acyltransferase (SOAT), known as acyl-CoA:cholesterol acyltransferase (ACAT), is located in the endoplasmic reticulum membrane. It plays an important role in cholesterol homeostasis and bile acid biosynthesis by catalyzing the conversion of cholesterol to cholesterol esters[7]. There are two SOAT isoforms in mammals, namely, SOAT1 and SOAT2. SOAT1 is a key enzyme with high expression levels. It is generally expressed in all tissues except the intestine and plays an important role by converting endoplasmic reticulum cholesterol into lipid droplet (LD) stored esters[8,9]. High SOAT1 expression has been shown in several tumor types (such as liver cancer, pancreatic cancer, and prostate cancer[10,11]) and associated with diagnosis and treatment[12-14]. Up-regulation of SOAT1 could further increase the expression levels of inflammatory factors and cause cardiovascular diseases such as atherosclerosis and coronary heart disease[15-17]. Cholesterol ester increases HCC growth by promoting the synthesis of phospholipids and hormones[18-21]. Proteomic evidence from early-stage HBV-HCC patients showed that HCC patients with more aggressive tumors and poor prognosis had disrupted cholesterol metabolism and increased SOAT1 expression[19]. The single nucleotide polymorphisms of SOAT1 have been closely related to cholesterol metabolism[22,23].

However, the relationship of SOAT1 expression with HCC remains unclear. In the current study, we explored whether SOAT1 is involved in the development of HCC, as well as the regulatory mechanisms of SOAT1[17]. Moreover, we further explored various biological processes and signaling pathways *via* which SOAT1 may potentially be involved in the pathogenesis of HCC.

MATERIALS AND METHODS

Microarray data and data processing

The RNA-seq and gene expression data of The Cancer Genome Atlas (TCGA)-liver hepatocellular carcinoma (LIHC) and pan-cancer, including unpaired samples and paired samples, were extracted, filtered to remove missing and duplicated results, and transformed by $\log_2$ (*TPM* + 1) using the Xiantao tool (www.xiantao.love). SOAT1 gene expression was also analyzed using Clinical Proteomic Tumor Analysis Consortium samples. $P < 0.05$ was regarded as significant.

Prognostic value of SOAT1 expression

To investigate the prognostic value of SOAT1 expression, Cox proportional hazard regression models were generated to describe patients' characteristics, including SOAT1 and SOAT2 expression levels and TNM stages. Overall survival (OS) and disease-specific survival (DSS) were also explored based on TCGA-LIHC data. *P* value < 0.05 was regarded as significant. To further investigate the prognostic value of SOAT1 expression, a nomogram and calibration curves were generated.

Diagnostic value of SOAT1 expression

Receiver operation characteristic curve analysis was conducted to explore the diagnostic value of SOAT1 expression in TCGA-LIHC with and without GTEx and the area under the receiver operating characteristic curve (AUC) was calculated using the "*pROC*" package.

Subgroup analysis

To validate the potential effects of SOAT1 expression on TCGA-LIHC progression, SOAT1 expression was determined in subgroups based on age, sex, and tumor stage. The RNA-seq data and related clinical data in level 3 HTSeq-fragments per kilobase per million mapped fragments formats were downloaded from the TCGA database, converted to transcripts per million formats, and then analyzed after log transformation. *P* value < 0.05 was considered as the cutoff criterion.

Association of SOAT1 expression with immune cells

To analyze the relationship between SOAT1 expression and immune cells, single sample gene set enrichment analysis (GSEA) (the "*GSVA*" package in R) was performed, providing a critical assessment and integration of 24 immune cells for RNA-seq samples from TCGA-LIHC.

Differentially expressed genes between SOAT1 high and low expression groups

The differentially expressed genes (DEGs) between groups with different SOAT1 expression (cut-off value: 50%) in TCGA-LIHC were identified. Utilizing Limma, $\log_2$ (*fold change*) > 2 and *P* value < 0.05 were applied as the cut-off criteria.

Enrichment analysis

Gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were conducted to investigate the DEGs between the high and low SOAT1 expression groups in TCGA-LIHC. GSEA was conducted utilizing the "*clusterProfiler*" package in R. *P* value < 0.05 was applied as the cut-off criterion.

Protein–protein interaction and the hub genes

To investigate the proteins that interact with SOAT1, the STRING database (https://string-db.org) was analyzed with a combined score of > 0.4. The nodes were analyzed with Cytoscape version 3.7.1. Protein–protein interaction (PPI) network analysis was conducted to obtain the hub genes using the Cytoscape plug-in MCODE.

Prognostic value of SOAT1 expression in TCGA-LIHC

Lasso regression and risk score analysis were performed to investigate the association between SOAT1 expression, hub genes, and patient status. The association between survival and hub genes was analyzed to further show the prognostic value of SOAT1 expression in TCGA-LIHC.

RESULTS

SOAT1 is highly expressed in LIHC patients

In the TCGA-LIHC cohort, SOAT1 and SOAT2 were highly expressed in unpaired samples, while only SOAT1 was highly expressed in paired samples (Figure 1A and B). The univariate analysis and multivariate analysis suggested that SOAT1 expression was an independent risk factor for HCC progression (Figure 1C; Supplementary Table 1). SOAT1 expression in pan-cancer, including unpaired and paired samples, was also investigated (Figure 1D and E).

Diagnostic and prognostic value of SOAT1 expression

To explore the diagnostic value of SOAT1 expression in HCC, we performed receiver operating characteristic curve analysis. The AUC of SOAT1 expression in tumor samples from LIHC patients compared with para-carcinoma tissues was 0.748, while the AUC of SOAT1 expression in tumor samples from LIHC patients compared with GTEx was 0.676,

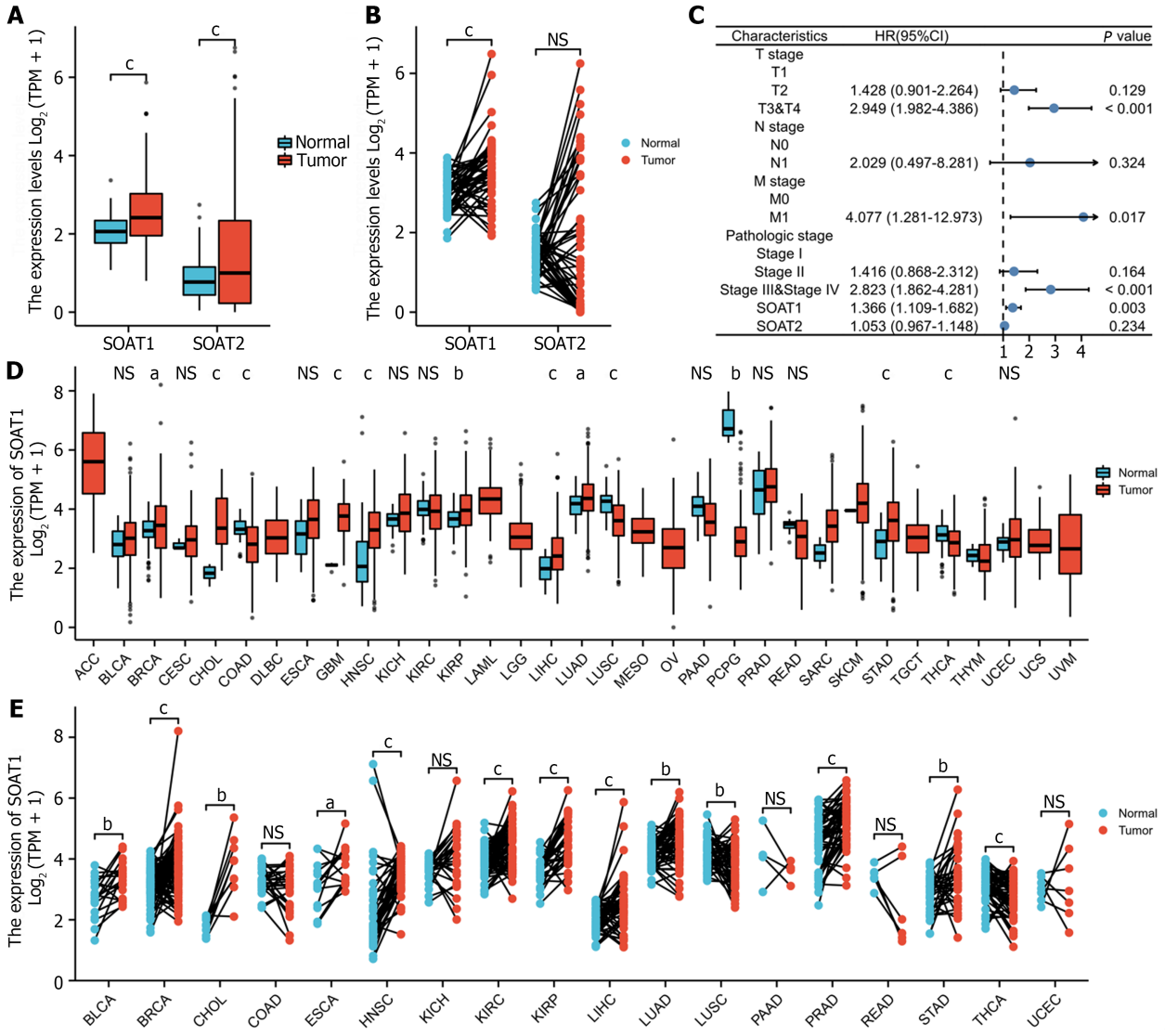

Figure 1 Expression of sterol O-acyltransferase 1 and sterol O-acyltransferase 2 in The Cancer Genome Atlas-liver hepatocellular carcinoma. A: Sterol O-acyltransferase 1 (SOAT1) and SOAT2 expression in the unpaired samples in The Cancer Genome Atlas (TCGA) liver hepatocellular carcinoma TCGA-LIHC; B: SOAT1 and SOAT2 expression in the paired samples in TCGA-LIHC; C: Forest diagram of univariate analysis of patients' characteristics and SOAT1 expression; D: SOAT1 expression in the unpaired samples in TCGA pan-cancer; E: SOAT1 expression in the paired samples (E) in TCGA pan-cancer. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$; NS: Not significant.

suggesting that SOAT1 may be a potential diagnostic biomarker for HCC invasion (Figure 2A and B).

To clarify the prognostic value of SOAT1 expression in HCC, OS and DSS were analyzed. Patients with higher SOAT1 expression had lower survival rates (Figure 2C and D). SOAT1 expression was also associated with age, gender, histologic grade, T stage, and N stage (Figure 3). In addition, 1-, 3-, and 5-year OS and DSS analysis demonstrated that higher SOAT1 expression was associated with a worse prognosis (Figure 4).

DEGs between groups with high and low SOAT1 expression

After log transformation, DEGs between the group with high and low expression of SOAT1 in LIHC were identified. GO enrichment analysis, KEGG pathway enrichment analysis, and GSEA showed that these DEGs are mainly involved in the PI3K/AKT signaling pathway, the IL-18 signaling pathway, the calcium signaling pathway, secreted factors, the Wnt signaling pathway, the Jak/STAT signaling pathway, the MAPK family signaling pathway, and cell–cell communication (Figure 5).

SOAT1 expression and immune cell analysis

Compared with healthy controls, patients with primary tumor showed significantly increased protein expression of SOAT1 (Supplementary Figure 1). To further analyze the association between SOAT1 expression and immune cells, single sample GSEA was conducted in LIHC, which showed that SOAT1 expression was positively associated with the abundance of macrophages, Th2 cells, T helper cells, $CD56^{bright}$ natural killer (NK) cells, and Th1 cells and negatively associated with the abundance of Th17 cells, dendritic cells, and cytotoxic cells (Supplementary Figure 2).

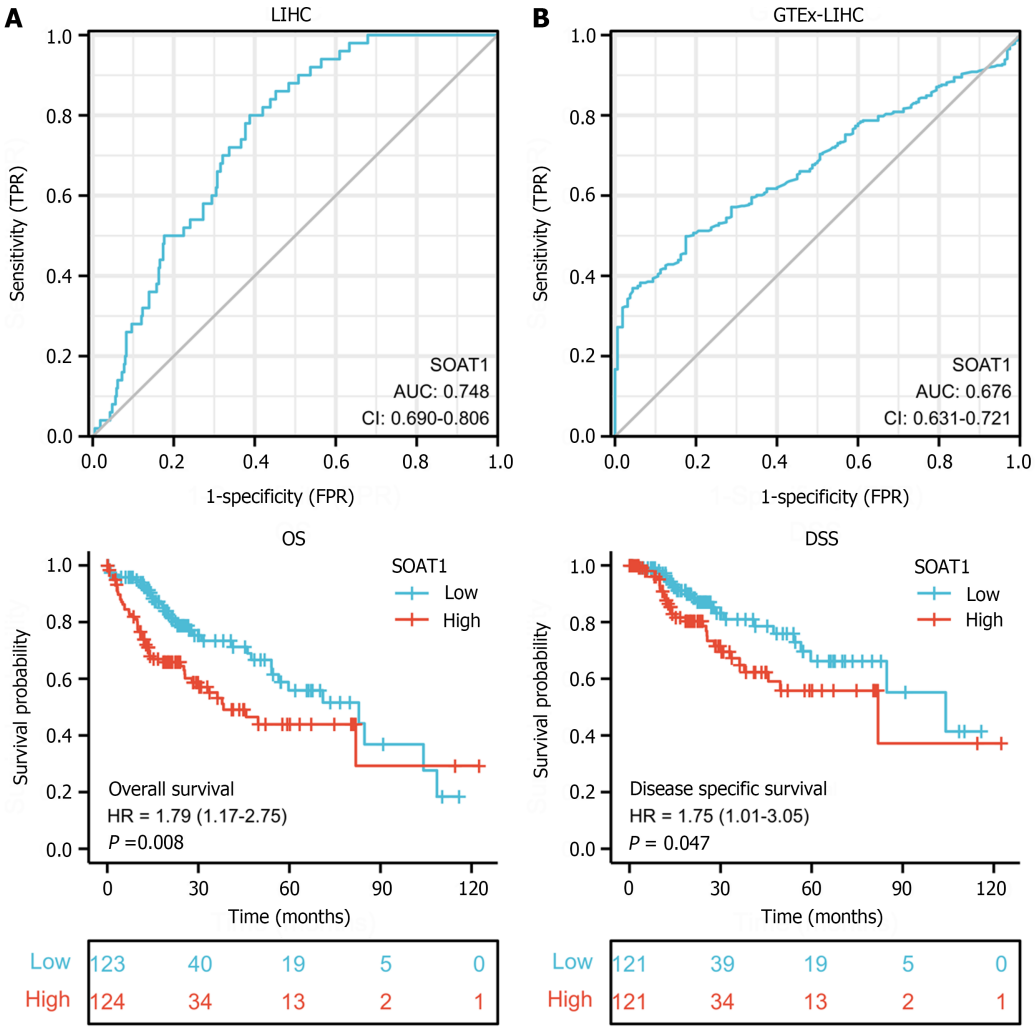

Figure 2 Diagnostic and prognostic value of sterol O-acyltransferase 1 expression in The Cancer Genome Atlas-liver hepatocellular carcinoma. A: Area under the curve (AUC) of sterol O-acyltransferase 1 (SOAT1) expression in tumor samples from liver hepatocellular carcinoma (LIHC) patients compared with para-carcinoma tissues; B: AUC of SOAT1 expression in tumor samples from LIHC patients compared with GTEx; C: Association between OS and SOAT1 expression demonstrated the prognostic value of SOAT1 expression in The Cancer Genome Atlas (TCGA)-LIHC; D: Association between DSS and SOAT1 expression demonstrated the prognostic value of SOAT1 expression in TCGA-LIHC.

PPI network and hub genes

To clarify the proteins that interact with SOAT1 in TCGA-LIHC, the nodes with a comprehensive score more than 0.4 were studied using the STRING database. The hub genes were obtained from the Cytoscape plug-in MCODE, which included two modules in the network (including *CYP19A1*, *CYP2A6*, *CYP1A2*, *CYP1A1*, *UGT1A10*, *KLK3*, *KRT19*, and *CEACAM5*). These genes might be potential targets for HCC treatment (Figure 6).

Effects of SOAT1 and hub genes on LIHC

To investigate the role of SOAT1 expression in LIHC progression, Lasso regression and risk score analysis were utilized. SOAT1 expression was highly correlated with survival time and with the expression of two hub genes, namely, *CYP19A1* and *UGT1A10* (Figure 7A and B). To further explore the prognostic value of these two hub genes, survival analysis was conducted, which showed that patients with higher expression of *CYP19A1* and *UGT1A10* had a worse prognosis, which was consistent with the prognostic value of SOAT1 expression.

DISCUSSION

Historically, chronic viral hepatitis was the main etiologies of HCC; however, nonalcoholic fatty liver disease and related metabolic factors have emerged as the fastest-growing risk factors for HCC in recent years. The relationship between lipids and HCC is complex, so more investigations are anticipated to continue over the next decade. Understanding the role of cholesterol in HCC development will contribute to developing new therapies. One way to further our understanding of the mechanisms that promote carcinogenesis is through analysis of the proteome[24]. Previously, a system-wide approach was adopted to reveal changes in DNA, protein expression, and phenotype in liver cancer tissue,

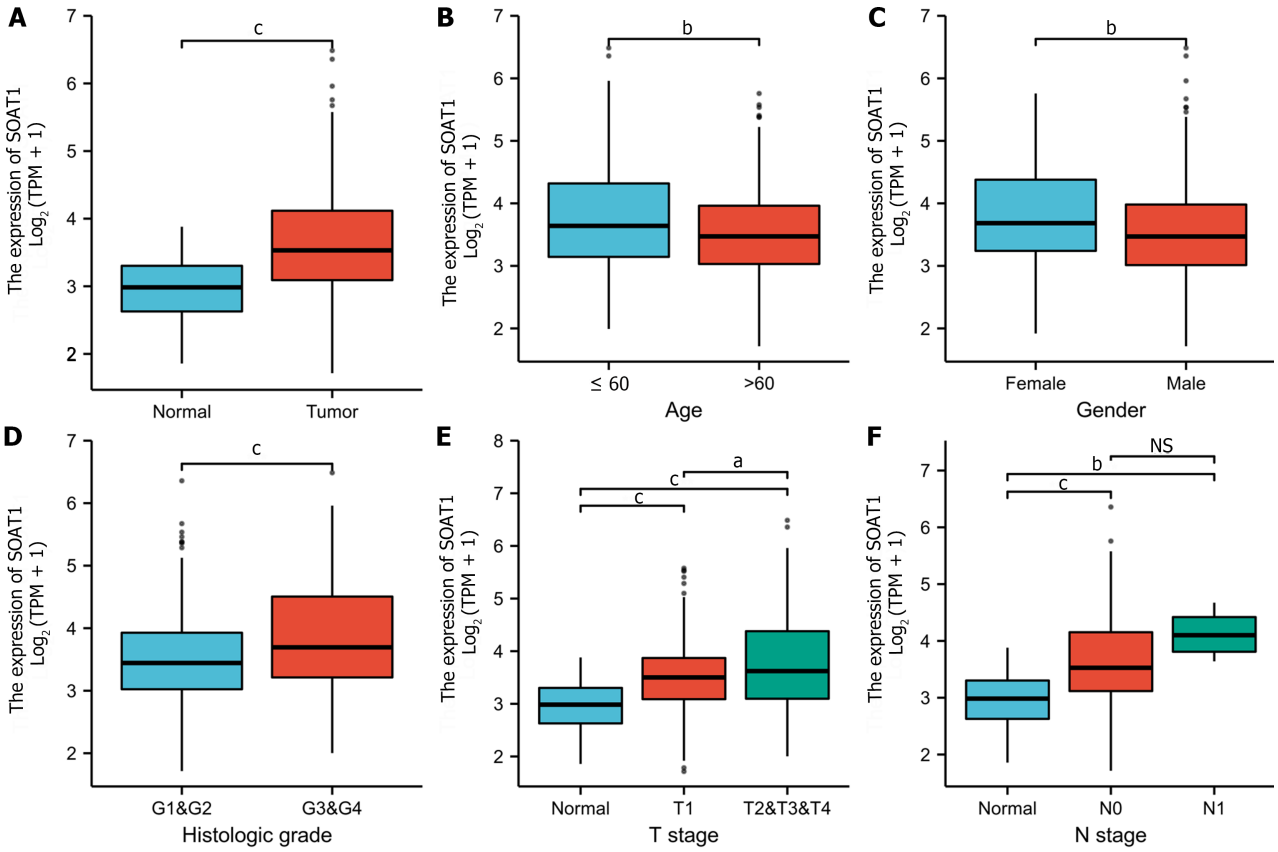

Figure 3 Subgroup analysis of sterol O-acyltransferase 1 expression in liver hepatocellular carcinoma. A: Sterol O-acyltransferase 1 (SOAT1) expression in tumor and normal tissues; B: Association between SOAT1 expression and age; C: Association between SOAT1 expression and gender; D: Association between SOAT1 expression and histologic grade; E: Association between SOAT1 expression and T stage; F: Association between SOAT1 expression and N stage (F). $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$; NS: Not significant.

identifying SOAT1 as a potential biomarker for early-stage HCC. SOAT1 was found to be overexpressed in HCC and to be an independent risk factor for HCC progression[19]. In fact, an increasing body of evidence demonstrates a strong relationship of the tumor metabolic microenvironment with immune microenvironment. In the current study, we found that in HCC, SOAT1 expression was positively linked to the abundance of macrophages, Th2 cells, T helper cells, CD56^{bright} NK cells, and Th1 cells, and negatively associated with the abundance of Th17 cells, dendritic cells, and cytotoxic cells.

Previous studies have shown lower lipid levels in HCC patients compared to healthy controls[23], suggesting that cholesterol metabolism plays a pivotal role in the development of HCC[25,26]. Evidence from proteomic studies have found that HCC patients with abnormal cholesterol metabolism and high SOAT1 expression seemed to have a worse prognosis[19], suggesting that SOAT1 may have an effect on HCC by regulating lipid metabolism. A recent study has found that extracellular lipid loading promoted glioma-associated macrophage infiltration and new blood vessel formation in tumors, which was increased by an elevated continuous supply of lipids throughout the body[27]. It is direct evidence that LD^{+} glioblastoma cells are related to immunosuppressive glioma-associated macrophage infiltration. Since LDs are formed due to the aggregation of cholesterol esters, it is not surprising that SOAT1 expression is associated with M2 macrophage infiltration in HCC. There is a complex relationship between lipids and HCC. Altered lipid metabolism may be a result of HCC development. Cachexia commonly exists in cancer patients, characterized by reduced fat storage, increased carbohydrate utilization, and elevated protein degradation. The high growth rate of cancer cells may lead to hypoxia and increased energy requirements, ultimately promoting fatty-acid oxidation and depleting fat stores[28,29]. In addition, dysregulation of lipid metabolism may contribute to the development of HCC, due to impaired pro-tumorigenic insulin and insulin-like growth factor 1 signaling[30,31]. Additionally, research in mice and humans has showed that liver cells without fatty acid synthase might support c-MET oncogene-mediated liver tumor formation through up-regulation of SREBP2 *via* the cholesterol synthesis pathway[32].

