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Treatment and prognostic assessment of liver cirrhosis and its complications

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Treatment and prognostic assessment of liver cirrhosis and its complications

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Editorial: Treatment and prognostic assessment of liver cirrhosis and its complications

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Editorial on the Research Topic
Treatment and prognostic assessment of liver cirrhosis and its complications

We are pleased to announce the success of the Research Topic entitled as "*Treatment and prognostic assessment of liver cirrhosis and its complications*" in the Frontiers, where a total of 10 high-quality papers have been published by 92 authors from Brazil, China, Germany, Italy, and Japan after internal editorial assessment, external peer review, and editorial decision processes. In this editorial, we summarize the contents of the 10 papers, which can be divided into two major sections, one about pathogenesis and risk factors, and another about prognostic assessment.

Pathogenesis and risk factors of liver cirrhosis and its complications

Liver cirrhosis

Zheng et al. from the Sun Yat-sen Memorial Hospital of Sun Yat-sen University, China, performed a meta-analysis to evaluate the association of the interferon-γ gene polymorphism rs2430561 and the tumor necrosis factor-α gene polymorphism rs361525 with the risk of liver cirrhosis. The currently available evidence demonstrated positive correlations of rs2430561 with alcoholic liver cirrhosis and hepatitis B virus related cirrhosis as well as that of rs361525 with hepatitis B virus related cirrhosis. This suggests the contribution of inflammation on cirrhosis. However, it should be acknowledged that the effect of the two gene polymorphisms on liver cirrhosis from other etiologies remains unclear.

Osteoporosis in cirrhosis

Zhao et al. from the Shandong University of Traditional Chinese Medicine, China, reported the results of a bidirectional two-sample Mendelian randomization to clarify the causal association between primary biliary cirrhosis and osteoporosis by using the data from two public databases of genome-wide association studies, one about primary biliary cirrhosis, and another about osteoporosis. The investigators confirmed the causal effect of primary biliary cirrhosis on the risk of osteoporosis; by contrast and as expected, the presence of osteoporosis is not a causal factor for primary biliary cirrhosis. Generally speaking, this study should be the first to explore the causal relationship between them by using the Mendelian randomization method, and strengthens the necessity of routine screening for bone density in such patients.

Hydrothorax in cirrhosis

Bai et al. from the Shandong University, China, conducted a retrospective study to evaluate the risk factors for hepatic hydrothorax in cirrhosis. It seemed that the severity of liver cirrhosis and portal hypertension, such as model for end-stage liver diseases, prothrombin activity, and portal vein diameter, as well as high-density lipoprotein cholesterol, would be closely associated with the development of this complication.

Venous thrombosis in cirrhosis

da Cruz Renó et al. from the University of São Paulo, Brazil, evaluated the prevalence and risk factors of deep venous thrombosis and chronic venous insufficiency in outpatients with cirrhosis. Unfortunately, they did not find any variables which significantly correlated with deep venous thrombosis, but edema and orthostatism might be positively associated with the severity of chronic venous insufficiency. Zhang Y. et al. from the General Hospital of Northern Theater Command, China, attempted to analyze whether C-type lectin-like receptor 2 and galectin-1, two proteins involved in the activation and aggregation of platelets, were associated with the presence of portal vein thrombosis in hepatitis B virus related liver cirrhosis. Their association was significant, especially when portal vein thrombosis was more severe or extended to the superior mesenteric vein. Thus, *in vivo* studies should be warranted to validate their effects on the mechanism of portal vein thrombosis in cirrhosis.

Prognostic assessment of liver cirrhosis and its complications

General assessment

Gülcicegi et al. from the University of Cologne, Germany, reviews the recent advances regarding prognostic assessment of liver cirrhosis with an emphasis on current staging system for clinical course of liver cirrhosis, including compensated cirrhosis, stable and unstable decompensated cirrhosis, pre-acute-on-chronic liver failure, and acute-on-chronic liver failure grades 1–3. Besides, some novel scoring systems and biomarkers for liver cirrhosis have been reviewed as well as non-invasive approaches for assessment of portal hypertension.

Decompensation in compensated cirrhosis

Saeki et al. performed a retrospective study to explore the prognostic impact of insulin-like growth factor, which can reflect liver function reserve, in patients with liver cirrhosis admitted to two medical centers in Japan. They found that a low insulin-like growth factor level should be an independent predictor of decompensation in compensated cirrhosis. Besides, a low insulin-like growth factor level was significantly associated with lower cumulative survival rates in compensated cirrhosis, but not decompensated cirrhosis.

Hepatic encephalopathy in decompensated cirrhosis

Riggio et al. analyzed the impact of hepatic encephalopathy on the mortality and readmission of patients decompensated cirrhosis based on the data prospectively collected from 25 Italian referral centers. Multivariate competing risk analysis demonstrated that hepatic encephalopathy should be an independent predictor of death in cirrhotic patients. Meanwhile, patients with hepatic encephalopathy had a significantly higher probability of hospital readmission, and their readmission was primarily related to the recurrence of hepatic encephalopathy. These findings strongly indicated that patients with hepatic encephalopathy should be actively listed for liver transplantation. Similarly, in a retrospective single-center study, Zhang L. et al. from the Peking University People's Hospital, China, observed that hepatic encephalopathy recurrence was the most common cause for hospital readmission in patients with hepatic encephalopathy. Additionally, they identified neutrophil-to-lymphocyte ratio as a predictor of 30-, 90-, and 180-day hospital admission in such patients.

Variceal rebleeding after in cirrhosis

Shi et al. selected patients with liver cirrhosis related to non-alcoholic steatohepatitis and hepatitis B virus infection who underwent hepatic venous pressure gradient measurement before and after transjugular intrahepatic portosystemic shunt at three medical centers in Chongqing, China. They identified a high hepatic venous pressure gradient of >17 mmHg after transjugular intrahepatic portosystemic shunt as an independent predictor for variceal rebleeding in non-alcoholic steatohepatitis related cirrhosis, but >20 mmHg in hepatitis B virus infection related cirrhosis.

Finally, we cordially thank all authors' contributions and editors' and peer-reviewers' efforts on the final publication of this Research Topic. Staff members from the Frontiers, especially Penerade Huang, should also be acknowledged because of their efficient and professional assistance on coordinating various issues involved in this Research Topic. We sincerely wish that the papers in the Research Topic can produce significant impact on clinical practice and academic research in future, and eagerly look forward to more high-quality submissions to the Research Topic Volume II in 2024.

Author contributions

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The *TNF-α* rs361525 and *IFN-γ* rs2430561 polymorphisms are associated with liver cirrhosis risk: a comprehensive meta-analysis

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Background: Inflammation serves as an essential driver of liver cirrhosis (LC) incidence. Accordingly, a meta-analysis was carried out to explore the association between specific polymorphisms in the interferon-γ (*IFN-γ*) and tumor necrosis factor-α (*TNF-α*) genes and the incidence of LC based on comparisons of genotype and allele frequencies.

Objectives: To study the relationship between *TNF-α* rs361525 and *IFN-γ* rs2430561 polymorphisms and the risk of LC.

Methods: A database search was performed for all studies published as of September 10, 2022. The strength of risk relationships was assessed based on odds ratios (ORs) with 95% confidence intervals (CIs).

Results: Pooled analyses were conducted for one common *TNF-α* polymorphism (rs361525) as well as one common *IFN-γ* polymorphism (rs2430561). Both of these SNPs were identified as LC-related risk factors. Specifically, rs361525 was related to LC incidence in both alcoholic liver cirrhosis (OR: 1.86, 95%CI: 1.03-3.34) and hepatitis B virus (HBV)-related cirrhosis cases (OR: 1.44, 95%CI: 1.00-2.06) when using an allelic contrast model. Moreover, rs2430561 was significantly related to LC in an Asian population (OR: 1.45, 95%CI: 1.13-1.86) and in the context of HBV-related cirrhosis (OR: 1.48, 95%CI: 1.13-1.93) when using an allelic contrast model.

Conclusion: These findings indicate that rs361525 and rs2430561 represent LC-related risk factors, although additional large-scale clinical and case-control studies will be vital to confirm these results.