Studies have demonstrated that SOAT1 plays a carcinogenic role through multiple pathways. Our OS and DSS analyses also showed that higher SOAT1 expression was associated with poor survival in patients with HCC. Therefore, further studies are warranted to explore the prognostic value of SOAT1 in HCC. Indeed, SOAT1 expression is associated with a poor prognosis in all HCC cases. Our 1-, 3- and 5-year OS and DSS analyses demonstrated that higher SOAT1 expression was associated with a worse prognosis (Figure 4), suggesting that SOAT1 may be a potential diagnostic biomarker for HCC invasion. Down-regulation of SOAT1 has been reported to inhibit proliferation and migration of HCC cells by reducing plasma membrane cholesterol content and inhibiting the integrin and TGF-β signaling pathways[19]. Consistently, integrin binding was also significantly enhanced, as determined by enrichment analysis of the GO and

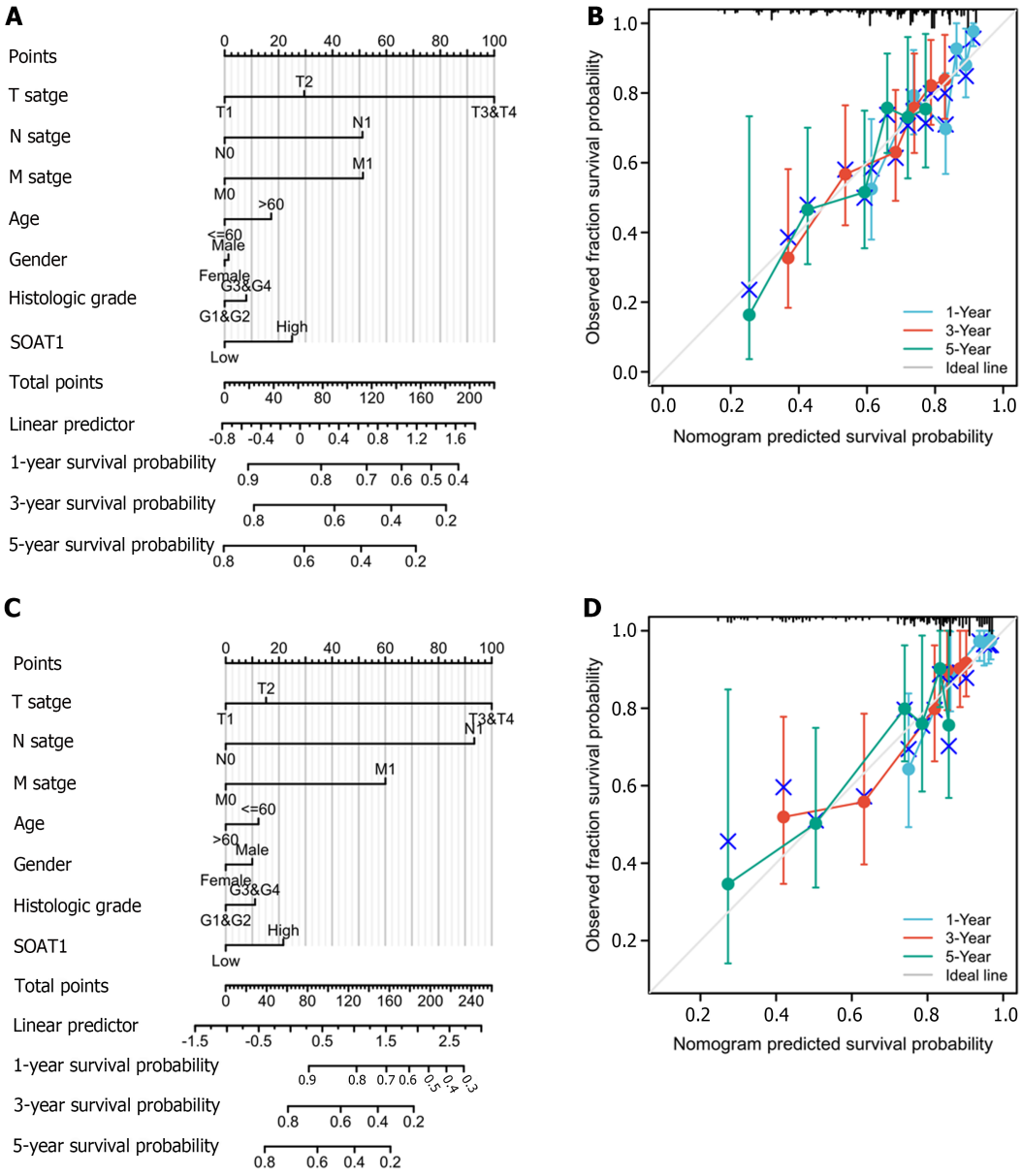

Figure 4 Prognostic value of sterol O-acyltransferase 1 expression in liver hepatocellular carcinoma. A: Nomograms for 1-, 3-, and 5-year overall survival in different subgroups based on sterol O-acyltransferase 1 (SOAT1) expression and other clinical characteristics in liver hepatocellular carcinoma (LIHC); B: Calibration for 1-, 3-, and 5-year overall survival in different subgroups based on SOAT1 expression and other clinical characteristics in LIHC; C: Nomograms for 1-, 3-, and 5-year disease specific survival in different subgroups based on SOAT1 expression and other clinical characteristics in LIHC; D: Calibration for 1-, 3-, and 5-year disease specific survival in different subgroups based on SOAT1 expression and other clinical characteristics in LIHC.

KEGG pathways of upregulated DEGs in HCC (Figure 5). Multiple genes, including *CYP19A1*, *CYP2A6*, *CYP1A2*, *CYP1A1*, *UGT1A10*, *KLK3*, *KRT19*, and *CEACAM5* (Figure 6), whose encoded proteins may interact with SOAT1 in HCC, were identified *via* PPI network and co-expression analyses, which may be potential targets for HCC treatment. The higher the expression of *CYP19A1* and *UGT1A10*, the worse the prognosis, which is consistent with the prognostic analysis of SOAT1 expression. SOAT1 expression was reported to be regulated by multiple mechanisms in tumors. Runt-related transcription factor 1 promotes SOAT1 expression in squamous cell carcinoma by binding to the promoter region of *SOAT1*[33]. Loss of p53 heterozygosity can promote the expression of SOAT1 by enhancing the transcription of *SOAT1* in pancreatic ductal adenocarcinoma[10]. In addition, β-catenin has been reported to be directly bind to the *SOAT1* promoter element and promote its transcription in colorectal cancer[21], as well.

CONCLUSION

The progression of HCC is complex and several factors are involved, including age, alcohol consumption, environmental toxins, HBV and HCV levels, and diet. In the present study, the prognostic value of SOAT1 in HCC was elucidated. Our findings suggest that SOAT1 may modestly alter the risk for HCC by regulating lipid metabolism, but the effect might be

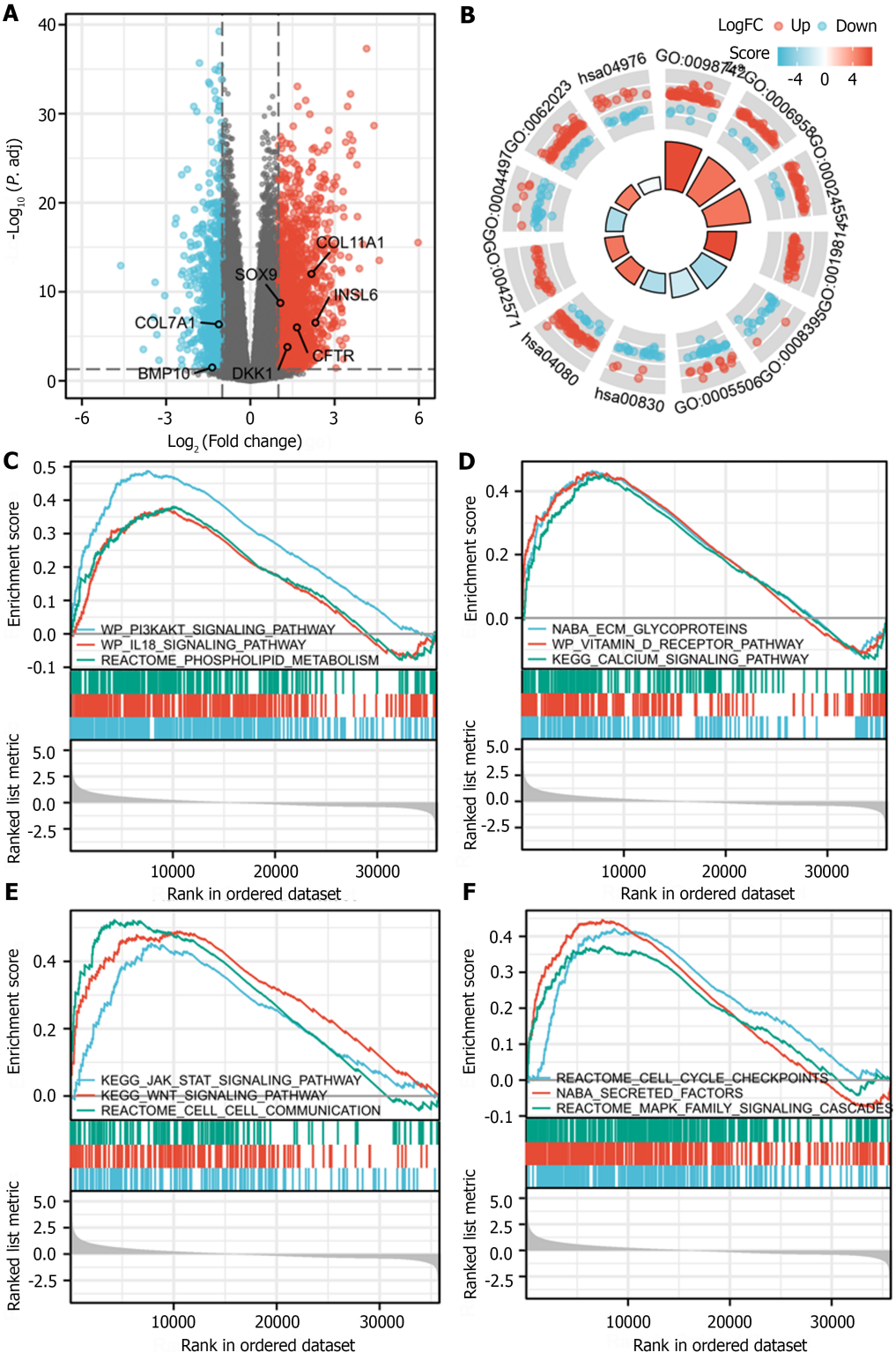

Figure 5 Differentially expressed genes between high and low sterol O-acyltransferase 1 expression groups in liver hepatocellular carcinoma. A: Volcano plot of differentially expressed genes (DEGs) between high and low sterol O-acyltransferase 1 (SOAT1) expression groups in liver hepatocellular carcinoma (LIHC); B: Top GO terms and KEGG pathways enriched by DEGs between high and low SOAT1 expression groups in LIHC; C-F: Gene set enrichment analysis of DEGs between high and low SOAT1 expression groups in LIHC.

limited. Further studies are warranted to validate our results. The identification of other HCC proteins involved in this multigenic heterogeneous cancer type is an important objective for future research. Since early diagnosis of HCC is of great benefit to patients, complementary studies using the most advanced proteomic techniques on HCC-related proteins in serum samples can be a very attractive research direction in the future. That is, SOAT1 may be recognized as a new target to advance the development of immunotherapy and metabolic therapy.

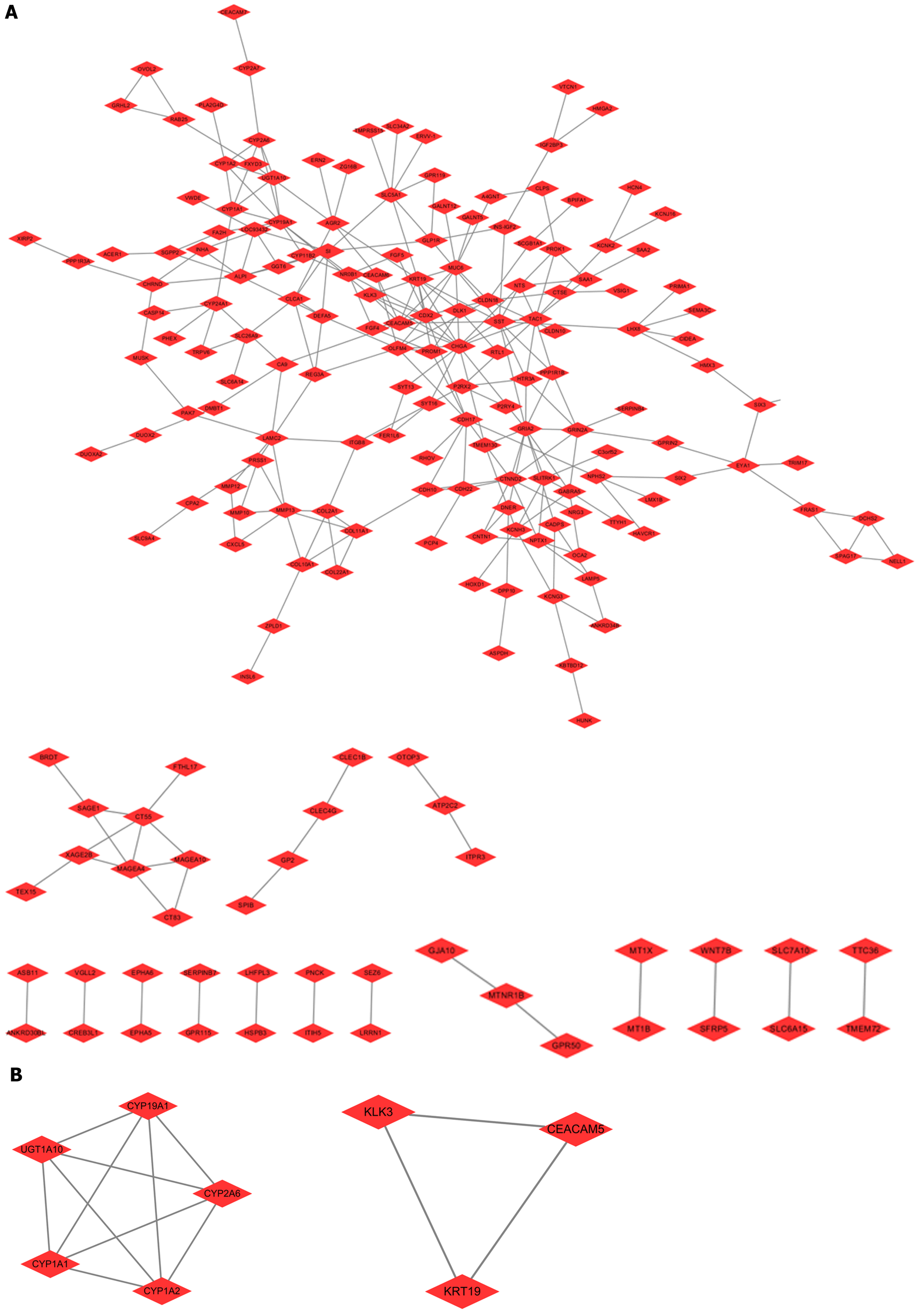

Figure 6 Protein–protein interaction network and hub genes of differentially expressed genes between the high and low sterol O-

acyltransferase 1 expression groups. A: Protein–protein interaction network of differentially expressed genes between high and low sterol O-acyltransferase 1 expression groups; B: Hub genes (two modules) screened using the Cytoscape plugin MCODE.

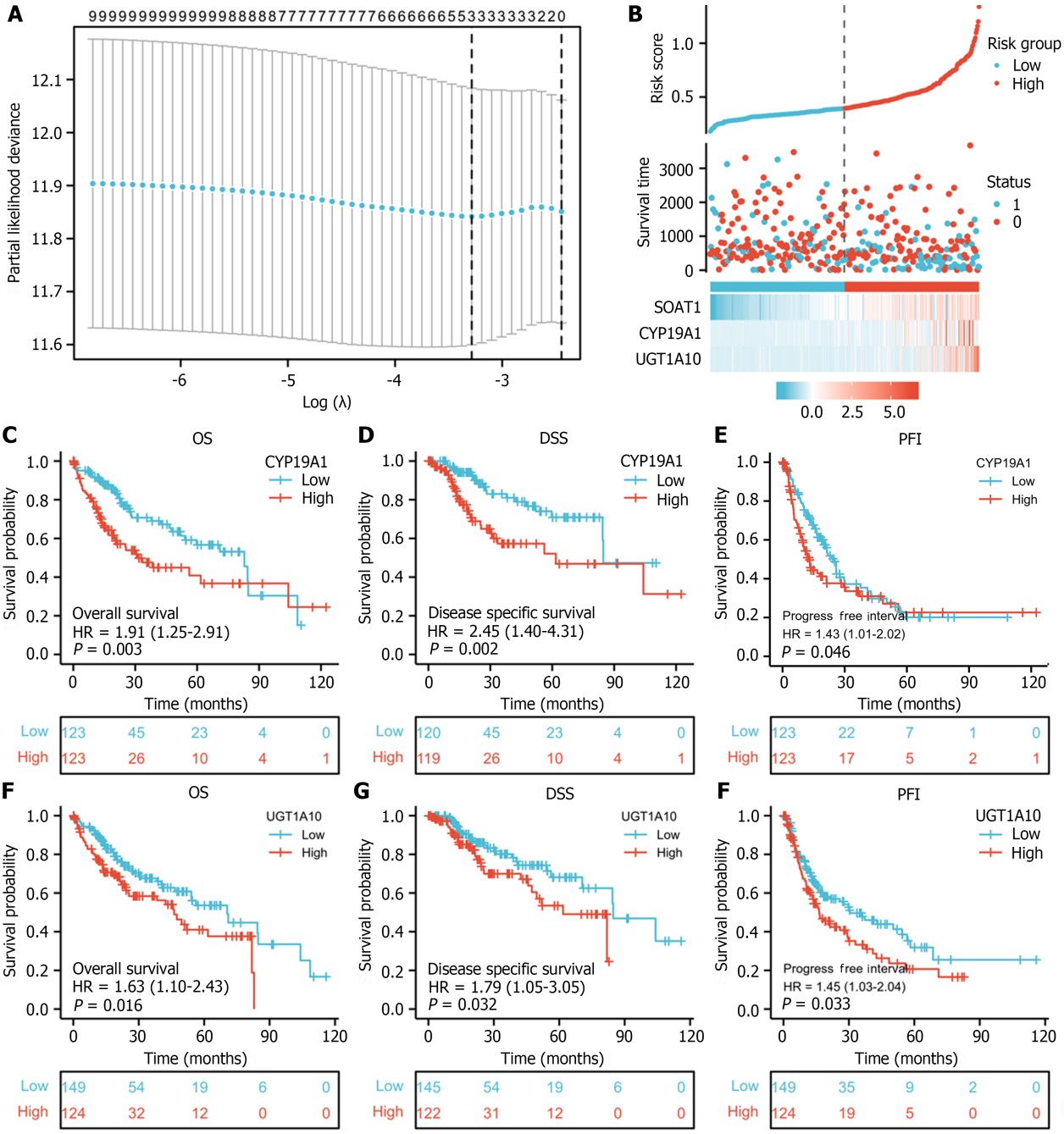

Figure 7 Effects of sterol O-acyltransferase 1 and hub genes on liver hepatocellular carcinoma. A: Lasso regression of survival time and sterol O-acyltransferase 1 (SOAT1) and hub gene expression levels in liver hepatocellular carcinoma (LIHC); B: Risk score analysis of survival time and SOAT1 and hub gene expression levels in LIHC. 1, survival; 0, dead; C: Overall survival (OS) of LIHC patients between high and low CYP19A1 expression groups; D: Disease-specific survival (DSS) of LIHC patients between high and low CYP19A1 expression groups; E: Progression free interval of LIHC patients between high and low CYP19A1 expression groups; F: OS of LIHC patients between high and low UGT1A10 expression groups; G: DSS of LIHC patients between high and low UGT1A10 expression groups: H: Progression free interval of LIHC patients between high and low UGT1A10 expression groups. OS: Overall survival; DSS: Disease specific survival; PFI: Progression free interval.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular carcinoma (HCC) has a poor prognosis and heavy disease burden, but its treatment methods are not satisfactory.

Research motivation

High sterol O-acyltransferase 1 (SOAT1) expression has been shown to be associated with several tumor types (liver cancer, pancreatic cancer, and prostate cancer) and with diagnosis and treatment. However, the relationship between SOAT1 expression and HCC remains unclear. As patients would greatly benefit from early detection of HCC, the complementary study of HCC-associated proteins in serum samples using state-of-the-art proteomics would also be a very attractive direction for future research. Therefore, SOAT1 may serve as a novel target that drives the development of immunotherapy and metabolic therapy.

Research objectives

This study aimed to investigate the correlation between SOAT1 expression and HCC, using RNA-seq and gene expression data of The Cancer Genome Atlas (TCGA)-liver hepatocellular carcinoma (LIHC) and pan-cancer. Our findings demonstrate that SOAT1 may serve as a new target for HCC treatment and promote the development of new strategies for immunotherapy and metabolic therapy.

Research methods

The correlation between SOAT1 expression and HCC was analyzed. Cox hazard regression models were used to investigate the prognostic value of SOAT1. Overall survival and disease-specific survival were also explored in TCGA-LIHC. Moreover, the biological processes and functional pathways regulated by SOAT1 were characterized using gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of differentially expressed genes. To better understand the regulatory mechanism of SOAT1 in HCC, protein–protein interaction network and co-expression analyses of SOAT1 in HCC were conducted.

Research results

SOAT1 and SOAT2 were highly expressed in unpaired samples, while only SOAT1 was highly expressed in paired samples. The area under the receiver operating characteristic curve of SOAT1 expression in tumor samples from LIHC patients compared with para-carcinoma tissues was 0.748, while the area under the curve of SOAT1 expression in tumor samples from LIHC patients compared with GTEx was 0.676. Patients with higher SOAT1 expression had lower survival rates. Results from GO/KEGG and gene set enrichment analyses suggested that the PI3K/AKT signaling pathway, the IL-18 signaling pathway, the calcium signaling pathway, secreted factors, the Wnt signaling pathway, the Jak/STAT signaling pathway, the MAPK family signaling pathway, and cell–cell communication were involved in such association. SOAT1 expression was positively associated with the abundance of macrophages, Th2 cells, T helper cells, $CD56^{bright}$ natural killer cells, and Th1 cells, and negatively linked to the abundance of Th17 cells, dendritic cells, and cytotoxic cells.

Research conclusions

As patients would greatly benefit from early detection of hepatocellular carcinoma, the complementary study of hepatocellular carcinoma-associated proteins in serum samples using state-of-the-art proteomics would be a very attractive direction for future exploration.

Research perspectives

The identification of other HCC proteins involved in this multigenic heterogeneous cancer type is an important objective for future research.

FOOTNOTES

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Author contributions: Gan CJ, Zheng Y, Cao LM, and Yang B conceptualized and designed the research; Gan CJ and Zheng Y performed data analysis; Gan CJ and Zheng Y wrote the paper. All the authors have read and approved the final manuscript. Both Gan CJ and Zheng Y have made crucial and indispensable contributions towards the completion of the project and thus are qualified as the co-first authors of the paper. Both Cao LM and Yang B have played important and indispensable roles in the study design, and manuscript preparation as the co-corresponding authors. Both Cao LM and Yang B applied for and obtained the funds for this research project. Cao LM conceptualized, designed, and supervised the whole process of the project. She searched the literature, and revised and submitted the early version of the manuscript. Yang B was instrumental and responsible for data re-analysis and re-interpretation, figure plotting, comprehensive literature search, and preparation and submission of the current version of the manuscript. This collaboration between Cao LM and Yang B is crucial for the publication of this manuscript and other manuscripts still in preparation.

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SYSTEMATIC REVIEWS

Update in lean metabolic dysfunction-associated steatotic liver disease

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Abstract

BACKGROUND

A new nomenclature consensus has emerged for liver diseases that were previously known as non-alcoholic fatty liver disease (NAFLD) and metabolic dysfunction-associated fatty liver disease (MAFLD). They are now defined as metabolic dysfunction-associated steatotic liver disease (MASLD), which includes cardiometabolic criteria in adults. This condition, extensively studied in obese or overweight patients, constitutes around 30% of the population, with a steady increase worldwide. Lean patients account for approximately 10%-15% of the MASLD population. However, the pathogenesis is complex and is not well understood.

AIM

To systematically review the literature on the diagnosis, pathogenesis, characteristics, and prognosis in lean MASLD patients and provide an interpretation of these new criteria.

METHODS

We conducted a comprehensive database search on PubMed and Google Scholar between January 2012 and September 2023, specifically focusing on lean NAFLD, MAFLD, or MASLD patients. We include original articles with patients aged 18

years or older, with a lean body mass index categorized according to the World Health Organization criteria, using a cutoff of 25 kg/m^2 for the general population and 23 kg/m^2 for the Asian population.

RESULTS

We include 85 studies in our analysis. Our findings revealed that, for lean NAFLD patients, the prevalence rate varied widely, ranging from 3.8% to 34.1%. The precise pathogenesis mechanism remained elusive, with associations found in genetic variants, epigenetic modifications, and adaptative metabolic response. Common risk factors included metabolic syndrome, hypertension, and type 2 diabetes mellitus, but their prevalence varied based on the comparison group involving lean patients. Regarding non-invasive tools, Fibrosis-4 index outperformed the NAFLD fibrosis score in lean patients. Lifestyle modifications aided in reducing hepatic steatosis and improving cardiometabolic profiles, with some medications showing efficacy to a lesser extent. However, lean NAFLD patients exhibited a worse prognosis compared to the obese or overweight counterpart.

CONCLUSION

MASLD is a complex disease comprising epigenetic, genetic, and metabolic factors in its pathogenesis. Results vary across populations, gender, and age. Limited data exists on clinical practice guidelines for lean patients. Future studies employing this new nomenclature can contribute to standardizing and generalizing results among lean patients with steatotic liver disease.

Key Words: Lean; Non-obese; Non-alcoholic fatty liver disease; Metabolic dysfunction-associated fatty liver disease; Metabolic dysfunction-associated steatotic liver disease; Guidelines; Diagnosis; Management; Pathogenesis; Treatment

Core Tip: Steatotic liver disease, extensively studied in overweight/obese patients, poses a unique challenge in lean individuals due to limited data on its pathogenesis, diagnosis, management, and risk factors. The lack of consensus in nomenclature impedes the comprehension and application of findings. To address this gap, we conducted a systematic review focusing on lean individuals with steatotic liver disease. This review interprets the new approach, introducing the term metabolic dysfunction-associated steatotic liver disease in alignment with current literature. We aim to enhance the understanding of steatotic liver disease in lean populations, contributing to a precise approach in research and clinical settings.

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INTRODUCTION

In 1980, Ludwig, Viggiano, McGill, and Oh introduced the term non-alcoholic fatty liver disease (NAFLD), defining the disease as the presence of hepatic fat in the absence of significant alcohol intake. It was characterized as hepatic steatosis observed through imaging or histology, excluding other causes of chronic liver disease and steatosis, such as substantial alcohol consumption, prolonged use of steatogenic medication, or hereditary monogenic disorders[1]. By utilizing this exclusionary criterion, the differential diagnosis of NAFLD was formed. In 2020, the concept of metabolic dysfunction-associated fatty liver disease (MAFLD) emerged, encompassing individuals previously excluded due to alcohol consumption or other liver diseases[2]. This represented a shift towards a "positive" diagnosis, moving away from an exclusory approach. However, even with this new terminology, patient stigmatization persisted due to the continued use of the term "fatty." Consequently, a collaborative effort involving the following groups: American Association for the Study of Liver Disease, European Association for the Study of the Liver, and Latin American Association for the Study of the Liver, utilizing the Delphi method, led to the development of a novel nomenclature metabolic dysfunction-associated steatotic liver disease (MASLD)[3]. The recent consensus reclassified NAFLD and MAFLD[4,5] to MASLD[3]. To meet the new MASLD criteria, individuals must exhibit at least 1 of 5 cardiometabolic risk factors linked to insulin resistance (IR). MASLD constitutes approximately 30% of the global population, and its prevalence is steadily increasing worldwide[6]. Despite this condition being extensively researched in overweight and obese individuals, 10%-15% of MASLD patients will exhibit normal weight and are classified as either lean or non-obese[7]. The categorization depends on ethnicity; the World Health Organization (WHO) categorizes a normal body mass index (BMI) for the general population with a cutoff of 25 kg/m^2 and 23 kg/m^2 for the Asian population[8]. Most studies have predominantly focused on BMI when investigating patients with lean MASLD. However, BMI has been proven to be an imperfect marker of adiposity[9-13]. Vilarinho *et al*[14] have proposed a classification system for patients with lean MASLD, distinguishing two phenotypes

based on epidemiological characteristics, natural history, and prognosis. Type 1 includes individuals with visceral adiposity and insulin resistance. While type 2 comprises of those with hepatic steatosis resulting from monogenic diseases, this requires a nuanced understanding of the pathophysiology.

The pathophysiology of MASLD is intricate and diverse. The clinical spectrum of this disease ranges from simple steatosis to cirrhosis and is influenced by diverse factors, including the overconsumption of carbohydrates and dietary sugars such as fructose, sucrose, and glucose[15]. Dysbiosis, bacterial translocation, and pro-inflammatory factors in the liver also contribute to its complexity[16]. It is proposed that the disease phenotype arises from intricate interactions between genetic and environmental factors[17]. Despite the various potential mechanism proposed, the literature supports that IR and lipotoxicity play a key role in the pathogenesis[18]. This interplay results in a chronic elevation of plasma levels of non-esterified fatty acids, which are ectopically deposited in the liver, promoting the development of steatosis. Additionally, triglycerides (TG) within hepatocytes further increase the accumulation of toxic lipids, such as ceramides and diacylglycerols, intensifying IR and activating inflammatory pathways. Furthermore, it has been reported that lean MASLD patients experience increased concentrations of serum bile acids and elevated farnesoid X receptor (FXR) activity as an initial metabolic response[16-19].

Genes have been identified as modulators of insulin sensitivity and regulators of the intracellular flow of fatty acids, TG, oxidative stress, endotoxin response, cytokine activity, and the development of fibrosis[18]. The most studied single nucleotide polymorphisms (SNPs) associated with steatosis across diverse ethnicities are rs58542926 in the *TM6SF2* gene (transmembrane 6 superfamily member 2)[20] and rs738409 in the *PNPLA3* gene (patatin-like phospholipase domain-containing protein 3)[21]. The I148M polymorphism of *PNPLA3* disrupts triglyceride lipolysis in lipid droplets[22]. Polymorphism in *TM6SF2* plays a pivotal role in hepatic and cholesterol metabolism[20]. Additionally, *MBOAT7* influences phospholipid metabolism[23].

Regarding the diagnosis of steatotic liver disease in lean patients, it is typically conducted through[18,24] imaging modalities such as abdominal ultrasound (US)[25,26], computed tomography (CT)[27,28], or magnetic resonance imaging (MRI)[29]. Additionally, FibroScan, assessing the controlled attenuation parameter (CAP)[30-32] and liver stiffness measurement (LSM)[31,33], is employed. However, liver biopsy is usually reserved for patients with an unclear diagnosis. Conversely, non-invasive scores are also utilized for diagnosis, which will be discussed later in this review.

The development of the new MASLD nomenclature consensus has been proven helpful for accurately classifying patients with liver steatosis, allowing individuals previously classified as "lean NAFLD" to be categorized as lean MASLD, facilitating uniform studies in the future, particularly for those presenting with cardiometabolic risk[34,35]. These new approaches broaden the focus regarding the metabolic pathogenesis of the disease. However, individuals not meeting these criteria and have no known cause of liver disease have been classified as having cryptogenic steatotic liver disease[3]. This distinction is significant because some patients previously labeled as NAFLD are now reclassified as cryptogenic steatotic liver disease. Discussing this reclassification is important because this new approach does not imply that other causes of steatosis should not be considered, and it also allows for a more in-depth characterization of fibrosis severity using a non-invasive test. Due to the homogenization of the concept of steatotic liver disease, this has been a significant step forward in understanding and addressing this complex disease. As establishing a consensus on how to categorize these patients is essential for future studies, ensuring that results are comparable across different research endeavors.

Considering the significant implication of this complex disease, we intended to conduct a systematic review of the literature pertaining to the diagnosis, pathogenesis, characteristics, and complications associated with lean MASLD patients. Additionally, our goal is to provide an interpretation of this new criteria.

MATERIALS AND METHODS

We conducted a database search on PubMed and Google Scholar, selecting papers published between January 2012 and September 2023 in the English language. The last access to PubMed and Google Scholar occurred on 25 September 2023. The keywords and terms utilized in our search were as follows: (1) NAFLD or non-alcoholic liver disease; (2) MASLD or metabolic dysfunction association steatotic liver disease; (3) guidelines; (4) management; (5) characteristics; and (6) lean. The specific search terms included "non-alcoholic fatty liver disease"[MeSH Terms] OR nafld [All Fields], "guideline"[Publication Type] OR "guidelines as topic"[MeSH Terms] OR "guidelines" [All Fields], "diagnosis"[Subheading] OR "diagnosis"[MeSH Terms] OR diagnosis [All Field], "organization and administration"[MeSH Terms] OR "disease management"[MeSH Terms] OR management[All Field], "therapy"[Subheading] OR "therapeutics"[MeSH Terms] OR treatment [All Field], characteristic[All Fields], and lean[All Fields].

We included original articles that featured patients aged 18 years or older, with BMI categorized by the WHO for both the general and Asian populations. In the general population, BMI was described as normal (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), and obese (> 30 kg/m^2). In the Asian population, BMI was described as normal (18.5-22.9 kg/m^2), overweight (23-24.9 kg/m^2), and obese (> 25 kg/m^2). In this review, normal BMI is referred to as lean, non-obese, or normal weight. We included studies that diagnose steatosis liver disease using abdominal US, abdominal CT, or MRI, in conjunction with FibroScan, which incorporates CAP and/or LSM, as well as histological diagnosis *via* biopsy. Diagnosis may also involve clinically identifying steatosis liver disease based on elevated liver enzymes, while ruling out other liver diseases.

We excluded systematic reviews, review articles, case reports, poster presentations, conference abstracts, editorials, letters to the editor, studies involving patients under 18 years old, studies which utilizes animals, and studies categorizing BMI differently than the WHO. After removing duplicates and applying our inclusion and exclusion criteria,

a total of 85 papers were identified. Refer to Figure 1 for more details.

RESULTS

Current guidelines

Only one expert review on clinical practice updates for lean MASLD patients was found in the literature[24]. The review offered practical advice for physicians. The evaluation of MASLD patients should include routine assessments for hypertension (HTN), type 2 diabetes mellitus (T2DM), dyslipidemia, and a comprehensive alcohol consumption history. Regarding screening lean patients, only patients older than 40 years old with T2DM require recommended evaluation. It is essential to investigate and rule out alternative causes of liver steatosis, starting with non-invasive methods such as serum scores or imaging; liver biopsy should be reserved for undetermined diagnosis. NAFLD fibrosis score (NFS) and fibrosis-4 score (FIB-4) were the two non-invasive scores recommended. The recommended imaging modalities were transient elastography (FibroScan) and magnetic resonance elastography. While no specific treatment exists for lean patients, it is recommended that lifestyle modifications advocating a modest weight loss of 3%-5% (less than in overweight or obese patients) be pursued. Surveillance for liver cancer is crucial, and it involves employing abdominal ultrasound, with or without alpha-fetoprotein, in patients with cirrhosis.

DISCUSSION

Pathogenesis

Genetic variants and epigenetic modifications have been correlated in lean NAFLD patients. However, the precise mechanisms have yet to be fully elucidated, and in some cases, have produced contradictory results. Zeng *et al*[36] described that in the Chinese population, there was no significant difference in SNPs in the *SIRT1*, *APOC3*, *PNPLA3*, *AGTR1*, and *PPARGC1A* genes between lean patients with and without NAFLD. They concluded that metabolic factors played a vital role in the occurrence and progression of NAFLD rather than genetic factors.

On the other hand, Wei *et al*[37] found that a SNP in *PNPLA3* (rs738409) had a higher prevalence in non-obese patients compared to obese patients with NAFLD. Carrying the GG allele in *PNPLA3* (rs738409) increases the risk of NAFLD in the general population, especially in patients without metabolic syndrome (MetS). This SNP appeared to be independent of dietary factors or metabolic conditions[38]. Despite these contradictory results, the GG variant of patatin-like phospholipase domain 3 (*PNPLA3*), encodes adiponutrin and plays a crucial role in lipid metabolism. It has been identified as an independent variable, and it has been associated with a higher risk of NAFLD and significant fibrosis in lean patients[37-39].

Alharthi *et al*[16] described an alteration in adaptive metabolic response characterized by elevated concentrations of serum bile acids and increased activity of the FXR in lean NAFLD patients. Models of metabolic maladaptation loss have been proposed for these patients[16,19]. The Western diet may alter intestinal permeability, increase exposure to bacterial products, and lipopolysaccharides. In lean patients with NAFLD, this could lead to higher endotoxemia, increased expression of macrophage TLR4, and higher production of inflammatory cytokines compared to healthy thin individuals.

Characteristic

The prevalence of lean NAFLD exhibits a wide range, varying from 3.8% to 34.1%[7,40-56]. Refer to Table 1, for more details.

Many studies have indicated that lean NAFLD occurs to people that are older than 40 years old[40,41,46,47,53,55-57]. However, conflicting findings exist, with some studies suggesting that patients are younger than 40 years old[7,42,58,59]. While other studies report patients being older than 60 years old[45,60]. One study demonstrated, by stratifying the prevalence of lean NAFLD by age and sex, that males under 50 years old have an increased likelihood of developing the lean NAFLD phenotype; however, beyond 50 years old, no significant differences between the sexes were observed[37].

When examining the sexes separately, some studies reported a high prevalence of lean NAFLD in males[40,41,45,46,59,61], while others indicated a higher prevalence in females[7,50,58,62]. Nevertheless, there are studies reporting no significant differences in prevalence between females and males[42,51,52,57,60,63].

These variations highlight the heterogeneity of lean NAFLD prevalence in different cohorts and across distinct populations.

Risk factors

Studies have compared lean patients with and without NAFLD. These studies have demonstrated that lean NAFLD patients are at a higher risk of atherogenic dyslipidemia[40,64], MetS, T2DM[41,46], dyslipidemia, and cardiovascular complications[46]. Additionally, these patients manifest elevated cardiovascular and all-cause mortality rates[65]. When laboratory values were compared, this revealed elevated levels of TG, total cholesterol, and fasting blood glucose (FBG) for patients with lean NAFLD[41]. Regarding anthropometric measurements, the studies showed higher waist circumference (WC)[40,41,44,46] and BMI[41] in lean NAFLD patients compared to those without NAFLD.

When comparing lean patients with NAFLD and overweight/obese patients with NAFLD, studies reported that lean NAFLD patients have a lower prevalence of T2DM[7,37,50,58,60-62,66], dyslipidemia[7,50,58,60] , HTN[7,49,50,52,56-58,60,63,66,67], MetS[49,52,62,66], cardiovascular disease[60], and cirrhosis[60,62]. Laboratory values were compared,

Table 1 Characteristics of lean non-alcoholic fatty liver disease patients

Ref.	Population	Prevalence	Characteristics	Cardiometabolic risk	Laboratory values	Anthropometric values
Younossi *et al*[7], 2012	Compared lean with overweight/obese NAFLD patients (*n* = 11613)	18%	< 40 yr, female	↓ T2DM, IR, HTN, Hypercholesterolemia	↓ AST, ALT, platelets	Not reported
Wei *et al*[37], 2015	Compared lean with overweight/obese NAFLD patients (*n* = 911)	19.3%	< 50 years: male, > 50 years: No difference between sexes	↓ T2DM, HTN, MetS and liver stiffness	↓ ALT, HOMA-IR, ↑ HDL	↓ WC, WHR
Fracanzani *et al* [66], 2017	Compare lean with overweight/obese NAFLD patients (*n* = 669)	21.3%	Not reported	↓ T2DM, MetS, HTN	↓ HOMA-IR, ↑ HDL, platelet	↓ WC
Golabi *et al*[65], 2019	Compare lean with and without NAFLD patients (*n* = 5375)	Not reported	Not reported	↑ Risk cardiovascular and all-cause of mortality	Not reported	Not reported
Shao *et al*[63], 2020	Compare lean with obese NAFLD patients (*n* = 543)	Not reported	No difference between sexes or age	↓ BP	↓ AST, ALT, LDL, total cholesterol, FBG, HOMA-IR, ↑ HDL	↓ BMI, WC, WHR
Aneni *et al*[40], 2020	Compared lean with and without NAFLD patients (*n* = 9137)	3.8%	> 40 yr, male	↑ Risk of AD, BP	↑ FBG, total cholesterol, LDL, TG, AST, ALTl; Low HDL	↑ WC
Rahman *et al* [41], 2020	Compared lean with and without NAFLD patients (*n* = 1305)	4.4%	> 40 yr, male	↑ MetS, T2DM	↑ TG, Total cholesterol, FBG	↑ Abdominal obesity, BMI
Semmler *et al* [46], 2021	Compared lean with and without NAFLD patients (*n* = 3043)	6.7%	> 40 yr, male	↑ Dyslipidemia, IR, T2DM, MetS, cardiovascular risk	Not reported	↑ WC
Weinberg *et al* [60], 2021	Compared lean with overweight/obese NAFLD patients (*n* = 3386)	Not reported	> 60 yr No difference between sexes	↓ Cirrhosis, CVD, HTN, T2DM, dyslipidemia	↓ AST, ALT; ↑ Albumin	Not reported
Aneni *et al*[58], 2022	Compared lean with overweight/obese NAFLD patients (*n* = 6513)	Not reported	< 45 yr, female	↓ HTN, T2DM, hyperlipidemia, MetS, AD, ↑ risk of all-cause of mortality	↓ FBG, total cholesterol, LDL, TG, AST, ALT; ↑ HDL	↓ WC
Razouki *et al*[45], 2022	Describe lean NAFLD (*n* = 1049)	5.8%	> 60 yr, male, Asian American	↑ MetS, Inadequate physical activity	↑ FBG, TG	
Zhang *et al*[56], 2022	Compare lean with obese NAFLD patients (*n* = 2708)	34.1%	> 40 yr	↓ BP	↓ HOMA-IR; ↑ HDL	↓ WC
Ahmed *et al*[50], 2022	Compared lean with overweight/obese NAFLD patients (*n* = 4834)	8.6%	Females, Asian and African American	↓ HTN, T2DM, hyperlipidemia	Not reported	Not reported
Nabi *et al*[42], 2023	Compared lean with non-lean NAFLD patients (*n* = 25753)	5.3%	< 40 yr, no difference between sexes	↑ Risk of CVD, liver-related events, CKD and all-cause of death	↑ AST	↓ WC
De *et al*[52], 2023	Compared lean with non-lean NAFLD patients (*n* = 1040)	14.3%	No difference between sexes and age	↓ HTN, MetS	No significant difference	↓ Central obesity
Wijarnpreecha *et al*[62], 2023	Compared lean with non-lean NAFLD patients (*n* = 18594)	11.4%	Female, no difference between age	↓ MetS, HTN, T2DM, CKD, cerebrovascular accident	↓ AST, ALT, total cholesterol, LDL and TG, ↑ HDL	Not reported
Biswas *et al*[59], 2023	Compared lean with overweight/obese NAFLD patients (*n* = 1051)	12.1%	< 40 yr, males	↓ HTN	Not difference in ALT and AST	↓ WC

Kawanaka *et al* [57], 2023	Compared lean with non-lean NAFLD patients (n = 782)	11%	> 50 yr, no difference between sexes	↓ HTN	↓ AST, ALT, TG, HOMA-IR, HbA1C	Not reported
Ishido *et al*[61], 2023	Compared lean with non-lean NAFLD patients (n = 581)	Not reported	Males, no difference between age	↓ HTN, T2DM	↓ AST, ALT, TG; ↑ HDL	↓ BMI

NAFLD: Non-alcoholic liver disease; T2DM: Type 2 diabetes mellitus; IR: Insulin resistance; HTN: Hypertension; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; MetS: Metabolic syndrome; HOMA-IR: Homoeostatic model assessment of insulin resistance; HDL: High density lipoprotein; WC: Waist circumference; WHR: Waist to hip ratio; BP: Blood pressure; LDL: Low density lipoprotein; FBG: Fasting blood glucose; BMI: Body mass index; AD: Atherogenic dyslipidemia; TG: Triglyceride; CVD: Cardiovascular disease; CKD: Chronic kidney disease; HbA1C: Hemoglobin A1C.