KEYWORDS

tumor necrosis factor-α, interferon-γ, liver cirrhosis, polymorphism, metaanalysis, risk

Introduction

Liver cirrhosis (LC) was responsible for an estimated 2.4% of global mortality in 2017 (1), more than 45% of all deaths in Western nations and over 1.32 million deaths throughout the globe (2).

Many different clinical trials have been developed and implemented in recent years with the goal of defining more effective anti-fibrotic therapies, with phase II and III trials having been performed for a range of agents including obethicolic acid, selonsertib, and elafibranor. While some of these studies have yielded promising findings, in general the benefits have been modest and the outlook for anti-fibrotic treatment remains poor. Accordingly, the most effective strategies currently available to protect against hepatic fibrosis center on its prevention and on alleviating known risk factors associated with this condition (3, 4).

The risk factors known to be most closely related to LC incidence include chronic infections with hepatitis B or C virus (HBV and HCV), which are respectively associated with 39.64 million and 30.36 million cases, as well as nonalcoholic fatty liver disease (NAFLD), which is linked to 10.26 million cases, and alcoholic liver disease (ALD), which is associated with 26.04 million cases. In addition, roughly 16.62 million cases are linked with other underlying causes (5, 6).

Both host genetic factors and immune activity can shape the progression of chronic viral hepatitis and associated infections, making them important determinants of LC risk. Single nucleotide polymorphisms (SNPs) are the most prevalent and extensively explored form of genetic variation (7, 8), and SNPs in genes such as PON1(9), GSTM1(10), and CTLA4(11) have been linked with LC.

Growth factors and cytokines such as members of the interferon (IFN) and tumor necrosis factor (TNF) family are vital components of host immune activity that can shape viral infection-related processes and the subsequent development of fibrosis (12, 13). In a prior study, our group conducted a meta-analysis examining the relationship between mutations in the IL-6 and IL-10 genes (rs1800871, rs1800872, rs1800795, rs1800796, rs1800797, rs1800896) and LC risk, ultimately identifying the IL-10 -592 and -1082 polymorphisms as being closely linked to LC susceptibility(14). Other reports have specifically focused on links between *IFN-γ* and *TNF-α* genes polymorphisms and cirrhotic disease (15).

TNF-α is a potent inflammatory and immunomodulatory cytokine, and the gene that encodes it is associated with the class III MHC region (16). Signaling through the TNF-α/NF-kB axis has been linked to damage, fibrotic activity, and cirrhosis in the liver (17, 18). IFN-γ is another pro-inflammatory cytokine that can influence hematopoietic stem cell development at baseline and serves as a major pathogenic factor associated with various forms of hematopoiesis-related disease stats such as hemophagocytic lymphohistiocytosis, aplastic anemia, and LC (19).

In one recent meta-analysis of 22 studies incorporating 2,638 LC patients and 2,905 control individuals, the *TNF-α* -308A/G polymorphism was found to be unrelated to the risk of developing LC (20). No similar analyses, however, have been performed for the common rs361525 (-238 A>G) polymorphism in this gene or for the common *IFN-γ* rs2430561 polymorphism (+874T/A).

To date, no studies have performed any comprehensive assessments of the *TNF-α* rs361525(21–33) and *IFN-γ* rs2430561 (8, 29, 34–40) polymorphisms. Given the above findings and in an effort to overcome limitations associated with small study sample sizes or ethnic/regional variation among study cohorts, the present meta-analysis was thus performed to attempt to more fully clarify the link between these two SNPs in inflammatory cytokine genes and LC risk in an effort to provide a foundation for future clinical reference.

Materials and methods

Study selection

In the current study, relevant papers published as of September 10, 2022, were retrieved from PubMed and other databases using the following search terms: "tumor necrosis factor-α or *TNF-α*", "interferon-γ or *IFN-γ*", "polymorphism or variation or mutation", "rs361525", "rs2430561" and "liver cirrhosis or nonalcoholic fatty liver disease or primary biliary cirrhosis". This analysis included the research with the largest sample size when several studies used the same set of clinical data.

Inclusion criteria

Research studies were only considered if they fulfilled the following requirements: (a) studies in which the criteria used to diagnose LC were clearly defined, including liver biopsy, CT, MRI, B-ultrasound, or endoscopic retrograde cholangiopancreatography, (b) studies of correlations between the designated *TNF-α* and *IFN-γ* gene polymorphisms (rs361525 or rs2430561) and LC risk, (c) studies using cohort or case-control designs, (d) studies with enough data to allow for the calculation of 95% confidence interval (95%CI) values, odds ratio (OR), and (e) studies were not duplicated.

Data extraction

Data extracted from individual studies included first author, country, subject ethnicity, number of cases, year of publication, number of controls, source of control subjects, HWE in the control group, genotyping approach, and LC type. Two investigators (Minghui Zheng, Hong Luo) independently extracted data, with any discrepancies being resolved through discussion and consensus.

Statistical analysis

Relationships between these *IFN-γ* and *TNF-α* polymorphisms of interest and LC risk were analyzed based on ORs and 95% CIs. Analyzing the significance of ORs was performed using the Z-test (41), while heterogeneity was assessed with the Q-test and I^2

statistic (42, 43). I^2 values < 25%, ~50%, and >75% were respectively associated with low, moderate, and high degrees of heterogeneity (44). When heterogeneity was absent, a fixed-effect Mantel-Haenszel model was used ($P_{heterogeneity} > 0.1$), whereas a random-effects DerSimonian-Laird model was instead employed in the presence of heterogeneity (45, 46). Sensitivity analyses were used to assess result stability through a leave-one-out approach (47). Pearson's chi-square test was used to assess whether polymorphism frequencies diverged from the expected HWE (48). The potential for publication bias was examined through a combination of Begg's funnel plots and Egger's regression test (49). Stata 11.0 (StataCorp LP, TX, USA) was used for all statistical analyses.

Protein-protein interaction network analysis

To better explore links between TNF-α and IFN-γ and LC pathogenesis, Protein-protein interaction networks involving these two genes were generated using the STRING database (http://string-db.org/) (50).

Results

Study selection

Initial literature searches of PubMed and other databases led to the identification of 659 studies (Figure 1), including 445 pertaining to the *TNF-α* rs361525 polymorphism and 214 pertaining to the *IFN-γ* rs2430561 polymorphism. Of these studies, 503 were excluded following title and abstract review (no relationship between above two polymorphisms and liver disease), 156 articles were left after reviewing full-text, additional 135 articles were excluded due to following reasons: duplicates (including 36 articles), meta-analyses or systematic reviews (including 11 articles), articles focused on other polymorphisms (including 38 articles), review (including 44 articles), or clinical trials (including 6 articles). The remaining 21 studies (8, 21–40) met with inclusion criteria for this analysis and were thus incorporated into this study, including 13 pertaining to the *TNF-α* rs361525 polymorphism (5, 5, and 2 case-control studies related to PBC, HBV, and ALC, respectively) and 9 pertaining to the *IFN-γ* rs2430561 polymorphism. In all studies, DNA was isolated from samples of patient blood (Table 1). Minor allele frequency (MAF) reports for these two SNPs were assessed in 6 different global populations with the 1000 Genomes Browser (https://www.ncbi.nlm.nih.gov/snp/) (Figure 2). The data of Figure 2 is from the online website (https://www.ncbi.nlm.nih.gov/snp/), which is objective and based on statistic data from several genome-wide association studies and published articles. In addition, the difference among different ethnicity may be from ethnicity themselves or other polymorphisms, including other IFN-γ and TNF-α polymorphisms.