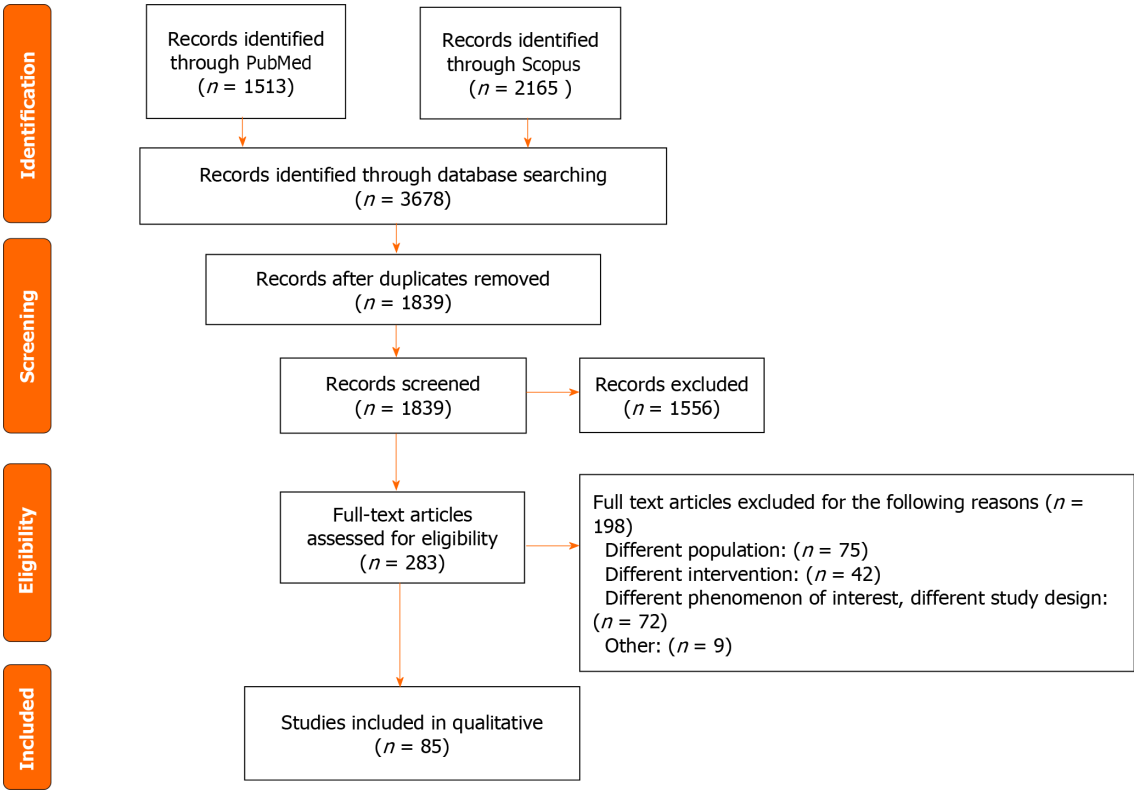

Figure 1 Flow chart of the systematic review. PUBMED: Publication from MEDLINE; Scopus: Society for cutting up of old publications.

indicating lower levels of aspartate aminotransferase (AST)[7,53,57,59,62,63,67], alanine aminotransferase (ALT)[7,53,57,59,62,63,67], platelet count[7,66], FBG[53,58,63], TG[53,57,58,61,62], homeostatic model assessment for insulin resistance (HOMA-IR)[57,63,68], and total cholesterol[57,58,61-63], as well as higher levels of high density lipoproteins (HDL)[56,61-63,66,69]. Regarding anthropometric measurements, the studies reported lower WC[52,56,63,66], BMI[63,70], and waist-to-hip ratio (WHR)[63,70] in lean NAFLD compared to overweight/obese counterparts.

In studies where BMI was compared, lean NAFLD patients exhibited a lower prevalence of comorbidities and a more favorable laboratory profile when compared to overweight or obese patients with NAFLD. Conversely, in studies comparing individuals with and without NAFLD, lean NAFLD patients displayed a worse profile with the highest rates of comorbidities and adverse laboratory values compared to healthy lean individuals without NAFLD. This consideration holds significant importance in the interpretation and application of risk factor concepts in clinical practice. These heterogeneous results underscore the need for regular monitoring in patients who are lean and have NAFLD, given the elevated risk of metabolic diseases compared to those who are lean and do not have NAFLD.

Histological characteristics and diagnosis scores

Patients with NAFLD are at risk of progressing to non-alcoholic steatohepatitis (NASH) and developing other complications[71]. We will now present literature that has evaluated and characterized NASH patients, refer to Table 2 for more details. The most used score in studies diagnosing NASH in patients is the NAFLD Activity Score (NAS), which has been proposed and validated by the NASH Clinical Research Network[72]. This score assesses three characteristics in liver histology: Steatosis Grade, Lobular Inflammation, and Hepatocellular Ballooning. The score ranges from 0 to 8, with a score < 3 correlating with not-NASH, and a score > 5 correlating with a diagnosis of NASH.

Table 2 Non-invasive scores accuracy and histology characteristics in lean patients with non-alcoholic fatty liver disease

Ref.	Population	Results
Leung *et al*[73], 2017	Histology (*n* = 307), lean *vs* obese NAFLD patients	Low NAS (steatosis and hepatocyte ballooning), low stiffness
Denkmayr *et al*[76], 2018	Histology (*n* = 466), lean *vs* overweight/obese NAFLD patients	High lobular inflammation and hepatocellular ballooning
Li *et al*[82], 2019	Scores (*n* = 898), lean *vs* overweight/obese NAFLD patients	WHR and FLI accurate in lean and obese patients, ZJU and HSI accurate in lean patients
Kim *et al*[75], 2019	Histology (*n* = 542), lean *vs* obese NAFLD patients	Low grade steatosis and NAS, high stage of fibrosis
Fu *et al*[81], 2020	Scores (*n* = 709), non-obese *vs* obese NAFLD patients	FIB-4, NFS, APRI, BARD score and AST-to-ALT ratio had similar accurate in obese and non-obese patients
Eren *et al*[79], 2022	Scores (*n* = 560), lean *vs* overweight *vs* severely and morbid obese NAFLD patients	FIB-4 and NFS cannot discriminate advance fibrosis in lean patients
Park *et al*[80], 2023	Scores (*n* = 1501), lean *vs* non-lean NAFLD patients	FIB-4 and NFS accurate in identify advance fibrosis in lean and non-lean NAFLD patients
Iwaki *et al*[74], 2022	Histology (*n* = 223), lean *vs* obese NAFLD patients	Low grade lobular inflammation, steatosis
Rastogi *et al*[77], 2022	Histology (*n* = 1273), lean vs overweight/obese NAFLD patients	High hepatocyte ballooning, early-stage fibrosis

NAFLD: Non-alcoholic liver disease; NAS: NAFLD activity score; WHR: Waist circumference; FLI: Fatty liver index; ZJU: Zhejiang University Index; HSI: Hepatic steatosis index; FIB-4: Fibrosis-4 index; NFS: NAFLD fibrosis score; APRI: AST-to-platelet; BARD: Bilirubin, albumin, INR and ascites; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

Leung *et al*[73] reported that non-obese patients with NASH exhibited lower NAS due to reduced steatosis and hepatocyte ballooning, along with lower liver stiffness. Furthermore, Iwaki *et al*[74] observed a low grade of lobular inflammation and fibrosis stage, with no significant differences in steatosis, ballooning, and overall NAS in non-obese compared to obese patients. Additionally, Kim *et al*[75] found that lean patients displayed a low grade of steatosis and overall NAS, but a higher stage of fibrosis compared to their obese counterparts with NAFLD.

On the contrary, Denkmayr *et al*[76] identified a higher proportion of lobular inflammation and hepatocellular ballooning, with a notable prevalence of cirrhosis in lean patients. However, the degree of steatosis was similar across the groups. Also, Rastogi *et al*[77] found a high proportion of hepatocyte ballooning but a high prevalence in none/early-stage fibrosis.

The results of histology in different studies are inconclusive. They indicate that histological characteristics could vary, showing either worse or better outcomes in lean *vs* overweight or obese individuals. However, this emphasizes the importance of careful evaluation for lean patients, similar to the rest of the population. These contradictory results may be influenced by the different types of patients undergoing liver biopsy. Leung, Kim, and Denkymar assessed histology in the following types of patients: those exhibiting abnormal liver enzyme levels, those with suspected NAFLD, and those with a confirmed diagnosis of NAFLD through non-invasive tools. In contrast, Iwaki examined the histology in a tertiary center where referrals were received, particularly for patients with more severe liver conditions. Moreover, the differences in study designs, including prospective, retrospective, and cross-sectional approaches, complicate the comparison of results. A limitation noted across all the studies was the relatively small sample size in the lean group compared to the overweight/obese groups.

In the context of interpreting non-invasive tools in lean patients with NAFLD or NASH, a critical consideration is the selection of the most suitable scoring system or algorithm for clinical application. We will now present literature that has evaluated accuracy of those scores, refer to Table 2 for more details.

The accuracy of FIB-4 and NFS was compared in patients who underwent liver biopsy[78]. FIB-4 assessed age, levels of AST, ALT, and platelets, while NFS considered age, BMI, impaired fasting glucose or diabetes, levels of AST, ALT, platelets, and albumin. In a study by Eren *et al*[79], it was observed that both FIB-4 and NFS were ineffective in discriminating against advanced fibrosis in both lean and morbidly obese patients. Contrastingly, a study by Park *et al*[80] revealed that the diagnostic performance of FIB-4 and NFS in identifying advanced hepatic fibrosis was comparable, irrespective of BMI. The sensitivity of NFS in lean patients was inferior to that of FIB-4. In addition to comparing FIB-4 and NFS, Fu *et al*[81] included AST-to-platelet, BARD score, and the AST-to-ALT ratio in the comparison. They found that all non-invasive scores performed equally for both obese and non-obese patients. The negative predictive value (NPV) was higher in non-obese patients due to the lower prevalence of advanced fibrosis. Moreover, Li *et al*[82] compared 8 NAFLD-related algorithms, finding that WHR and Fatty Liver Index exhibited diagnostic accuracy for NAFLD in both lean and overweight/obese populations, but Zhejiang University Index and Hepatic Steatosis Index demonstrated exclusively positive associations in lean patients.

In summary, the review of accuracy and performance across different non-invasive tools in patients with NAFLD revealed that FIB-4 outperformed NFS in this specific population. However, it is crucial to note that this result was observed in only one study. Nonetheless, this finding does hold significance, considering that the only clinical guideline for lean MASLD recommends FIB4 and NFS equally. Thus, it is imperative that new studies compare these non-invasive

tools in patients with MASLD due to the updated guidelines.

Treatment

Clinical trials were conducted to explore potential treatments for NAFLD. In the literature reviewed, we found two types of treatment: pharmacological and non-pharmacological.

Pharmacological: In a one-year follow-up study involving 8 lean patients with NAFLD, half received ursodeoxycholic acid, and the other half received 10 mg of the Niemann-Pick C1 Like 1 (NPC1L1) inhibitor, ezetimibe. The findings revealed that patients treated with ezetimibe for 12 months experienced decreased levels of AST and low-density lipoprotein, but no significant changes were observed in HDL, TG, HOMA-IR, or liver fat attenuation in abdominal US [83]. In another study involving 50 patients, 25 received a synbiotic capsule, and 25 received a placebo capsule. Both groups received advice on maintaining a balanced diet and engaging in physical activity. After 28 wk of treatment and follow-up, both groups exhibited reduced hepatic steatosis and inflammatory markers, with the synbiotic group having a higher mean reduction in FBS, TG, and AST[84].

Pemafibrate, a selective peroxisome proliferator-activated receptor-αmodulator, dosed at 0.1 mg twice daily was studied. The first study by Shinozaki *et al*[85] treated 71 patients for 6 months, finding that lean patients experienced a greater reduction in ALT and serum mac-2 binding protein glycosylation isomer than obese patients. The second study by Suzuki *et al*[86] treated 38 patients for 12 months and found a strong association in the decrease of ALT, AST, hepatic steatosis, and fibrosis in both lean and obese patients. Canagliflozin at a dosage of 100 mg once daily was evaluated in 20 patients with T2DM and NAFLD, but due to only one patient being lean, the results were inconclusive in this population [87].

Various pharmacological treatments and interventions have been investigated in patients with lean MASLD, demonstrating some degree of efficacy in improving the metabolic profile or reducing hepatic steatosis. However, longitudinal clinical trials with large study populations are still warranted to identify a promising drug for treating both lean MASLD and MASH. On the other hand, the literature supports that lifestyle modification is an effective therapy in lean patients with MASLD, similarly to overweight/obese patients.

Non-pharmacological: Lifestyle changes such as exercise and diet modification were evaluated in lean patients with NAFLD. Jin *et al*[88] followed patients for 14 years and found a reduction in hepatic steatosis, total cholesterol levels, and body weight. Wong *et al*[89] followed patients for 12 months and found that 50% of non-obese patients achieved NAFLD remission with a 3%-5% weight reduction, which was maintained over 6 years of follow-up. However, 50% of the obese group achieved remission with a higher percentage of weight loss (7%-10%). Hamurcu *et al*[90] and Sinn *et al*[91] found a decrease in body weight and hepatic steatosis, as well as improvement in anthropometric parameters in both lean and obese patients.

Outcomes/prognosis

A retrospective study compared post-transplant outcomes in lean and obese patients with NASH from the United Network for Organ Sharing (UNOS)[92]. The study concluded that lean individuals experienced lower survival rates and graft survival at 10 years follow up compared to their obese counterparts. Although no distinguishable trends in the cause of death based on BMI were identified, early multiorgan failure was more prevalent in lean patients[92]. A recent retrospective study including NAFLD patients of the UNOS, found that patients with normal weight and who maintained a stable weight during the wait period for a liver transplant had a worse survival rate than patients with stable obesity during this period at 3 and 5 years. Also, patients with stable normal weight compared to stable obese, had high risk of all-cause mortality and graft failure[93].

Overall, the findings of these studies reveal a poorer survival rate and graft failure in lean patients compared to their overweight/obese counterparts. However, this may have been influenced by the baseline conditions of these individuals. For example, conditions such as sarcopenia, which demonstrated a strong correlation in lean patients[9-12], were not assessed in these studies due to the exclusive consideration of BMI rather than skeletal muscle mass. Sarcopenia could serve as a potential contributor to the worse prognosis in lean patients. Another factor highlighted in the study is that lean patients exhibited a higher rate of ascites and worse functional status, necessitating total assistance. These factors could potentially explain the heightened risk of complications during and post liver transplant. While these variables could explain the worse outcomes in lean patients, there remains a gap in knowledge concerning the exact reasons underlying the adverse outcomes. Further research is needed to elucidate the specific mechanisms and factors that contribute to the observed disparities in transplantation outcomes between lean and overweight/obese patients.

CONCLUSION

MASLD is a complex disease that comprised of epigenetic, genetic, and metabolic factors in its pathogenesis. The prevalence varies among populations, ranging from approximately 4% to 34%. The current literature reveals disparities in sex and age, with older male patients being the most at-risk group. Furthermore, when metabolic conditions were examined in lean patients with NAFLD *vs* without NAFLD, lean patients with NAFLD were associated with a higher prevalence of metabolic diseases and a worse metabolic profile. However, when BMI was compared among NAFLD patients, lean patients showed a lower prevalence of metabolic disease, a better metabolic profile, but in some cases, worse histologic results with advanced fibrosis. In evaluating the accuracy and performance of non-invasive tools for diagnosing steatotic liver disease in this population, FIB-4 appears to be the most ideal score to use. Regarding prognosis

and outcomes, lean patients with NAFLD have a better metabolic profile and clinical characteristics than overweight/obese patients. However, lean NAFLD patients experience a higher mortality rate, primarily due to cardiovascular disease or all-cause mortality, and faster progression to advanced liver disease. It is important to note that metabolic diseases were a significant variable in past studies of NAFLD patients, indicating that the new concept of MASLD that includes cardiometabolic risk criteria provides a more accurate diagnosis for patients with liver steatosis. Future studies utilizing this new nomenclature can contribute to standardizing and generalizing study results among lean patients with steatotic liver diseases.

ARTICLE HIGHLIGHTS

Research background

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the new nomenclature of non-alcoholic fatty liver disease (NAFLD) and metabolic dysfunction-associated fatty liver disease (MAFLD). It is a complex condition, and its mechanism is poorly understood. There are several studies involving overweight/obese patients but there is very limited literature available regarding lean patients.

Research motivation

Only one clinical guideline is available for physicians to diagnosis and manage lean patients with MASLD. However, the pathogenesis, accurate treatment, risk factor and outcomes remain unknown.

Research objectives

The aim of this systematic review is to report literature of diagnosis, pathogenesis, characteristics, and prognosis in lean MASLD patients in diverse populations, and provide an interpretation of the new MASLD criteria.

Research methods

A search on two large databases was conducted, PubMed and Google Scholar, selecting original articles published between January 2012 and September 2023 specifically focusing on lean NAFLD, MAFLD, or MASLD patients.

Research results

85 articles met the eligibility criteria and underwent further analysis. The prevalence of lean MASLD among diverse populations ranges from 4% to 34%. The pathogenesis of lean MASLD involves genetic, epigenetic, and metabolic factors; however, the mechanism remains elusive. Although adequate treatment remains challenging to identify, lifestyle modifications have proven effective in reducing hepatic steatosis and improving cardiometabolic profiles. Some medications have shown efficacy to a lesser extent.

Research conclusions

MASLD is a complex condition that requires attention, especially in lean patients. Risk factors and metabolic conditions are associated with this condition independently of BMI. Therefore, investigations aimed at decreasing the risk of future complications, such as cirrhosis or the development of hepatocellular carcinoma in lean MASLD patient, are necessary with the same relevance as in overweight/obese counterparts.

Research perspectives

Future studies using this new nomenclature of MASLD can contribute to standardizing and generalizing study results in lean patients with steatotic liver diseases. It is also important to take into consideration other values, such as muscle mass or waist circumference and not only BMI, to make a more accurate evaluation of the lean patients.

FOOTNOTES

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META-ANALYSIS

Influence of nonalcoholic fatty liver disease on response to antiviral treatment in patients with chronic hepatitis B: A meta-analysis

Shi-Yi Liu, Dian Wang, Jing Liu, Lu-Ping Yang, Gong-Ying Chen

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Abstract

BACKGROUND

Although hepatitis B virus infection is the leading cause of chronic liver injury globally, nonalcoholic fatty liver disease (NAFLD) is gradually gaining attention as another major chronic liver disease. The number of patients having chronic hepatitis B (CHB) with concomitant hepatic steatosis has increased.

AIM

To analyze the effect of NAFLD on the response to antiviral treatment in patients with CHB.

METHODS

Relevant English studies were systematically searched across PubMed, EMBASE, Web of Science, and Cochrane Library until October 2023. Studies in which the treatment outcomes were compared between patients with CHB only and those with CHB and hepatic steatosis were included.

RESULTS

Of the 2502 retrieved studies, 11 articles were finally included. Biochemical response until 48 wk (OR = 0.87, 95%CI: 0.50–1.53, P = 0.000) and 96 wk (OR = 0.35, 95%CI: 0.24–0.53, P = 0.24) and virological response until 96 wk (OR = 0.80, 95%CI: 0.43–1.49, P = 0.097) were lower in patients with hepatic steatosis than in patients with CHB alone.

CONCLUSION

Hepatic steatosis lowers the biochemical response to antiviral treatment in patients with CHB.

Key Words: Nonalcoholic fatty liver disease; Hepatitis B virus; Antiviral treatment; effect; Meta-analysis

Core Tip: No consensus is available in the literature about which effect of nonalcoholic fatty liver disease on the response to antiviral treatment in patients with chronic hepatitis B (CHB). This is a systematic review and meta-analysis comparing the response to antiviral treatment between patients with CHB only and those with CHB and hepatic steatosis were included. We investigated these two groups in terms of biochemical responses, serological responses, virological responses the incidence of hepatocellular carcinoma.

Citation: Liu SY, Wang D, Liu J, Yang LP, Chen GY. Influence of nonalcoholic fatty liver disease on response to antiviral treatment in patients with chronic hepatitis B: A meta-analysis. *World J Hepatol* 2024; 16(3): 465-476
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INTRODUCTION

Chronic hepatitis B (CHB) infection is an important disease globally, particularly in Asia. Epidemiological data indicate that nearly 400 million people have CHB worldwide[1]. If left untreated, approximately one-third of these patients progress to severe end-stage liver diseases, which manifest as liver failure, cirrhosis, and hepatocellular carcinoma (HCC). Therefore, antiviral therapy is crucial for the clinical management of CHB. The currently available antiviral drugs, such as nucleoside/nucleotide analogs (NAs) and interferons (IFNs), can reduce the progression of liver disease, thereby improving the long-term outcomes in CHB patients[2].

Nonalcoholic fatty liver disease (NAFLD) is a common clinicopathologic condition characterized by lipid deposition without or with inflammation in hepatocytes. NAFLD comprises a wide spectrum of liver damage, including simple steatosis, nonalcoholic steatohepatitis, and fibrosis[3]. In recent years, owing to the epidemic of obesity and lifestyle changes, NAFLD has become a common chronic liver disease. The worldwide prevalence of NAFLD in the adult population has been reported to be approximately 25%[4-6]. Hence, there is a surge in patients having CHB with NAFLD. Moreover, the complexity of liver disease has increased, which poses new challenges in clinical diagnosis and treatment. In this scenario, a specific antiviral strategy is warranted for patients having CHB with NAFLD.

Considering the several conflicting observations in the literature on the effect of NAFLD in patients with CHB who are under antiviral treatment, a meta-analysis was conducted to explore the impact of NAFLD on the treatment response in antiviral-treated patients with CHB.

MATERIALS AND METHODS

This study was conducted and reported in accordance with the preferred reporting items for systematic reviews and meta-analyses statement[7] and in accordance with the meta-analysis of observational studies in epidemiology guidelines for the meta-analysis of observational studies.

Search strategy and study selection

A systematic search was conducted across PubMed, EMBASE, Web of Science, and Cochrane Library databases for articles published until October 2023. The following keywords were used in the search: "chronic hepatitis B" or "hepatitis B antigens" or "hepatitis B virus" or "hepatitis B, chronic"; "fatty liver" or "hepatic steatosis" or "NAFLD"; "antiviral agents" or "nucleoside" or "peginterferon." Furthermore, the reference lists of key articles were manually and independently reviewed. The potentially eligible studies were reviewed entirely following selection from the initial search.