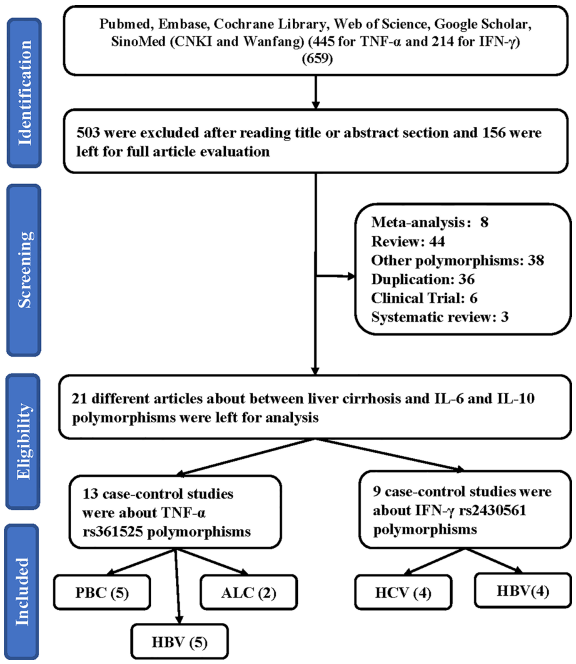

FIGURE 1
A flowchart outlining the search strategy utilized for the identification of association studies related to the relationship between the *TNF-α* rs361525/*IFN-γ* rs2430561 polymorphisms and the risk of LC.

Quantitative result synthesis

TNF-α rs361525 polymorphism

In an overall analysis, no significant association with LC risk was detected when using an allelic contrast model (OR: 1.22, 95% CI:0.98-1.52, P = 0.233 for heterogeneity, P = 0.081, I^2 = 20.9%). Subgroup analyses also failed to detect any significant association between this polymorphism and LC based on ethnicity or source of control subjects. However, positive relationships between rs361525 and LC risk were detected in the ALC (OR: 1.86, 95%CI:1.03-3.34, P = 0.420 for heterogeneity, P = 0.040, I^2 = 7.1%) and HBV (OR: 1.44, 95%CI:1.00-2.06, P = 0.404 for heterogeneity, P = 0.047, I^2 = 0.3%) subgroups using allelic contrast models (Figure 3; Table 2).

IFN-γ rs2430561 polymorphism

No overall association was detected between rs2430561 and risk of LC, and the same was true when assessing patients based on the source of control subjects. However, this polymorphism was linked to elevated LC risk in Asian individuals (allelic contrast: OR: 1.45, 95%CI:1.13-1.86, P = 0.197 for heterogeneity, P = 0.003, I^2 = 33.6%, Figure 4; dominant model: OR: 1.57, 95%CI:1.05-2.35, P = 0.046 for heterogeneity, P = 0.028, I^2 = 0.0%) and in patients with HBV (allelic contrast: OR: 1.48, 95%CI:1.13-1.93, P = 0.116 for

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TABLE 1 Characteristics of included studies about polymorphisms in *TNF-α* and *IFN-γ* genes and cirrhosis of liver risk.

Author	Year	Country	Ethnicity	Case	Control	SOC	HWE	Genotype	Type of liver cirrhosis
rs361525									
Bernal	1999	UK	Caucasian	98	93	PB	0.051	PCR	PBC
Pastor	2005	Spain	Caucasian	65	90	PB	0.534	PCR	ALC
Gordon	1999	UK	Caucasian	91	205	PB	0.706	PCR-RFLP	PBC
Sghaier	2016	Tunisia	African	36	200	PB	0.371	PCR-RFLP	HBV
Osterreicher	2005	Austria	Caucasian	55	94	HB	0.708	PCR-RFLP	HBV/HCV
Nguyen-Khac	2008	France	Caucasian	45	47	PB	0.473	PCR-RFLP	ALC
Niro	2009	Italy	Caucasian	107	141	HB	0.776	PCR-SSP	PBC
Jones	1999	UK	Caucasian	168	145	HB	0.667	PCR	PBC
Zhang	2008	China	Asian	80	61	HB	0.804	AS-PCR	HBV
Li	2014	China	Asian	30	40	HB	0.805	PCR-RFLP	HBV
Fan	2004	China	Asian	57	160	HB	0.763	PCR-RFLP	PBC
Zhang	2013	China	Asian	28	50	HB	0.943	PCR-RFLP	HBV
Ma	2009	China	Asian	163	132	HB	0.009	PCR-RFLP	HBV
rs2430561									
Bahgat	2015	Egypt	African	50	25	HB	0.298	allele-specific PCR	HCV
Sun	2015	China	Asian	126	173	HB	0.087	SSP-PCR	HBV
Wu	2008	China	Asian	32	50	HB	0.276	PCR	HBV
Ma	2009	China	Asian	150	141	HB	0.491	PCR	HBV
Bouzgarrou	2011	Tunisia	African	58	42	HB	0.002	PCR-RFLP	HCV
Sheneef	2017	Egypt	African	50	50	HB	0.004	PCR-RFLP	HCV
Talaat	2022	Egypt	African	69	106	PB	<0.001	SSP-PCR	HCV
Tang	2015	China	Asian	58	56	HB	0.002	PCR-RFLP	HBV
Gao	2010	China	Asian	24	74	PB	<0.001	PCR	HBV/HCV