Selection criteria

A total of 11 studies were screened for relevance based on the title, abstract, and entire manuscript. In this study, studies that included patients with CHB and NAFLD with CHB who underwent antiviral treatment (including NAs and IFNs) for at least 96 wk were assessed. Articles were excluded if their subjects were under the age of 18 years if they did not have the reported outcomes, if they did not contain usable primary data, or if they did not setting CHB complicated fatty liver patients. Each article was reviewed by two investigators independently (Liu SY and Wang D). Data were extracted from studies meeting both the inclusion and exclusion criteria following the review of the entire contents of each paper. Any differences were resolved by a third investigator (Chen GY), discussion, or revision.

Data extraction and quality assessment

Data were extracted independently by two authors, and any discrepancies were resolved *via* consensus. The following information was extracted from each trial: publication details (title, first author, and place of the study), study design (inclusion and exclusion criteria), participant details (number of patients enrolled and their age), intervention details (including type and dose of IFNs, NAs, and mode of administration), duration of treatment, follow-up, and outcomes. Quality assessment of the included studies was performed by two authors using an improved Newcastle–Ottawa Scale (the Newcastle–Ottawa Scale for assessing the quality of nonrandomized studies in meta-analyses). Studies that scored ≥ 9 points were deemed to be of high quality and those with 5–8 points and < 5 points were deemed to be of moderate and low quality, respectively. The risk of bias was rated independently by two authors (Liu SY and Wang D).

Outcome definition

The following outcomes were included in the study, biochemical response [time taken for the alanine aminotransferase (ALT) level to return to normal], virological response [time taken for the hepatitis b virus (HBV) DNA to become undetectable], serological response [time taken for the disappearance of hepatitis B e-antigen (HBeAg) and the appearance of anti-HBe], and the incidence of HCC.

Statistical analysis

Heterogeneity between individual studies was assessed by using the I^2 test. The random-effects model was selected a priori due to the anticipated heterogeneity of the included studies. A value of ≥ 75% was considered indicative of substantial heterogeneity, ≥ 50 as moderate heterogeneity, ≥ 25% as mild heterogeneity, and < 25% as the absence of heterogeneity. Publication bias was assessed by constructing a funnel plot of each study's effect size against the standard error. Funnel plot asymmetry was evaluated using Egger's test, and $P < 0.1$ was defined as having a significant publication bias. The Stata 16.0 software was employed for all analyses.

RESULTS

Search results and characteristics of the included studies

During the initial literature search, a total of 2502 articles were retrieved, of which 533 were eliminated because of duplication. After a careful review of the remaining 1969 titles and abstracts, 617 were excluded because they were not published as full reports (like conference abstracts or letters to the editor); 49 were excluded as they involved animal or cellular experiments; 80 were excluded because they only included patients with CHB not complicated by fatty liver; 615 were excluded because of a lack of correlation; and 517 were excluded due to the presence of other diseases. After a careful review of the 91 full-text articles, 37 were excluded for data insufficiency and 43 because of the absence of correlation. Finally, a total of 11 articles were included in the meta-analysis (Figure 1)[8-18].

General information pertaining to the included studies is presented in Table 1. Of these studies, one was conducted in Turkey[8],six in China[9-12,16,18], three in Korea[13-15], and one in the United States[17]. All studies were published in English. Patients were treated with IFN-α in three studies[8-10] and NAs in eight studies[9,11-17]. Three trials comprised a 48-week IFN-α treatment[8,10,12], with two involving a 48-week follow-up[1,3] and one involving a 96-week follow-up [18]. One trial included NAs and a 24-week follow-up[12], and seven trials included NAs and a > 48-week follow-up[9,11-17]. Five studies were prospective cohort studies[8-11,18], whereas the other six were retrospective cohort studies[12-17].

Baseline data, including ALT, aspartate aminotransferase, gamma-glutamyl transferase, total cholesterol, triglyceride, total bilirubin, albumin, high-density lipoprotein, low-density lipoprotein, and glucose, are shown in Table 1.

Biochemical responses

Six trials[9-12,17,18] had a combined study population of 459 patients having CHB plus steatosis and 695 patients had only CHB reported data on biochemical responses until 48 wk. The result is shown in Figure 2A. Moderate substantial heterogeneity was observed among these studies (I^2 = 60.7%, P = 0.026), and a random-effects model was applied for the analysis. Patients with CHB plus steatosis demonstrated a lower rate of biochemical response until 48 wk when compared to those with only CHB [odds ratio (OR) = 0.43, 95%CI: 0.28–0.77, P = 0.03, Figure 2A].

Six studies[9-12,17,18] reported data on biochemical response until 96 wk, which displayed heterogeneity (I^2 = 25.9%, P = 0.024). The P value indicated a significantly lower sustained biochemical response in patients with CHB and steatosis than in those with only CHB (OR = 0.35, 95%CI: 0.24–0.53, P = 0.47, Figure 2B).

Virological responses

Seven studies[8-12,17,18] reported data on virological response until 48 wk. Substantial heterogeneity was noted (I^2 = 75.6%, P = 0.000), and the random effects model was applied. No significant between-groups difference was observed with respect to the sustained virological response until 48 wk (OR = 0.87, 95%CI: 0.50–1.53, P = 0.112, Figure 3A).

Data on virological response until 96 wk was available for seven trials[8-12,17,18]. The P value indicated no significant between-group difference (OR = 0.80, 95%CI: 0.43–1.49, P = 0.097). Heterogeneity was observed among these studies (I^2 = 75.90%, P = 0.000), and the random-effects model was applied (Figure 3B).

Table 1 The basic characteristics of the enrolled studies included in this meta-analysis

Ref.		Ateş *et al*[8]	Jin *et al*[9]	Gong *et al*[10]	Liu *et al*[11]	Zhu *et al*[12]	Kim *et al*[13]	Cho *et al*[14]	Lee *et al*[15]	Chen *et al*[16]	Li *et al*[17]	Liang *et al*[18]
Year		2011	2012	2015	2015	2016	2019	2019	2019	2020	2020	2021
Location		Turkey	China	China	China	China	Korea	Korea	Korea	China	United States	China
Study		Prospective study	Prospective study	Prospective study	Prospective study	Retrospective study	Retrospective study	Retrospective study	Retrospective study	Retrospective study	Retrospective study	Prospective study
Journal		*World J Gastroenterol*	*PLoS One*	*Transplant Proc*	*J Interferon Cytokine Res*	*Drug Des Devel Ther*	*Clin Mol Hepatol*	*J Clin Gastroenterol*	*Clin Mol Hepatol*	*BMC Gastroenterol*	*Liver Int*	*Gastroenterol Res Pract*
Therapy regimen		PEG-IFN	Nucleos(t)ide analogues	PEG-IFN	Nucleos(t)ide analogues	Nucleos(t)ide analogues	Nucleos(t)ide analogues	Nucleos(t)ide analogues	Nucleos(t)ide analogues	Nucleos(t)ide analogues	Nucleos(t)ide analogues	PEG-IFN
Male	**CHB with NAFLD**	95	32	20	23	42	91	171	50	43	126	95
	CHB	82	85	38	12	41	119	333	146	43	211	82
Famale	**CHB with NAFLD**	12	33	11	17	19	69	89	20	13	61	12
	CHB	3	63	20	8	23	69	233	105	13	157	37
Age	**CHB with NAFLD**	50.5 ± 8.7	39.56 ± 11.87	30.3 ± 7.4	37.70 ± 7.90	39.26 ± 10.39	51.0 (42.0-56.3)	52.0 ± 9.4	45 (36–51)	39 (31-34)	47.7 ± 12.5	20.87 ± 1.96
	CHB	35.2 ± 8.9	39.55 ± 7.83	25.1 ± 7.8	37.35 ± 8.49	39.61 ± 10.87	51.0 (43.3-57.0)	54.0 ± 8.8	41 (32–48)	38.5 (31–44)	45.5 ± 14.6	29.50 ± 5.47
BMI	**CHB with NAFLD**	32.9 ± 3.1	26.35 ± 4.19	25.3 ± 2.1	28.16 ± 1.43	26.29 ± 3.99	24.7 (22.3-26.7	24.5 ± 3.5	NA	25.0 ± 3.0	25.4 ± 4.3	NA
	CHB	25.7 ± 3.3	24.26 ± 3.41	22.5 ± 2.9	22.06 ± 1.02	22.50 ± 2.85	22.5 (20.4-24.4)	23.8 ± 3.2	NA	23.0 ± 3.0	23.8 ± 4.0	20.87 ± 1.96
ALT	**CHB with NAFLD**	128.3 ± 18.9	143.3 ± 82.1	171.68 ± 46.23	227.70 ± 121.14	179.87 ± 78.50	56 (38-94)	NA	71 (32–114)	99 (68-154)	60 (15 - 1525)	NA
	CHB	139.2 ± 52.5	157.1 ± 108.3	159.18 ± 45.12	229.95 ± 137.36	187.95 ± 79.88	56 (34-90)	NA	94 (45–171)	124 (79–216)	50 (14 - 1079)	257.39 ± 175.17
AST	**CHB with NAFLD**	90.7 ± 34.8	70.2 ± 33.7	59.66 ± 13.81	183.23 ± 103.70	NA	NA	NA	53 (36–86)	61 (38-84)	37 (13 - 1465)	NA
	CHB	107.0 ± 40.3	93.5 ± 72.3	56.63 ± 13.13	167.60 ± 85.07	NA	NA	NA	85 (51–153)	72 (45–137)	35 (12 - 863)	126.72 ± 79.55
GGT	**CHB with NAFLD**	49.8 ± 29.8	95.2 ± 73.2	42.92 ± 14.83	98.75 – 19.90	NA	NA	NA	NA	NA	NA	NA
	CHB	50.0 ± 44.0	77.2 ± 89.7	46.05 ± 11.36	51.41 – 5.46	NA	NA	NA	NA	NA	NA	NA
TB	**CHB with NAFLD**	NA	NA	NA	NA	NA	0.8 (0.7-1.1)	1.0 ± 0.4	NA	0.8 (0.6-1.0)	1.3 ± 3.0	NA

	CHB	NA	NA	NA	NA	NA	0.9 (0.7-1.2)	1.1 ± 0.4	NA	1.0 (0.7–1.2)	1.3 ± 2.7	NA
ALB	**CHB with NAFLD**	NA	NA	NA	NA	NA	4.2 (4.0-4.4)	4.18 ± 0.3	4.2 (3.8–4.4)	NA	4.0 ± 0.6	NA
	CHB	NA	NA	NA	NA	NA	4.2 (3.9-4.4)	4.25 ± 0.4	4.0 (3.7–4.3)	NA	3.9 ± 0.6	NA
TC	**CHB with NAFLD**	192.2 ± 28.0	84.6 ± 14.4	80.28 ± 7.92	115.02 ± 9	68.04 ± 1.26	178 (151-204)	NA	176 (155–203)	NA	181 ± 40	NA
	CHB	178.0 ± 27.4	81.0 ± 14.4	78.66 ± 5.76	81.18 ± 8.1	66.96 ± 17.46	170 (151-190)	NA	159 (137–179)	NA	177 ± 41	0.56 ± 0.45
TG	**CHB with NAFLD**	188.3 ± 52.0	34.2 ± 14.4	27.54 ± 6.84	48.06 ± 10.98	32.4 ± 16.2	NA	NA	103 (81–137)	NA	189 ± 153	NA
	CHB	114.2 ± 44.2	21.6 ± 9	19.98 ± 7.02	25.92 ± 2.88	23.94 ± 11.52	NA	NA	80 (63–109)	NA	86 ± 42	4.68 ± 0.80
HDL	**CHB with NAFLD**	NA	NA	NA	NA	15.48 ± 6.12	NA	NA	44.4 (35.4–54.4)	NA	40 ± 9	NA
	CHB	NA	NA	NA	NA	19.26 ± 6.3	NA	NA	48.3 (39.4–59.4)	NA	58 ± 19	NA
LDL	**CHB with NAFLD**	NA	NA	NA	NA	41.58 ± 12.96	113 (93-129)	NA	105 (87–129)	NA	111 ± 37	NA
	CHB	NA	NA	NA	NA	38.88 ± 12.24	101 (80-126)	NA	90 (75–108)	NA	106 ± 32	NA
Glucose	**CHB with NAFLD**	102.7 ± 27.7	4.8 ± 0.6	5.46 ± 1.37	NA	5.11 ± 0.85	96.0 (89.3-107.0)	NA	103 (92–117)	NA	115 ± 37	NA
	CHB	96.7 ± 19.0	4.5 ± 0.5	5.07 ± 0.92	NA	4.89 ± 0.80	9 4.0 (87.3-102)	NA	98 (90–108)	NA	105 ± 26	NA
Quality scores		6	7	7	6	6	7	8	6	7	6	6

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; TC: Total cholesterol; TG: Triglyceride; TB: Total bilirubin; ALB: Albumin; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; NA: Not available.

Serological responses

Six trials[9-12,16,18] examined the serological response to antiviral treatment until 48 wk. No statistically significant heterogeneity was observed among these studies (I^2 = 51.6%, P = 0.067), and the P value showed no significant between-group difference (OR = 0.68, 95%CI: 0.41–1.15, P = 0.51, Figure 4A).

Six studies[9-12,16,18] documented the results of serological response until 96 wk. As no substantial heterogeneity was detected, the random-effects model was applied for the analysis (I^2 = 40.8%, P = 0.1333). The P value showed a significantly lower sustained biochemical response in patients with CHB and steatosis than in those with only CHB (OR = 0.63, 95%CI: 0.40–0.99, P = 0.047, Figure 4B).

Incidence of HCC until 5 years

Three trials[13-15] reported data on the incidence of HCC until 5 years, which showed no heterogeneity (I^2 = 60.7%, P = 0.079). The estimated pooled OR value showed no significant between-group difference (OR = 1.33, 95%CI: 0.85–2.06, P = 0.15, Figure 5).

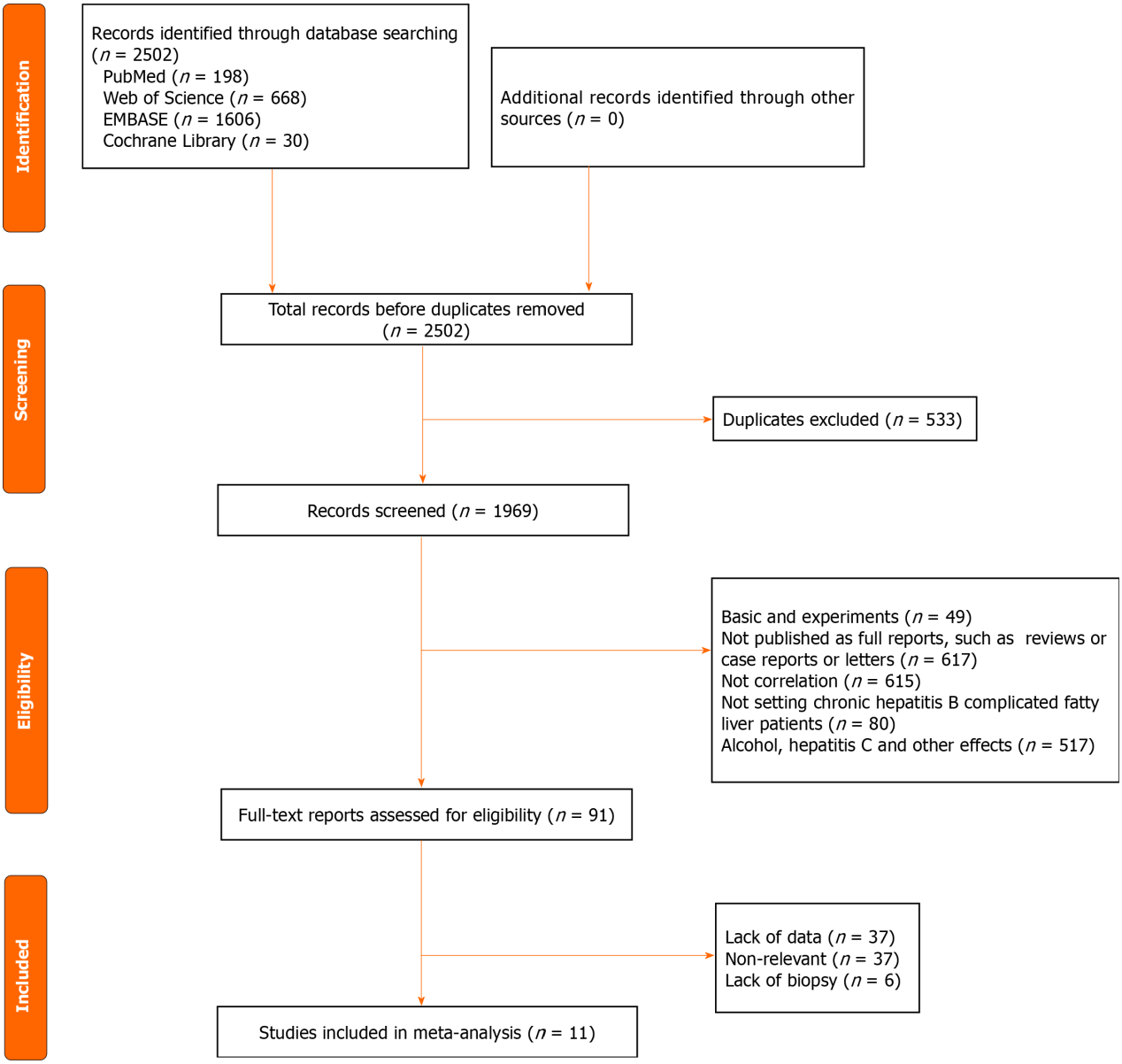


Figure 1 The flow diagram depicting the preferred reporting items for systematic reviews and meta-analyses in study selection.

Subgroup analysis based on the treatment regimens

Three studies[8,10,18] treating patients using IFNs and four studies[7,11,12,17] using NAs achieved a virological response until 48 wk and 96 wk, respectively. Thus, subgroup analysis was performed according to the treatment regimens: NAs or IFNs. Subgroup analysis implied that if patients were treated with NAs, there was no significant difference in the virological response until 48 wk (OR = 0.70, 95%CI: 0.42–1.17, P = 0.80, Figure 3C) and 96 wk (OR = 0.75, 95%CI: 0.45–1.25, P = 0.75, Figure 3D). No significant differences were observed in the virological response until 48 wk (OR = 1.23, 95%CI: 0.39–3.89, P = 0.96, Figure 3C) and 96 wk (OR = 0.85, 95%CI: 0.17–1.25, P = 1.01, Figure 3D) between the two groups if the patients were treated with IFNs.

Publication bias

Funnel plots of publication bias based on biochemical, virological, and serological responses did not demonstrate any obvious asymmetry. The interpretation of these plots was limited by the limited number of studies. Egger's tests for biochemical (P = 0.434), virological (P = 0.328), and serological responses (P = 0.429) until 48 wk were not significant. Egger's tests for biochemical (P = 0.517), virological (P = 0.231), and serological responses (P = 0.985) until 96 wk were also not significant.

DISCUSSION

The incidence of NAFLD is on the rise owing to the increase in the consumption of a fat-rich diet coupled with a

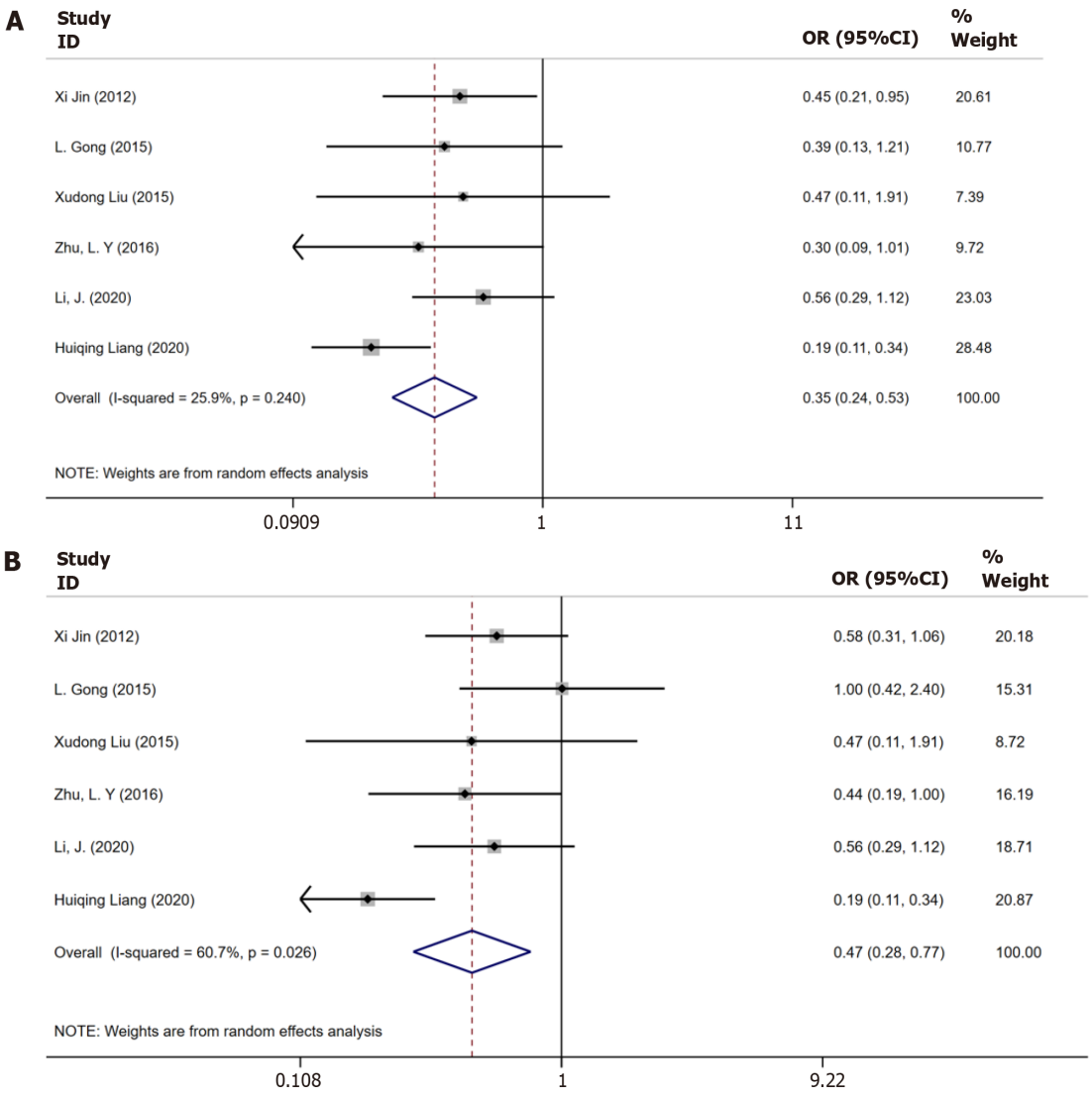
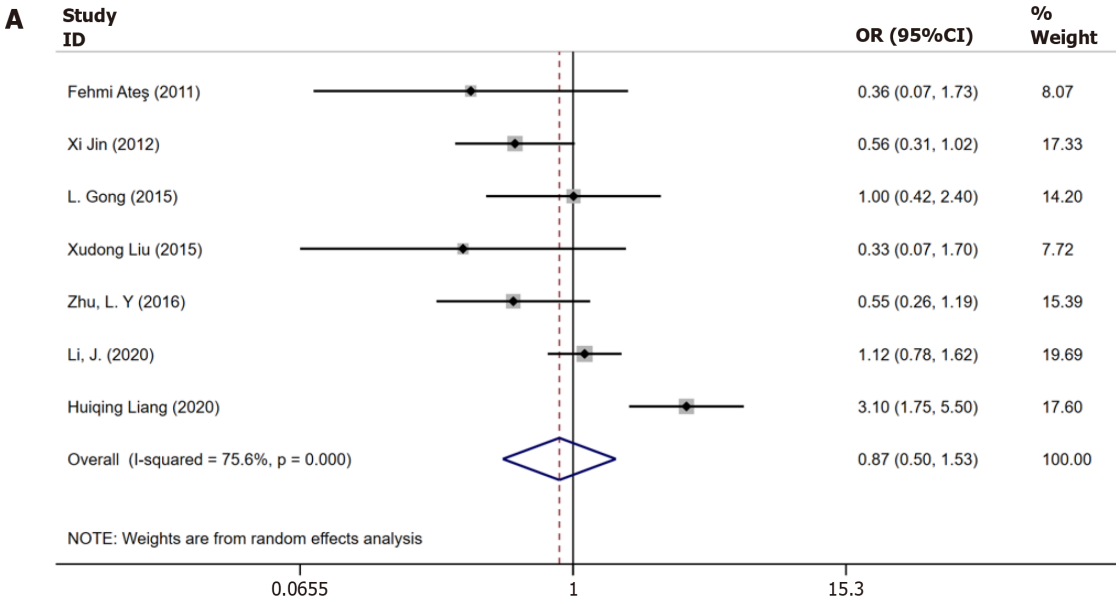