HB, hospital-based; PB, population-based; SOC, source of control; PCR-RFLP, polymerase chain reaction followed by restriction fragment length polymorphism; SSP, sequence specific primer; AS, allele specific primer; HWE, Hardy-Weinberg equilibrium of control group; PBC, primary biliary cirrhosis; ALC, alcoholic liver cirrhosis; HBV, hepatitis B virus; HCV, hepatitis C virus.

heterogeneity, $P = 0.004$, $I^2 = 9.2\%$, Figure 5; heterozygote comparison: OR: 1.61, 95%CI:1.03-2.52, $P = 0.694$ for heterogeneity, $P = 0.038$, $I^2 = 0.0\%$; dominant model: OR: 1.73, 95%CI:1.13-2.64, $P = 0.718$ for heterogeneity, $P = 0.011$, $I^2 = 0.0\%$) (Table 2).

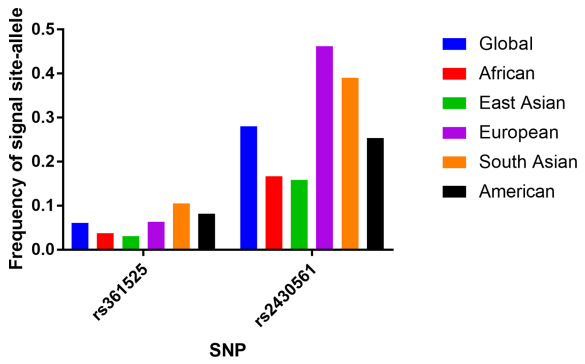

FIGURE 2
Minor (mutant) allele frequencies for rs361525 and rs2430561 polymorphisms based on data from the online 1000 Genomes database.

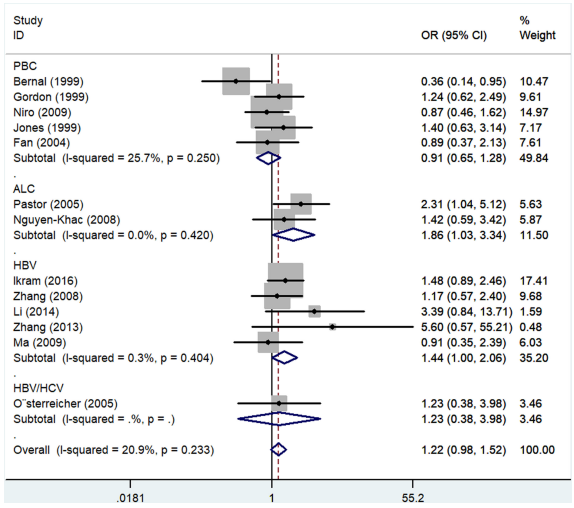

FIGURE 3
Forest plots depicting the association between rs361525 polymorphisms and LC risk in both the ALC and HBV LC subgroups generated using an allelic contrast model.

10.3389/fimmu.2023.1129767

TABLE 2 Stratified analyses of *TNF-α* and *IFN-γ* genes' common polymorphisms on cirrhosis of liver risk.