Figure 2 Meta-analysis of the biochemical responses in only chronic hepatitis B patients and in chronic hepatitis B with nonalcoholic fatty liver disease patients. A: Biochemical response in only chronic hepatitis B (CHB) patients and in CHB with nonalcoholic fatty liver disease (NAFLD) patients until 48 wk; B: Biochemical response in only CHB patients and in CHB with NAFLD patients until 96 wk.

sedentary lifestyle. Thus, hepatic steatosis is encountered frequently in patients with CHB. Definitive evidence is not available for the effect of hepatic steatosis on the efficacy of antiviral therapy in patients with CHB. In this meta-analysis, 11 cohort studies published between 2011 and 2020 with a combined population of 1903 patients having CHB plus hepatic steatosis and 1042 patients with only CHB were included. Almost all patients received IFNs or NAs for > 96 wk. In the meta-analysis, hepatic steatosis lowered the biochemical response until 48 wk and 96 wk and serological response until 96 wk to antiviral treatment in patients with CHB. On the contrary, virological responses until 48 wk and 96 wk, serological response until 48 wk, and the incidence of HCC until 5 years were not significantly different in patients with hepatic steatosis than in those without the condition. Our finding signifies that hepatic steatosis lowers the response to antiviral therapy in patients with CHB.

With regard to the biochemical response, hepatic steatosis and inflammation could also cause an elevation in ALT, which may mask the real ALT change caused by HBV activation, thereby resulting in the misclassification of patients with CHB into antiviral therapy[9]. Therefore, suitable criteria for antiviral therapy are required for patients having CHB with hepatic steatosis. Whether NAFLD should be first treated until selecting patients having CHB with NAFLD for anti-HBV therapy is an interesting question that warrants further investigation.

In addition, subgroup analysis was performed based on the treatment regimens. The results indicated that when patients were treated with IFNs or NAs, those with CHB and hepatic steatosis did not exhibit any significant difference from those with only CHB. The outcome of treatment with oral antivirals or interferons appears to be unaffected by HS [19]. Some studies have reported that fat accumulation in hepatocytes may minimize the contact area between drugs and hepatocytes, which can result in a low antiviral response to Nas[20]. Moreover, the declined activity of hepatic cytochromes in steatotic hepatocytes may hamper drug metabolism[21,22]. Concurrently, some studies have indicated the antiviral mechanism of IFNs, which enhanced the antiviral effect by activating the immune cells, which is completely different from that of Nas[10].


A
Study ID
OR (95%CI)
% Weight
Fehmi Ateş (2011) 0.36 (0.07, 1.73) 8.07
Xi Jin (2012) 0.56 (0.31, 1.02) 17.33
L. Gong (2015) 1.00 (0.42, 2.40) 14.20
Xudong Liu (2015) 0.33 (0.07, 1.70) 7.72
Zhu, L. Y (2016) 0.55 (0.26, 1.19) 15.39
Li, J. (2020) 1.12 (0.78, 1.62) 19.69
Huiqing Liang (2020) 3.10 (1.75, 5.50) 17.60
Overall (I-squared = 75.6%, p = 0.000) 0.87 (0.50, 1.53) 100.00
NOTE: Weights are from random effects analysis
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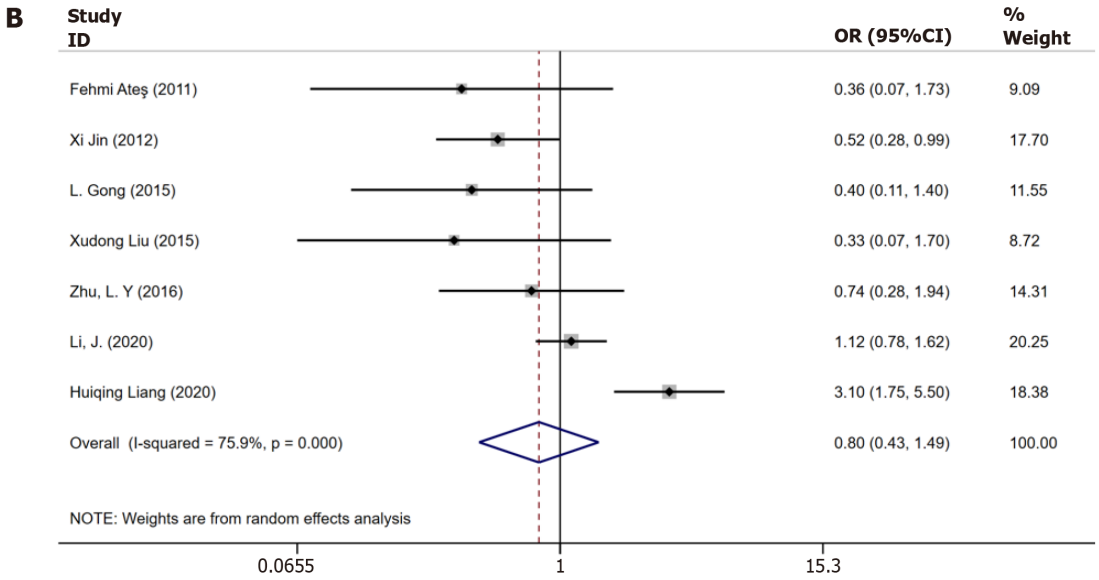
15.3


B
Study ID
OR (95%CI)
% Weight
Fehmi Ateş (2011) 0.36 (0.07, 1.73) 9.09
Xi Jin (2012) 0.52 (0.28, 0.99) 17.70
L. Gong (2015) 0.40 (0.11, 1.40) 11.55
Xudong Liu (2015) 0.33 (0.07, 1.70) 8.72
Zhu, L. Y (2016) 0.74 (0.28, 1.94) 14.31
Li, J. (2020) 1.12 (0.78, 1.62) 20.25
Huiqing Liang (2020) 3.10 (1.75, 5.50) 18.38
Overall (I-squared = 75.9%, p = 0.000) 0.80 (0.43, 1.49) 100.00
NOTE: Weights are from random effects analysis
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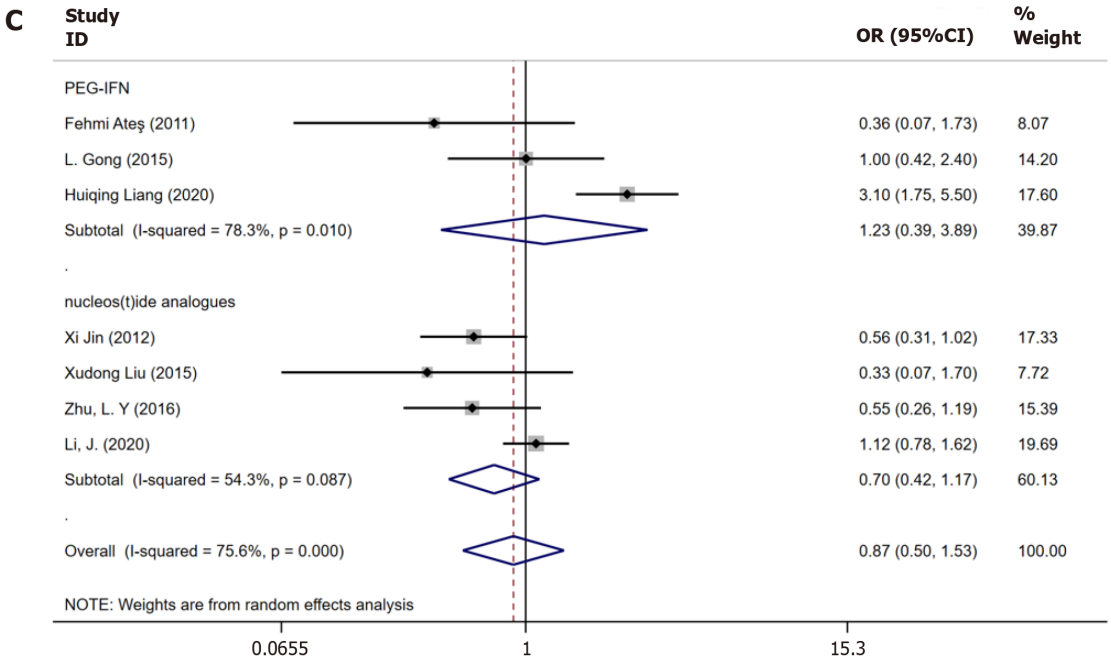
C
Study ID
OR (95%CI)
% Weight
PEG-IFN
Fehmi Ateş (2011) 0.36 (0.07, 1.73) 8.07
L. Gong (2015) 1.00 (0.42, 2.40) 14.20
Huiqing Liang (2020) 3.10 (1.75, 5.50) 17.60
Subtotal (I-squared = 78.3%, p = 0.010) 1.23 (0.39, 3.89) 39.87
nucleos(t)ide analogues
Xi Jin (2012) 0.56 (0.31, 1.02) 17.33
Xudong Liu (2015) 0.33 (0.07, 1.70) 7.72
Zhu, L. Y (2016) 0.55 (0.26, 1.19) 15.39
Li, J. (2020) 1.12 (0.78, 1.62) 19.69
Subtotal (I-squared = 54.3%, p = 0.087) 0.70 (0.42, 1.17) 60.13
Overall (I-squared = 75.6%, p = 0.000) 0.87 (0.50, 1.53) 100.00
NOTE: Weights are from random effects analysis
0.0655
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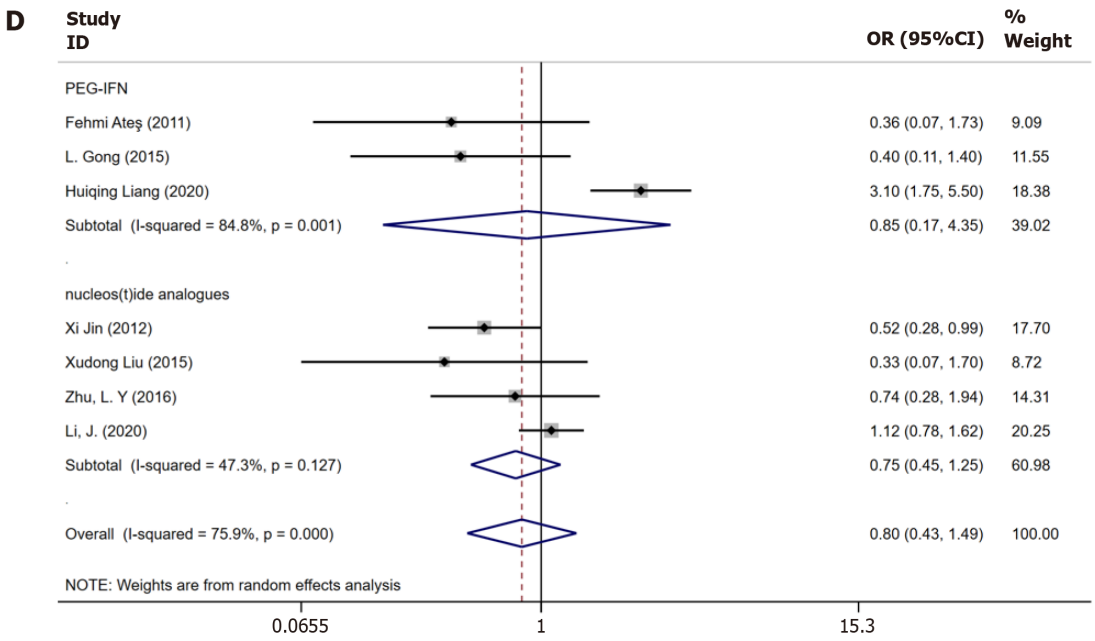

Figure 3 Meta-analysis of virological response in only chronic hepatitis B patients and in chronic hepatitis B with nonalcoholic fatty liver disease patients. A: Virological response in only chronic hepatitis B (CHB) patients and in CHB with nonalcoholic fatty liver disease (NAFLD) patients until 48 wk; B: Virological response in only CHB patients and in CHB with NAFLD patients until 96 wk; C: Subgroup analysis according to the treatment regimens until 48 wk; D: Subgroup analysis according to the treatment regimens until 96 wk.

HBeAg seroconversion is one of the therapeutic goals in patients with HBeAg-positive CHB. Considering the HBeAg seroconversion rate, past studies have shown that NAFLD may affect the rate of long-term serological response in CHB but does not affect the serological response rate during early treatment. In these studies, the degree of hepatic steatosis was unclear in most cases and the duration of therapy was short in patients with an HBeAg-positive status. Hence, drawing a definitive conclusion was difficult.

The findings from this study suggested that the coexistence of fatty liver associated with an increased risk of HCC development in patients with CHB was unclear. Nevertheless, only three trials reported data on the incidence of HCC; therefore, the relationship between hepatic steatosis and HCC warrants further investigations with more numbers of subjects. Notably, some studies suggested that NAFLD promotes HCC development *via* direct and indirect mechanisms. NAFLD not only directly affects hepatocytes but also immensely alters the local microenvironment in the liver and enhances HCC development. Dysregulation of lipid metabolism and accumulation of lipids in the liver causes the selective loss of intrahepatic $CD4^+$ T lymphocytes and results in accelerated hepatocarcinogenesis[15]. Inflammatory cytokines, endoplasmic reticulum stress, and circadian dysregulation mediate hepatocyte injury and progression of NAFLD. Furthermore, reshaped local immune systems with altered microbial metabolites foster a tumor-promoting environment and contribute to NAFLD-mediated hepatocarcinogenesis. The association between HCC in hepatic steatosis and HBV is unclear, but the influence of NAFLD on HCC development may have additive effects in patients with CHB. Although CHB affects the incidence of NAFLD, there is no conclusive evidence linking HS to liver fibrosis, cirrhosis, and HCC in patients with CHB infection. NASH, a severe form of NAFLD, shows a rapid progression in fibrosis, and it is the major cause of liver fibrosis, cirrhosis, and HCC in advanced NAFLD. However, the degree of hepatic steatosis was unclear in most studies, with no reference to NASH. The coexistence of NAFLD may independently increase the risk of HCC development, which is likely to be the same mechanism through which NAFLD alone induces HCC[19].

There are several limitations to this meta-analysis. First, most studies included in the meta-analysis were retrospective, single-center studies. Second, the sample size in certain studies was small. Both factors could have introduced an element of bias and affected the results of the meta-analysis. Hence, more prospective, multicenter observational studies are needed to validate the current findings.

CONCLUSION

Hepatic steatosis lowers the biochemical response to antiviral treatment in patients with CHB. This condition might become a protective factor of disease progression when present in patients affected by HBV. The significant effect of hepatic steatosis on the therapeutic response in patients with CHB should be demonstrated through larger prospective studies.

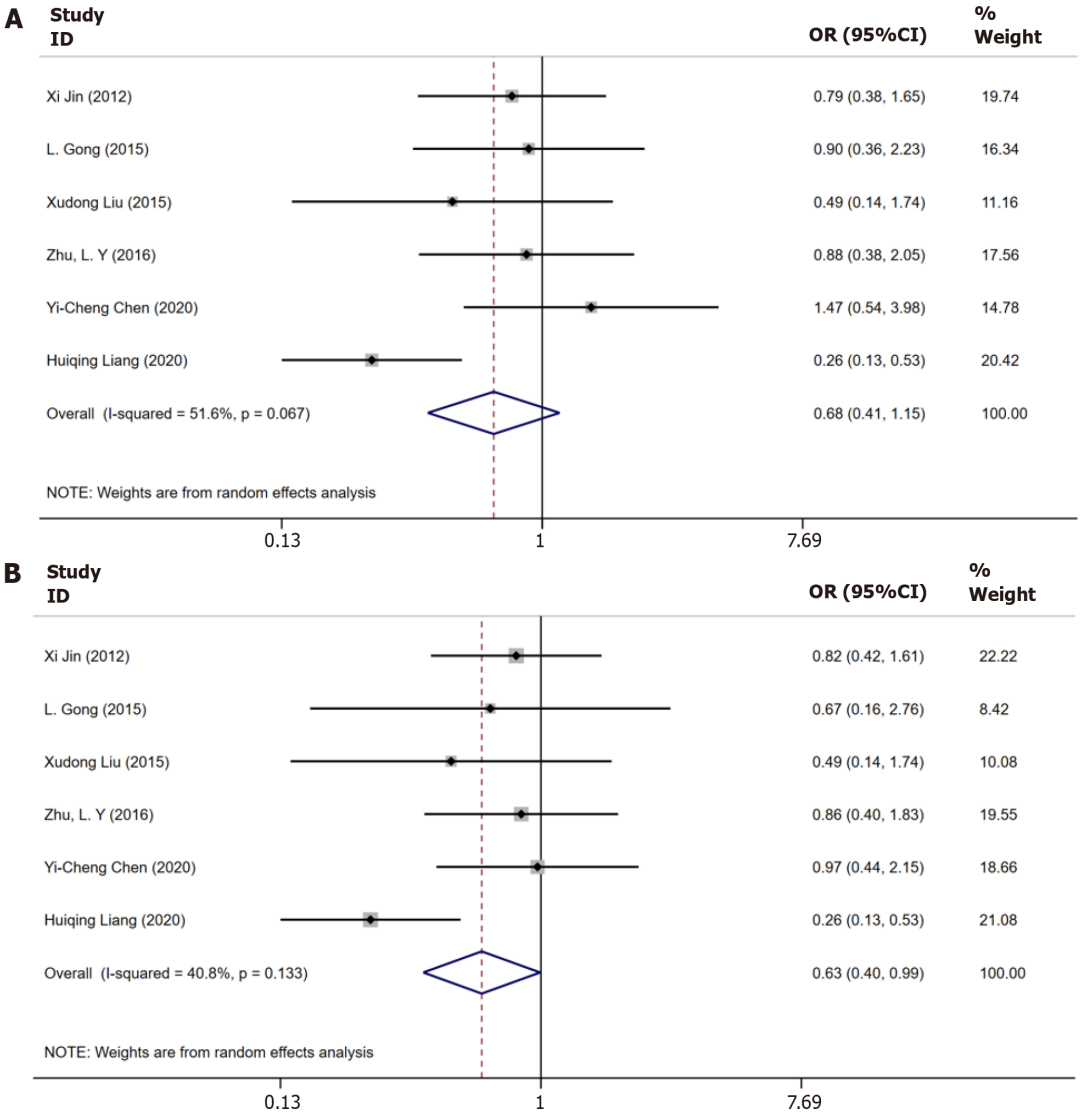

Figure 4 Meta-analysis of the serological responses in only chronic hepatitis B patients and in chronic hepatitis B with nonalcoholic fatty liver disease patients. A: Serological response in only chronic hepatitis B (CHB) patients and in CHB with nonalcoholic fatty liver disease (NAFLD) patients until 48 wk; B: Serological response in only CHB patients and in CHB with NAFLD patients until 96 wk.

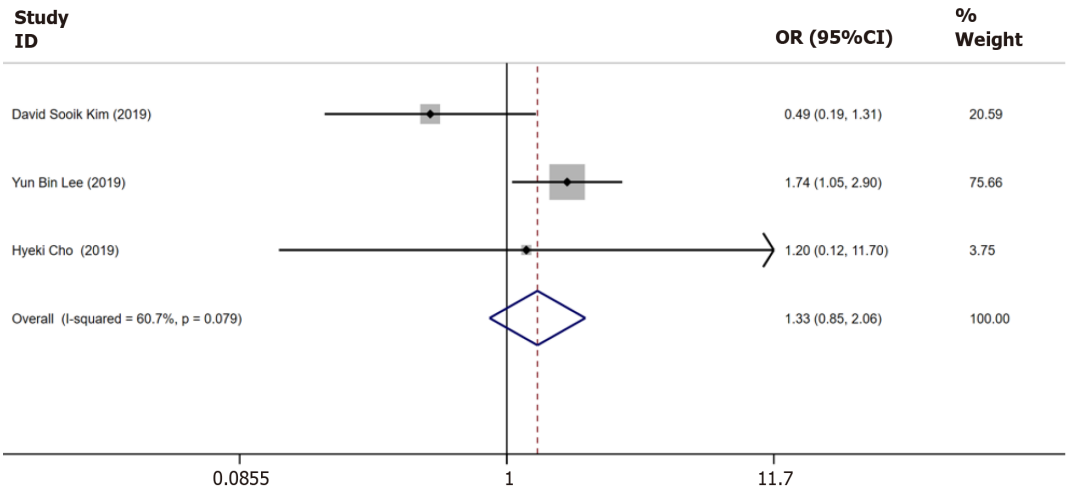

Figure 5 Meta-analysis of the incidence of hepatocellular carcinoma in only chronic hepatitis B patients and in chronic hepatitis B with nonalcoholic fatty liver disease patients.