Variables	N	Case/ Control	Allelic contrast OR(95%CI) P_h *P* I^2	Heterozygote comparison OR(95%CI) P_h *P* I^2	Dominant model OR(95%CI) P_h *P* I^2
rs361525					
Total	13	1023/1458	1.22(0.98-1.52)0.233 0.081 20.9%	1.14(0.88-1.47)0.392 0.338 5.4%	1.18(0.92-1.52)0.299 0.195 14.5%
Ethnicity					
Asian	5	358/443	1.24(0.80-1.92)0.325 0.326 14.0%	1.38(0.86-2.22)0.379 0.186 4.8%	1.32(0.83-2.10)0.343 0.242 11.0%
Caucasian	7	629/815	1.12(0.83-1.51)0.135 0.441 38.6%	1.01(0.72-1.41)0.272 0.952 0.6%	1.07(0.78-1.47)0.190 0.679 31.2%
SOC					
HB	8	688/823	1.15(0.84-1.58)0.571 0.369 0.0%	1.15(0.82-1.62)0.585 0.423 0.0%	1.16(0.83-1.61)0.583 0.389 0.0%
PB	5	335/635	1.24(0.75-2.04)0.059 0.406 56.0%	1.12(0.75-1.67)0.131 0.594 3.6%	1.19(0.67-2.11)0.080 0.563 51.9%
Disease type					
PBC	5	521/744	0.91(0.65-1.28)0.250 0.592 25.7%	0.85(0.59-1.24)0.454 0.410 0.0%	0.88(061-1.26)0.344 0.490 10.9%
ALC	2	110/137	**1.86(1.03-3.34)0.420 0.040 7.1%**	1.60(0.82-3.14)0.242 0.16827.1%	1.78(0.93-3.39)0.323 0.080 0.0%
HBV	5	337/483	**1.44(1.00-2.06)0.404 0.047 0.3%**	1.50(0.93-2.43)0.465 0.598 0.0%	1.52(0.96-2.41)0.433 0.073 0.0%
rs2430561					
Total	9	617/717	1.03(0.71-1.47)0.000 0.891 73.4%	1.26(0.91-1.76)0.270 0.16419.5%	1.14(0.84-1.54)0.048 0.404 1.8%
Ethnicity					
Asian	5	390/494	**1.45(1.13-1.86)0.197 0.00333.6%**	1.43(0.93-2.18)0.463 0.380 0.0%	**1.57(1.05-2.35)0.046 0.028 0.0%**
African	4	227/223	0.65(0.40-1.07)0.023 0.091 68.5%	1.05(0.62-1.78)0.219 0.86932.2%	0.72(0.45-1.16)0.112 0.173 49.9%
SOC					
HB	7	524/537	0.98(0.60-1.57)0.000 0.920 79.5%	1.26(0.88-1.80)0.410 0.213 1.9%	1.02(0.60-1.73)0.044 0.935 53.7%
PB	2	93/180	1.20(0.84-1.72)0.779 0.326 0.0%	1.07(0.18-6.21)0.050 0.93974.0%	1.42(0.61-3.31)0.111 0.410 60.5%
Disease type					
HCV	4	227/223	0.65(0.40-1.07)0.023 0.091 68.5%	1.05(0.62-1.78)0.219 0.86932.2%	0.72(0.45-1.15)0.112 0.073 49.9%
HBV	4	366/420	**1.48(1.13-1.93)0.116 0.004 9.2%**	**1.61(1.03-2.52)0.694 0.038 0.0%**	**1.73(1.13-2.64)0.718 0.011 0.0%**

P_h, value of Q-test for heterogeneity test; P, Z-test for the statistical significance of the OR; HB, hospital-based; PB, population-based; SOC, source of control; PBC, primary biliary cirrhosis; ALC, alcoholic liver cirrhosis; HBV, hepatitis B virus; HCV, hepatitis C virus.
The bold values stand for the significant relationships.

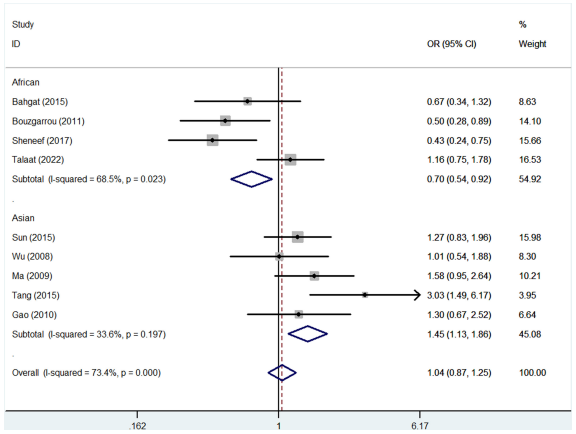

FIGURE 4
Forest plots corresponding to the association between LC risk and rs2430561 polymorphisms using an allelic contrast model in different ethnic subgroups.