ARTICLE HIGHLIGHTS

Research background

There is a surge in patients having chronic hepatitis B (CHB) with nonalcoholic fatty liver disease (NAFLD). However, there are several conflicting observations in the literature on the effect of NAFLD in patients with CHB who are under antiviral treatment.

Research motivation

A meta-analysis was conducted to explore the impact of NAFLD on the treatment response in antiviral-treated patients with CHB.

Research objectives

The complexity of liver disease has increased, which poses new challenges in clinical diagnosis and treatment. In this scenario, a specific antiviral strategy is warranted for patients having CHB with NAFLD.

Research methods

This is a systematic review and meta-analysis that compared the response to antiviral treatment between patients with CHB alone and those with CHB and hepatic steatosis. We investigated these two groups in terms of biochemical responses, serological responses, and virological responses to the incidence of hepatocellular carcinoma (HCC).

Research results

In the meta-analysis, hepatic steatosis lowered the biochemical response until 48 wk and 96 wk and serological response until 96 wk to antiviral treatment in patients with CHB. On the contrary, virological responses until 48 wk and 96 wk, serological response until 48 wk, and the incidence of HCC until 5 years were not significantly different in patients with hepatic steatosis than in those without the condition. Our finding signifies that hepatic steatosis lowers the response to antiviral therapy in patients with CHB.

Research conclusions

Hepatic steatosis lowers the biochemical response to antiviral treatment in patients with CHB. This condition might become a hazard factor of disease progression when present in patients affected by HBV.

Research perspectives

The significant effect of hepatic steatosis on the therapeutic response in patients with CHB should be demonstrated through larger prospective studies.

FOOTNOTES

Co-first authors: Shi-Yi Liu and Dian Wang.

Author contributions: Liu SY and Wang D reviewed each article independently; Data were extracted from studies meeting both inclusion and exclusion criteria following a review of the entire contents of each paper; Chen GY as a third investigator, discussion, or revision to resolve any differences.

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META-ANALYSIS

Prognostic value of neutrophil-to-lymphocyte ratio in end-stage liver disease: A meta-analysis

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Abstract

BACKGROUND

The neutrophil-to-lymphocyte ratio (NLR) is commonly utilized as a prognostic indicator in end-stage liver disease (ESLD), encompassing conditions like liver failure and decompensated cirrhosis. Nevertheless, some studies have contested the prognostic value of NLR in ESLD.

AIM

To investigate the ability of NLR to predict ESLD.

METHODS

Databases, such as Embase, PubMed, Web of Science, Cochrane Library, China National Knowledge Infrastructure, Weipu, and Wanfang, were comprehensively searched to identify studies published before October 2022 assessing the prognostic ability of NLR to predict mortality in patients with ESLD. Effect sizes were calculated using comprehensive meta-analysis software and SATAT 15.1.

RESULTS

A total of thirty studies involving patients with end-stage liver disease (ESLD) were included in the evaluation. Among the pooled results of eight studies, it was observed that the Neutrophil-to-Lymphocyte Ratio (NLR) was significantly higher in non-survivors compared to survivors (random-effects model: standardized mean difference = 1.02, 95% confidence interval = 0.67-1.37). Additionally, twenty-seven studies examined the associations between NLR and mortality in ESLD patients, reporting either hazard ratios (HR) or odds ratios (OR). The combined findings indicated a link between NLR and ESLD mortality (random-effects model; univariate HR = 1.07, 95%CI = 1.05-1.09; multivariate HR = 1.07, 95%CI = 1.07-1.09; univariate OR = 1.29, 95%CI = 1.18-1.39; multivariate OR = 1.29, 95%CI = 1.09-1.49). Furthermore, subgroup and meta-regression analyses revealed regional variations in the impact of NLR on ESLD mortality, with Asian studies demonstrating a more pronounced effect.

CONCLUSION
Increased NLR in patients with ESLD is associated with a higher risk of mortality, particularly in Asian patients. NLR is a useful prognostic biomarker in patients with ESLD.

Key Words: Neutrophil-to-lymphocyte ratio; End stage liver diseases; Prognosis; Meta-analysis; Mortality

Core Tip: This meta-analysis examines the association between neutrophil-to-lymphocyte ratio (NLR) and mortality in patients with end-stage liver disease (ESLD). It finds that elevated NLR is correlated with higher risk of death. Specifically, NLR levels were higher in non-survivors than survivors, and high NLR predicted increased mortality risk as indicated by univariate and multivariate hazards ratios and odds ratios. Moreover, NLR had stronger prognostic value in Asian populations, suggesting it may be a useful biomarker for identifying high-risk ESLD patients, particularly in Asia.

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INTRODUCTION

End-stage liver disease (ESLD) is defined as the final stage of liver disease caused by various factors. Globally, cirrhosis and liver cancer are ranked as the eleventh and sixteenth leading causes of death, respectively, accounting for 3.5% of all deaths each year worldwide[1]. The burden of ESLD is expected to increase in the future[2]. Because liver transplantation remains the only curative treatment for ESLD, it is crucial to identify predictors of ESLD prognosis to differentiate between patients who require immediate transplantation and those who can be managed with intensive medical care for a longer period.

The neutrophil-to-lymphocyte ratio (NLR) is a readily measurable parameter that has been shown to reflect disease severity[3]. NLR has been widely used as a biomarker for prognostic evaluation of patients with various diseases and has diagnostic value in distinguishing among certain conditions[4]. For example, NLR has shown promise in predicting poor prognosis in cancer patients[4]. Because Kupffer cells and inflammatory cells, such as macrophages, T lymphocytes, neutrophils, and dendritic cells, have been found to contribute to liver inflammation and fibrosis in patients with liver disease[5], NLR is often utilized as a prognostic factor in these patients. NLR has also been associated with prognosis in patients with hepatocellular carcinoma, suggesting its potential as a prognostic indicator after liver transplantation[6,7]. Moreover, NLR has been used to predict the prognosis of patients with other liver diseases, such as acute-on-chronic liver failure (ACLF) and decompensated liver cirrhosis (DC)[8-10], although the prognostic value of NLR in patients with ACLF and DC remains unclear. Most studies indicate that NLR is linked to poor prognosis in patients with ACLF or DC, although other studies have reported no association[11]. Most of these studies, however, focused solely on patients with ACLF or DC, with few examining whether NLR is a prognostic factor for ESLD, the broader condition.

The objective of this systematic review and meta-analysis was to thoroughly assess the correlation between NLR and prognosis in patients with ESLD. The aim was to identify a reliable and easily measurable parameter that could help identify patients in need of immediate liver transplantation.

MATERIALS AND METHODS

Literature search

Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) 2009 statement guidelines[12] were followed to report the results of this systematic review. The protocol was registered in the Prospective Register of Systematic Review [CRD42022367423].

The databases OVID Embase, PubMed, Web of Science, Cochrane Library, China National Knowledge Infrastructure (CNKI), Weipu, and Wanfang were systematically searched for studies on the associations of NLR with ESLD published from 1 January 1980 to 30 October 2022 in English or Chinese. Search terms included "end-stage liver disease"tOR "liver cirrhosis"rOR "hepatic cirrhosis"rOR "liver fibrosis"bOR "liver failure"iOR "hepatic failure"iOR "liver transplantation"aOR "hepatic transplantation"aOR rliver transplant"aAND "neutrophil-lymphocyte ratio"lOR "neutrophil-to-lymphocyte"tOR ro-lympThe full search strategy is described in Appendix 1.

Study Selection

Studies were selected if they were (1) observational studies, including cross-sectional, cohort, and case-control studies; (2) included adults aged ≥18 years; (3) involved patients who were diagnosed with ESLD; and (4) measured NLR in both survivors and non-survivors or reported a hazard ratio (HR) or odds ratio (OR) reflecting the association between NLR and mortality. Conference abstracts, case reports, systematic reviews, dissertations, expert opinions, and editorials or commentaries were excluded, as were studies that included fewer than 100 participants and studied published in Chinese journals limited to the Chinese Scientific and Technical Papers and Citation Database, the Chinese Science Citation Database, and the Chinese core journal criterion of Peking University. If multiple studies involved the same dataset, the study with the larger number of participants was included. After removing duplicates, two authors (CXH and TYM) independently reviewed the titles and abstracts to remove irrelevant studies. The full texts of the remaining studies were examined with a record of reasons for exclusion. A third author (LXH) resolved disagreements when necessary.

Definition of ESLD

ESLD was defined as chronic or acute-on-chronic liver failure according to the standard criteria of the Asian Pacific Association for the Study of the Liver (APASL)[13] or the European Association for the Study of the Liver[14]. Included were patients with liver cirrhosis who were diagnosed pathologically or by clear ultrasound with at least an index clinical complication of decompensation and candidates for liver transplantation due to liver failure or cirrhosis. Patients aged < 18 years and patients with acute liver failure or other terminal diseases were excluded.

Data extraction

Data were extracted from included articles using a standardized form in Microsoft Excel. Data extracted from these studies included the name of the first author; the year of publication; the location of the study; the number of patients analyzed, as well as their sex and mean or median age; the etiology of ESLD; the mean or median NLR and NLR cutoff value; the primary outcome of the study; and univariate and/or multivariate HRs or ORs, along with their associated 95% confidence intervals (CIs). Two authors (CXH and TYM) independently extracted these daga, with disagreements resolved by consensus.

Evaluation of study quality

Two authors (CXH and TYM) independently assessed the quality of each study using the Newcastle-Ottawa Scale. This tool consists of three items, selection, comparability and outcome/exposure, which included four, two, and three sub-items, respectively, to which star-based scores were assigned. Studies with scores ≥ 6 were considered high-quality studies, those with scores of 4-5 were regarded as having a moderate risk of bias, and those with scores < 4 were regarded as having a high risk of bias.

Statistical analyses

Statistical analyses were performed using Stata 15.1 (Stata Corp, College Station, TX, United States) and Comprehensive Meta-analysis software (2.0). The main pooled outcomes were the HRs or ORs with their 95%CIs of the associations between NLR and ESLD. HRs and ORs were analyzed separately, as were univariate and multivariate HRs and ORs.

The heterogeneity of the studies was assessed using I^2 statistics, with I^2 values of 25%, 50%, 75%, and ≥ 75% indicating low, moderate, high, and very high heterogeneity, respectively[15]. If heterogeneity was high or very high, a random-effects model was used. Study heterogeneity and some potential moderators were explored using subgroup analyses and meta-regression. These variables included the mean age of the patients (categorized as < 50 or > 50 years), location (categorized as Asia or non-Asian regions), etiology, and duration of follow-up. Publication bias was assessed by visual inspection of funnel plots, and by Begg'egand Eggersed ons). When necessary, trim-and-fill analyses and sensitivity analyses were performed.

All statistical tests were two-sided, with the level of significance set at $P < 0.05$.

RESULTS

Literature search

A search of the databases yielded 5510 studies. Analysis using EndNote Version 9.0 software found that 1132 of these studies were duplicates. The remaining 4378 studies were screened by reading their titles and abstracts, resulting in the removal of 4247 studies. A full-text review of the remaining 131 studies resulted in the inclusion of 30 of these studies. The literature search strategy is described in a PRISMA flow diagram (Figure 1).

Characteristics of eligible studies

The 30 studies consisted of 21 published in English and nine published in Chinese. Table 1 shows the main characteristics of the included studies.

All studies were published after 2014, with the largest number, seven, published in 2021. The studies included were from three continents, with the largest number, 22, from Asia. Sixteen studies included patients with ACLF, 13 included patients with acute decompensation (AD), and one included patients with both ACLF and AD. Eighteen studies analyzed patients with hepatitis B virus (HBV)-related ESLD. The mean quality assessment score of the 30 studies was 7.4 (range: 5–9).

Table 1 Characteristic of included studies

Ref.	Year	Location	Population	Patient number (male)	Mean age	Outcome	Etiology	NLR cutoff value	Analysis	NOS scores
Agiasotelli *et al*[8]	2016	Greece	ACLF patients	108 (80)	60.5	30-d & 180-d mortality	Mixed	NR	HR (Univariate & Multivariate)	8
Bernsmeier *et al*[27]	2020	Britain	DCC & ACLF patients	617 (386)	NR	90-d mortality	Mixed	30	OR (Univariate & Multivariate)	8
Cai *et al*[28]	2018	China	ACLF patients	203 (151)	51.14	90-d mortality	HBV	5.09	HR (Univariate & Multivariate)	8
Cai *et al*[18]	2017	China	ACLF patients	637 (486)	54	6-month, 1-yr & 3-yr mortality	Mixed	5.7	HR (Multivariate)	8
Chiriac *et al*[29]	2020	Romania	ACLF patients	70 (49)	62	In-hospital mortality	Mixed	5	NR	7
Fan *et al*[30]	2017	China	ACLF patients	560 (487)	44.9	30-d mortality	HBV	NR	OR (Multivariate)	8
Gao *et al*[31]	2017	China	ACLF patients	573 (478)	43.5	90-d mortality	HBV	NR	HR (Univariate & Multivariate)	8
Guan *et al*[32]	2019	China	ACLF patients	174 (135)	49.60	Mortality	HBV	6.5	OR (Univariate)	6
Li *et al*[33]	2022	China	LC patients with UGIB	376 (235)	60.25	1-yr mortality	Mixed	3.76	OR (Univariate)	7
Li *et al*[10]	2020	China	DCC patients	174 (139)	53.6	28-d mortality	HBV	3.78	HR (Univariate & Multivariate)	8
Liang *et al*[34]	2020	China	ACLF patients	227 (202)	46.4	90-d mortality	HBV	5.38	HR (Univariate)	6
Lin *et al*[35]	2018	China	DCC patients	235 (133)	60	30-d mortality	Mixed	NR	HR (Multivariate)	9
Liu *et al*[36]	2014	China	ACLF patients	216 (183)	45.58	8-wk mortality	HBV	6.12	NR	8
Liu *et al*[37]	2021	China	ACLF patients	160 (145)	46.1	28-d mortality	HBV	4.5	OR (Univariate)	7
Maccali *et al*[38]	2021	Brazil	DCC patients	320 (235)	55.67	90-d mortality	Mixed	NR	HR (Univariate & Multivariate)	8
Moreau *et al*[39]	2018	Belgium	ACLF patients	105 (72)	58	90-d mortality	Mixed	6.2	HR (Univariate & Multivariate)	7
Oikonomou *et al*[11]	2020	Greece	DCC patients	132 (NR)	NR	10-month mortality	Mixed	NR	HR (Univariate)	7
Qi *et al*[26]	2021	China	DCC patients	144 (115)	54.0	30-d mortality	HBV	3.78	OR (Univariate & Multivariate)	8
Qiang *et al*[40]	2021	China	ACLF patients	577 (494)	48.20	90-d mortality	HBV	4.09	HR (Univariate & Multivariate)	7
Shi *et al*[41]	2022	China	LC patients with HE	402 (323)	52	30-d mortality	HBV	4	HR (Univariate & Multivariate)	7
Sun *et al*[9]	2021	China	ACLF patients	412 (351)	NR	28-d & 90-d mortality	HBV	4.79	OR (Univariate & Multivariate)	9
Sun *et al*[42]	2021	China	ACLF patients	290 (252)	44	90-d mortality	HBV	4.78	HR (Univariate & Multivariate)	9
Wang *et al*[43]	2019	China	ACLF patients	270 (228)	46.56	90-d mortality	HBV	NR	OR (Univariate)	6
Wang *et al*[44]	2020	China	ACLF patients	102 (75)	42.9	90-d mortality	HBV	4.22	OR (Univariate)	6
Wu *et al*[45]	2018	China	ACLF patients	100 (89)	47.3	28-d mortality	HBV	NR	NR	6
Xue *et al*[46]	2021	China	LC patients with HE	116 (74)	60	30-d mortality	Mixed	4.4	OR (Univariate)	6
Zhang *et al*[47]	2016	China	DCC patients	148 (118)	53.2	30-d mortality	HBV	5	HR (Univariate & Multivariate)	7
Zhang *et al*[48]	2018	China	ACLF patients	133 (108)	44.9	90-d mortality	HBV	2.06	OR (Univariate)	5
Zhang *et al*[49]	2022	United State	DCC patients	264 (122)	58.31	30-d & 90-d mortality	Mixed	10.6	HR (Univariate & Multivariate)	9

Zhou *et al*[50]	2022	China	LC patients with acute UGIB	676 (398)	62.29	6-wk mortality	Mixed	5.04	OR (Univariate)	9

ACLF: Acute-on-chronic liver failure; DCC: Decompensated cirrhosis; HE: Hepatic encephalopathy; UGIB: Upper gastrointestinal bleeding; LC: Liver cirrhosis; NLR: Neutrophil-to-lymphocyte ratio; NOS: Newcastle-Ottawa Quality Assessment Scale.

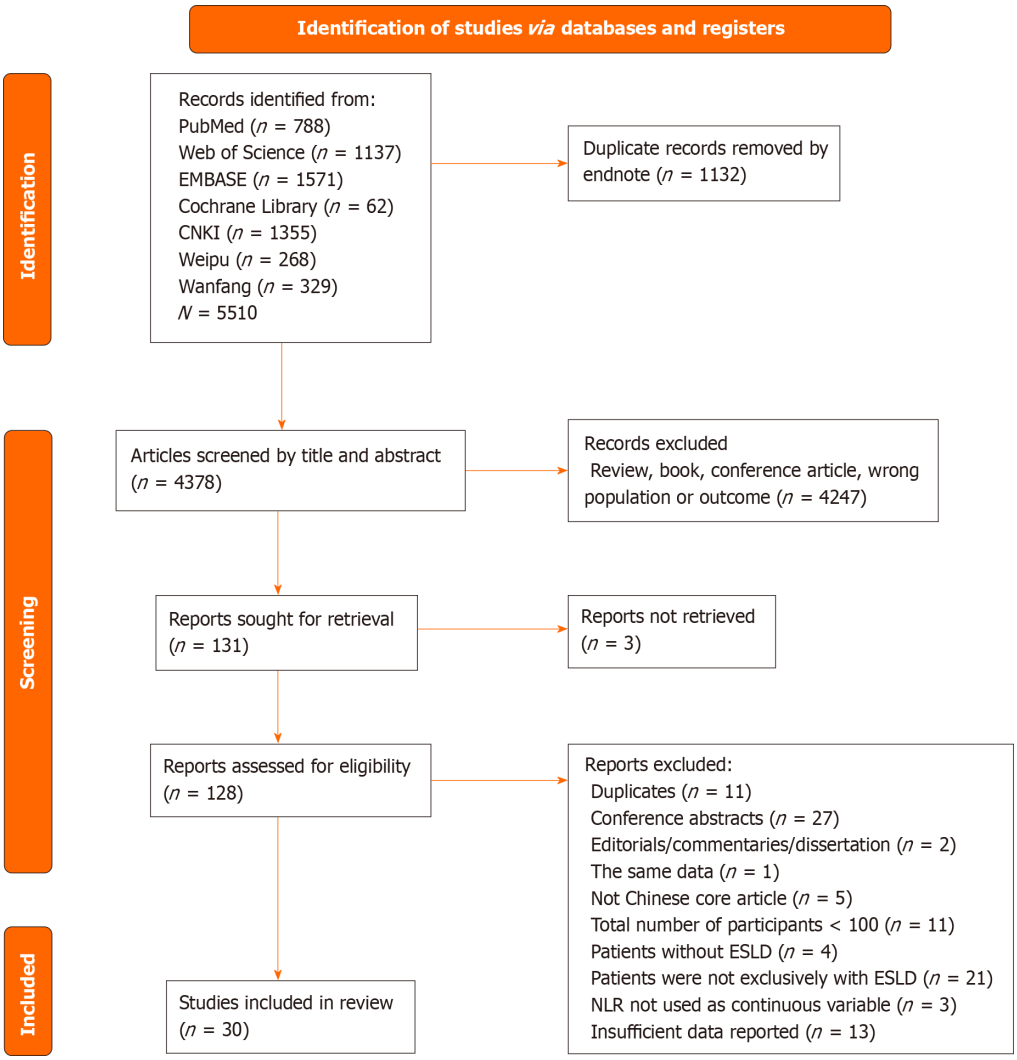


Figure 1 PRISMA flowchart outlining the study search. NLR: Neutrophil-to-lymphocyte ratio; ESLD: End-stage liver disease.

Eight studies provided NLR data for both survivors and non-survivors. Twelve studies used logistic regression analysis to determine the association between NLR and mortality in patients with ESLD, whereas 15 studies used Cox regression analysis to determine this association.

Effect of NLR

Univariate HR: Thirteen studies reported the association between NLR and mortality as univariate HR, with a meta-analysis finding that increased NLR was predictive of increased mortality (Figure 2, Panel A, HR = 1.07, 95%CI = 1.05-1.09). There was significant heterogeneity among these studies (I^2 = 89.4%, P < 0.001). Subgroup (Table 2) and meta-regression (Supplementary Table 1) analyses showed that patient age, sex ratio, region, population, primary outcome, and etiology of ESLD did not affect the prognostic value of NLR. On publication bias tests, Begg's test was non-significant, whereas Egger linear regression indicated possible bias (Supplementary Figure 1, P < 0.05). Using trim-and-fill analyses, two studies were imputed into the meta-analysis, but this did not significantly change the results (Supplementary Figure 2, HR = 1.06, 95%CI = 1.04-1.08). Sensitivity analysis showed similar results when each study was excluded.

Multivariate HR: Thirteen studies also reported the association between NLR and mortality as multivariate HR, with a meta-analysis finding that increased NLR was predictive of increased mortality (Figure 2, Panel B, HR = 1.07, 95%CI = 1.04-1.09). There was significant heterogeneity among these studies (I^2 = 89.1%, P < 0.001). Similar to the results of

Table 2 Subgroup analyses

	Number of subgroup data from studies	Effect size	95%CI	I^2	Q between subgoup
Univariate HR					
Mean age					
> 50	9	1.072	1.043-1.101	85.63[c]	0.492
≤ 50	3	1.056	1.016-1.097	92.65[c]	
Study location					
Not Asian	6	1.049	1.018-1.082	63.92[a]	2.168
Asian	8	1.082	1.054-1.110	91.32[c]	
Population					
AD	8	1.076	1.044-1.110	85.60[c]	0.376
ACLF	6	1.062	1.032-1.093	92.03[c]	
Primary outcome					
≤ 30 mortality	5	1.097	1.057-1.139	80.06[c]	2.852
Long term mortality	9	1.057	1.034-1.080	90.95[c]	
Etiology					
HBV	8	1.082	1.054-1.110	91.95[c]	2.168
Mixed	6	1.049	1.018-1.082	63.92[a]	
Multivariate HR					
Mean age					
> 50	12	1.082	1.051-1.113	90.10[c]	0.621
≤ 50	2	1.052	0.986-1.121	83.28[b]	
Study location					
Not Asian	6	1.030	1.000-1.061	70.153[b]	7.728[b]
Asian	11	1.087	1.061-1.113	87.451[c]	
Population					
AD	11	1.046	1.038-1.054	91.00[c]	3.472
ACLF	6	1.031	1.022-1.040	82.00[c]	
Mortality					
≤ 30 mortality	6	1.087	1.044-1.131	77.08[c]	1.297
Long term mortality	11	1.058	1.033-1.083	91.38[c]	
Etiology					
HBV	7	1.069	1.030-1.110	78.04[c]	0.864
Mixed	10	1.065	1.036-1.095	92.38[c]	
Univariate OR					
Mean age					
> 50	4	1.323	1.077-1.625	78.80[b]	0.036
≤ 50	5	1.289	1.080-1.538	85.73[c]	
Population					
AD	4	1.329	1.058-1.669	78.80[b]	0.095
ACLF	7	1.388	1.179-1.634	92.12[c]	
Mortality					
≤ 30 mortality	3	1.329	1.127-1.567	87.61[b]	0.301

Long term mortality	7	1.256	1.117-1.412	93.79[c]	
Etiology					
HBV	8	1.375	1.247-1.515	90.93[c]	3.558
Mixed	4	1.166	1.013-1.081	76.32[b]	

[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$.
AD: Acute decompensation; ACLF: acute-on-chronic liver failure; HR: Hazard ratios; HBV: Hepatitis B virus.

univariate HR analysis, subgroup (Table 2), and meta-regression (Supplementary Table 1) analyses showed that age, sex ratio, population, primary outcome, and etiology of ESLD did not affect the prognostic value of NLR. In contrast, subgroup analysis revealed that studies in Asia (HR = 1.87, 95%CI = 1.06-1.11) and studies not in Asia (HR = 1.03, 95%CI = 1.00-1.06) yielded significant effects (P = 0.005). Both Begg and Egger test showed possible publication biases (Supplementary Figure 3, $P < 0.05$). By trim-and-fill analyses, two studies were imputed into the meta-analysis, but this did not significantly change the results (Supplementary Figure 4, HR = 1.06, 95%CI = 1.04-1.08). Sensitivity analysis showed similar result when each study was excluded.

Univariate OR: Eleven studies reported the association between NLR and mortality as univariate OR, with a meta-analysis showing that increased NLR was predictive of increased mortality (Figure 2, Panel C, OR = 1.29, 95%CI = 1.18-1.39). There was significant heterogeneity among these studies (I^2 = 91.3%, $P < 0.001$). Subgroup (Table 2) and meta-regression (Supplementary Table 1) analyses showed that age, sex ratio, region, population, primary outcome, and etiology of ESLD did not affect the prognostic value of NLR. In the publication bias test, Begg's test was non-significant (p=0.81). However, Egger's linear regression showed the possible presence of bias (Supplementary Figure 5, $P < 0.05$). No study was imputed into the meta-analysis by trim-and-fill analyses (Supplementary Figure 6). Because the number of studies was small, the possibility of publication bias could not be completely excluded. Sensitivity analysis showed similar results when each study was excluded.

Multivariate OR: Four studies reported the association between NLR and mortality as multivariate OR, with a meta-analysis indicating that increased NLR was predictive of increased mortality (Figure 2, Panel D, OR = 1.29, 95%CI = 1.09-1.49). There was significant heterogeneity among these studies (I^2 = 93.4%, $P < 0.001$). Because the number of studies was not adequate, subgroup and meta-regression analyses were not performed. In publication bias tests, Begg test was not significant (P = 0.81), whereas Egger linear regression showed possible bias (Supplementary Figure 7, $P < 0.05$). No study was imputed into the meta-analysis by trim-and-fill analyses. Because the number of studies was small, the possibility of publication bias could not be excluded completely. Sensitivity analysis showed similar result when each study was excluded.

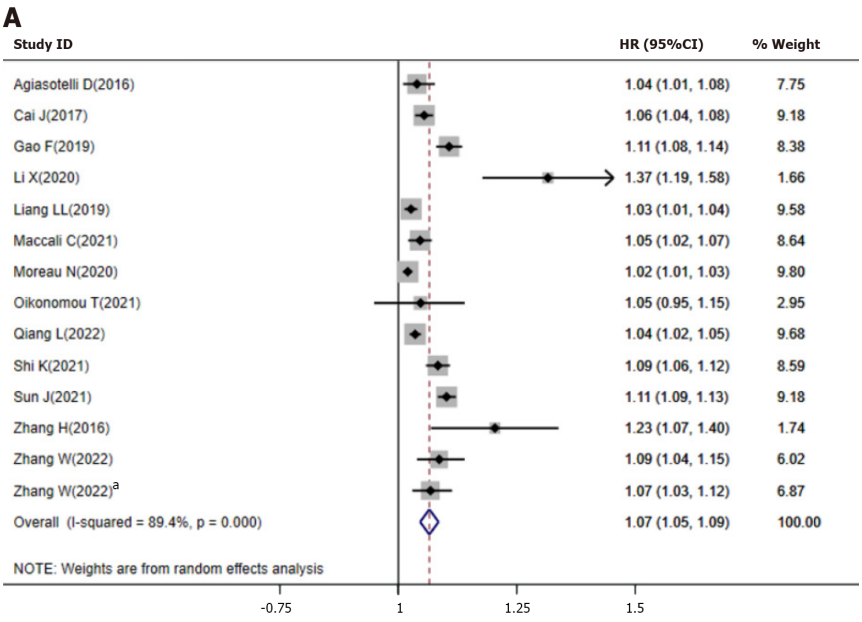
Comparison of NLR in survivors and non-survivors

Eight studies compared NLR in surviving and non-surviving patients with ESLD. A meta-analysis showed that NLR was significantly higher in non-survivors than in survivors (Supplementary Figure 8, random-effects model: SMD = 1.02 95%CI; 0.67–1.37).

DISCUSSION

To our knowledge, this systematic review is the first to report a relationship between NLR and mortality in patients with ESLD. The pooled results of this study indicated that NLR was associated with mortality (random-effects model; univariate HR = 1.07, 95%CI = 1.05-1.09; multivariate HR = 1.07, 95%CI = 1.07-1.09; univariate OR = 1.29, 95%CI = 1.18-1.39; multivariate OR = 1.29, 95%CI = 1.09-1.49). Furthermore, the pooled results of eight studies showed that NLR levels were significantly higher in non-survivors than in survivors with ESLD (random-effects model: SMD = 1.02, 95%CI = 0.67-1.37).

Mortality rates are high in patients with ESLD, such as liver failure and decompensated cirrhosis. Systemic inflammatory reactions are closely related to the severity and prognosis of liver disease in patients with severe cirrhosis, with the occurrence of systemic inflammatory response syndrome increasing mortality rates in patients with cirrhosis[16]. It is therefore crucial to identify and treat infections and systemic inflammation in patients with ESLD. Although routine tests, including measurements of C-reactive protein and procalcitonin (PCT) concentrations and white blood cell (WBC) counts, are commonly used to assess bacterial infection and systemic inflammation, these tests may not fully meet the demands of patients with ESLD. High serum total bilirubin concentrations in these patients can influence the diagnostic sensitivity of PCT[17]. Additionally, patients with ESLD often have lower baseline WBC counts, which can impair the predictive value of WBC in detecting infections. A study included in this review confirmed that NLR is superior to WBC or PCT for assessing infection in patients with ACLF[9]. NLR may also be a useful indicator of systemic inflammatory response syndrome or infection in patients with decompensated cirrhosis[18]. Taken together, these findings suggest that NLR


A
Study ID
HR (95%CI)
% Weight
Agiasotelli D(2016) 1.04 (1.01, 1.08) 7.75
Cai J(2017) 1.06 (1.04, 1.08) 9.18
Gao F(2019) 1.11 (1.08, 1.14) 8.38
Li X(2020) 1.37 (1.19, 1.58) 1.66
Liang LL(2019) 1.03 (1.01, 1.04) 9.58
Maccali C(2021) 1.05 (1.02, 1.07) 8.64
Moreau N(2020) 1.02 (1.01, 1.03) 9.80
Oikonomou T(2021) 1.05 (0.95, 1.15) 2.95
Qiang L(2022) 1.04 (1.02, 1.05) 9.68
Shi K(2021) 1.09 (1.06, 1.12) 8.59
Sun J(2021) 1.11 (1.09, 1.13) 9.18
Zhang H(2016) 1.23 (1.07, 1.40) 1.74
Zhang W(2022) 1.09 (1.04, 1.15) 6.02
Zhang W(2022)[a] 1.07 (1.03, 1.12) 6.87
Overall (I-squared = 89.4%, p = 0.000) 1.07 (1.05, 1.09) 100.00
NOTE: Weights are from random effects analysis
-0.75
1
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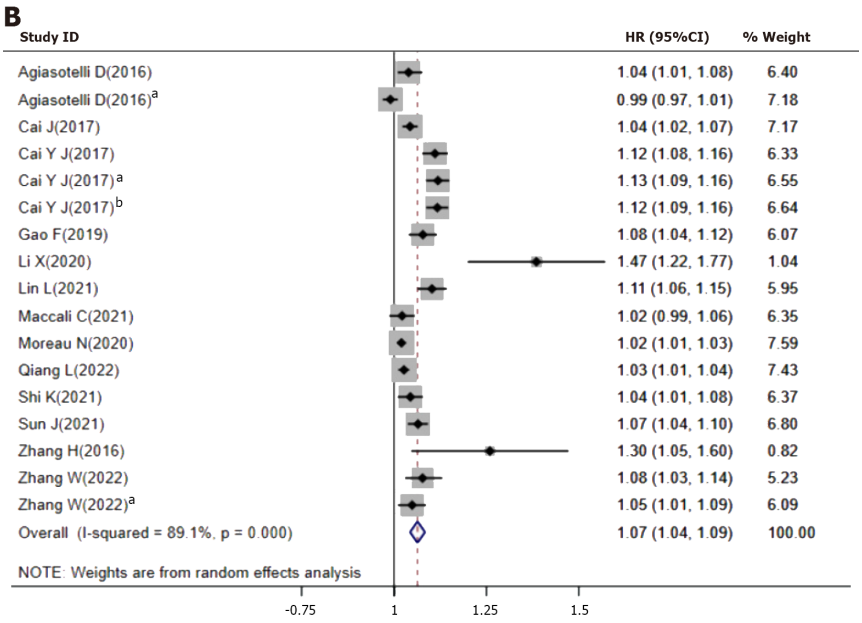
1.5


B
Study ID
HR (95%CI)
% Weight
Agiasotelli D(2016) 1.04 (1.01, 1.08) 6.40
Agiasotelli D(2016)[a] 0.99 (0.97, 1.01) 7.18
Cai J(2017) 1.04 (1.02, 1.07) 7.17
Cai Y J(2017) 1.12 (1.08, 1.16) 6.33
Cai Y J(2017)[a] 1.13 (1.09, 1.16) 6.55
Cai Y J(2017)[b] 1.12 (1.09, 1.16) 6.64
Gao F(2019) 1.08 (1.04, 1.12) 6.07
Li X(2020) 1.47 (1.22, 1.77) 1.04
Lin L(2021) 1.11 (1.06, 1.15) 5.95
Maccali C(2021) 1.02 (0.99, 1.06) 6.35
Moreau N(2020) 1.02 (1.01, 1.03) 7.59
Qiang L(2022) 1.03 (1.01, 1.04) 7.43
Shi K(2021) 1.04 (1.01, 1.08) 6.37
Sun J(2021) 1.07 (1.04, 1.10) 6.80
Zhang H(2016) 1.30 (1.05, 1.60) 0.82
Zhang W(2022) 1.08 (1.03, 1.14) 5.23
Zhang W(2022)[a] 1.05 (1.01, 1.09) 6.09
Overall (I-squared = 89.1%, p = 0.000) 1.07 (1.04, 1.09) 100.00
NOTE: Weights are from random effects analysis
-0.75
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1.25
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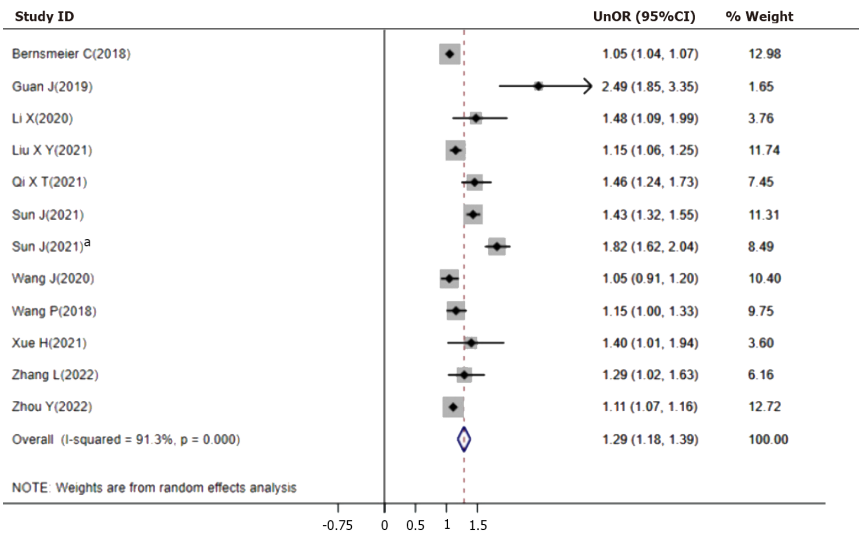
C
Study ID
UnOR (95%CI)
% Weight
Bernsmeier C(2018) 1.05 (1.04, 1.07) 12.98
Guan J(2019) 2.49 (1.85, 3.35) 1.65
Li X(2020) 1.48 (1.09, 1.99) 3.76
Liu X Y(2021) 1.15 (1.06, 1.25) 11.74
Qi X T(2021) 1.46 (1.24, 1.73) 7.45
Sun J(2021) 1.43 (1.32, 1.55) 11.31
Sun J(2021)[a] 1.82 (1.62, 2.04) 8.49
Wang J(2020) 1.05 (0.91, 1.20) 10.40
Wang P(2018) 1.15 (1.00, 1.33) 9.75
Xue H(2021) 1.40 (1.01, 1.94) 3.60
Zhang L(2022) 1.29 (1.02, 1.63) 6.16
Zhou Y(2022) 1.11 (1.07, 1.16) 12.72
Overall (I-squared = 91.3%, p = 0.000) 1.29 (1.18, 1.39) 100.00
NOTE: Weights are from random effects analysis
-0.75
0
0.5
1
1.5

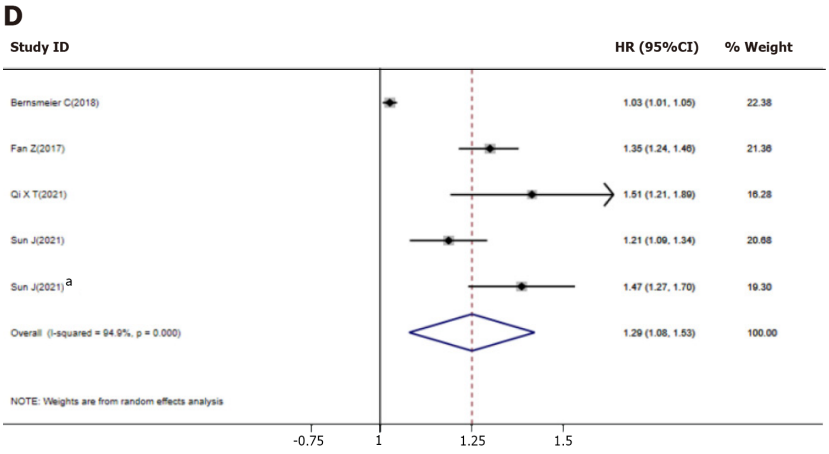

Figure 2 Forest plot of association between neutrophil-to-lymphocyte ratio and end-stage liver disease mortality. A: Univariate hazard ratios (HR); B: Multivariate HR; C: Univariate odds ratios (OR); D: Multivariate OR. Different subgroup data extracted in the same literature were distinguished using the letter ([a]) and ([b]).

strongly correlates with infection and systemic inflammatory response syndrome in patients with ESLD and that NLR may be predictive of mortality. These findings are consistent with the majority of the included studies and the final pooled results.

NLR has also been shown to be an indicator of inflammation in other conditions, such as colorectal cancer and myocardial infarction[19]. Peripheral neutrophil counts have been reported to serve as markers for both acute and chronic inflammation[20]. Activation of these neutrophils can inhibit T lymphocyte activation through the production of reactive oxygen and arginase[21]. Peripheral T-lymphocyte subsets were found to be significantly lower in ACLF patients than in healthy controls[22], and lower lymphocyte cell counts have been associated with poorer immune responses in patients with chronic liver disease[23]. These findings suggest that NLR may be a practical indicator that reflects the balance between inflammation and immune reactions. Furthermore, the inflammatory process has been shown to play a significant role in the development of liver fibrosis and cirrhosis. A meta-analysis suggested that NLR may be a marker of the degree of fibrosis and predictor of prognosis in patients with chronic liver disease[24]. NLR may also be predict of for prognosis in patients with ESLD.

Subgroup and meta-regression analyses revealed that the predictive value of NLR was not influenced by patient age, sex ratio, or the etiology of ESLD, suggesting that NLR is a reliable predictor of ESLD prognosis across different patient populations. NLR is considered a cost-effective and practical tool for predicting mortality in critically ill patients with liver failure and for screening patients with severe liver disease. Unlike other prognostic biomarkers, neutrophils and lymphocytes can be easily obtained and measured in clinical practice. Subgroup analysis of multivariate HR from 13 studies showed that NLR was strongly associated with mortality in Asian patients with ESLD, possibly due to the high prevalence of hepatitis B infection in Asian populations. HBV-ACLF patients exhibit lower levels of circulating lymphocytes and significantly higher levels of liver infiltrating lymphocytes[25]. Subgroup analysis, however, did not find significant differences in NLR between patients with HBV and those with mixed etiology. This may have been due to confounding factors and high heterogeneity in the mixed etiology group.

It is worth mentioned, the severity of the neutropenia and the overall status of the patient should be taken into account. Profound neutropenia may signify a more severe inflammatory or immunocompromised state, potentially affecting the NLR's ability to reflect the underlying inflammatory process accurately. In these patients, it can potentially impact the accuracy of NLR as a marker of systemic inflammation. Future research should focus on large-scale longitudinal studies to assess the predictive value of the NLR in ESLD patients with neutropenia, subgroup analyses to account for specific clinical characteristics, mechanistic studies to understand the underlying pathophysiology.

While NLR was identified as the strongest independent predictor in this study, other ratios such as platelet-to-lymphocyte ratio (PLR) and platelet-to-neutrophil ratio (PNR) have also been investigated for prognosis in liver diseases. However, study have shown that NLR had good predictive ability for mortality, higher than PNR[26]. In the setting of ESLD, PLR and PNR may be less reliable due to various thrombocytopenia mechanisms associated with advanced liver dysfunction. This is aruably a more direct assessment of the disease stage and prognosis in decompensated cirrhosis patients. For this reason, this article focused on NLR rather than PLR or PNR, though future studies could explore whether a combination of ratios provides even stronger predictive ability than individual markers alone.

The present review and meta-analysis had several limitations. First, there was high heterogeneity among the studies included in this analysis, similar to other prognostic reviews, despite the use of a random-effects model. Second, most of the included studies reported positive results, which may have introduced latent publication bias, although Begg's test and Egger's test did not show significant biases. Moreover, the number of studies that utilized multivariate OR analysis to assess the association between NLR and mortality was too small for determination of publication bias. Third, the critical cut-off value of NLR for determining prognosis remains unclear. Due to limitations in the original studies, the present analysis could not determine an exact ideal cut-off value.

CONCLUSION

This meta-analysis highlights the significance of NLR as a valuable prognostic biomarker in patients with ESLD, with higher NLRs indicating an increased risk of mortality. These findings especially emphasize the strong association between higher NLRs and prognosis in the Asian patients with ESLD. The continuing absence of a critical cut-off value of NLR for determining prognosis suggests the need for additional research to clarify this matter.

ARTICLE HIGHLIGHTS

Research background

End-stage liver disease (ESLD) carries a high mortality risk. Identifying reliable prognostic factors is important to guide management, but studies on the prognostic value of neutrophil-to-lymphocyte ratio (NLR) in ESLD have reported conflicting results.

Research motivation

To comprehensively evaluate the association between NLR and ESLD prognosis through a systematic review and meta-analysis of existing literature.

Research objectives

To establish whether NLR is a useful prognostic biomarker for predicting mortality in patients with ESLD.

Research methods

A systematic literature search was conducted through multiple databases. Studies evaluating the relationship between NLR and mortality in ESLD patients were selected and their data extracted. Pooled effect sizes were calculated using meta-analysis.

Research results

Higher NLR levels were associated with increased mortality risk in ESLD based on meta-analysis of 27 studies reporting hazard/odds ratios. NLR also distinguished survivors from non-survivors. The prognostic value of NLR was not influenced by patient characteristics but differed regionally.

Research conclusions

NLR is clinically useful for prognostic assessment in ESLD patients, especially Asian populations, but optimal cut-off values require further investigation.

Research perspectives

NLR represents a promising, readily available prognostic tool for risk stratifying ESLD patients. Future research should establish standardized NLR cut-offs and evaluate its utility accounting for potential confounders like severity of neutropenia.

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FOOTNOTES

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Co-corresponding authors: Xin-Hua Li and Hong Cao.

Author contributions: Li XH and Cao H contributed to study concept and design; Cai XH and Tang YM contributed to drafting of the manuscript; and Pang JH collected data of including articles; Cai XH and Chen SR performed the analysis of the project; Pang JH compiled the figures and tables. Li XH and Cao H contributed to critical revision of the manuscript for important content; Chong YT, Li XH, and Cao H contributed to obtaining funding. Cai XH and Tang YM are co-first authors due to their significant involvement in drafting the manuscript and collecting data, showcasing their strong understanding of the research findings. Li XH and Cao H are likely the corresponding authors because of their significant contributions to the study's concept, design, and funding acquisition. The pivotal roles of Li XH and Cao H in shaping the study's foundation and ensuring its continued support position them as the lead corresponding authors in this collaborative effort. Chong YT and Li XH contributed to obtained funding. This collaboration between Li XH and Cao H is crucial for the publication of this manuscript. Their combined efforts reflect their shared responsibility for the scholarly work and ensure that their individual contributions are duly recognized and attributed. All authors approved submission.

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