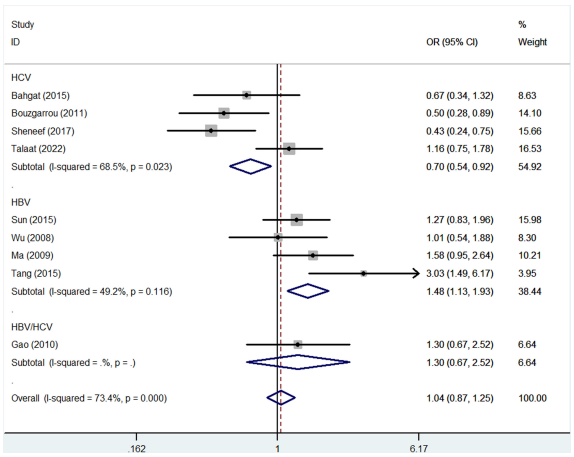

FIGURE 5
Forest plots corresponding to the association between LC risk and rs2430561 polymorphisms in different LC subgroups generated using an allelic contrast model.

Risk of bias and sensitivity analyses

Publication bias was evaluated using Begg's funnel plots and Egger's test. Allele-by-allelic comparisons in funnel plots showed asymmetry for these two polymorphisms, which is not attributable to publication bias. When using an allelic contrast model, Egger's test also showed that there was no publication bias (rs361525: t = 0.6, $P = 0.564$ for Egger's test; z = 0.18, $P = 0.855$ for Begg's test; Figures 6A, B; rs2430561: t = -0.18, $P = 0.861$ for Egger's test; z = 0.31, $P = 0.754$ for Begg's test; Figures 7A, B) (Table 3).

Additionally, sensitivity analysis was performed to explore factors with the potential to impact the stability of these overall analyses, and none of the included case-control studies were found to alter overall study conclusions under the three tested models for either of the analyzed polymorphisms (Figures 6C, 7C).

Protein-protein interaction network

Accordingly, the STRING database was used to identify other genes potentially associated with TNF-α or IFN-γ, with these genes then being used to establish putative protein-protein interaction networks (Figure 8). The majority of genes identified through this approach were associated with cytokines (TNFRSF1A, TNFRSF1B, TRADD, TRAF2, IL-10, TNFAIP3, IFNGR1, IFNGR2, IRF1, SOCS1), exhibiting interaction scores> 0.9 consistent with very close relationships.

Discussion

Cirrhosis is the final progressive stage of hepatic fibrosis, and most cases of LC develop in patients with major risk factors such as chronic alcohol abuse or hepatitis caused by viral infections. Histologically, LC is characterized by extensive extracellular matrix material accumulating within the liver parenchyma including very high levels of fibrillar collagen, ultimately compromising the structural integrity and functional utility of this tissue (51).

LC is also closely associated with an elevated risk of hepatocellular carcinoma (HCC), with is the most prominent form of liver cancer in humans, resulting in high rates of morbidity and mortality owing to a lack of effective therapeutic interventions (52). The present study was developed to explore efforts to more effectively diagnose LC and reduce the medical burden associated with this disease by lowering patient mortality rates.

Short–chain fatty acids can influence the production of TNF-α and diverse other inflammatory mediators in response to signaling through the TLR4 pathway, playing important roles in inflammatory and fibrotic activity in the liver through the upregulation of TIMP-1 in activated hepatic stellate cells (HSCs) and through the inhibition of HSC apoptosis (53).

NK-derived IFN-γ can suppress the fibrotic differentiation of HSCs (54), in addition to suppressing proliferative activity, as well as the expression of α-SMA TGF-β (55). In certain contexts, IFN-γ can also promote activated HSC death through mechanisms

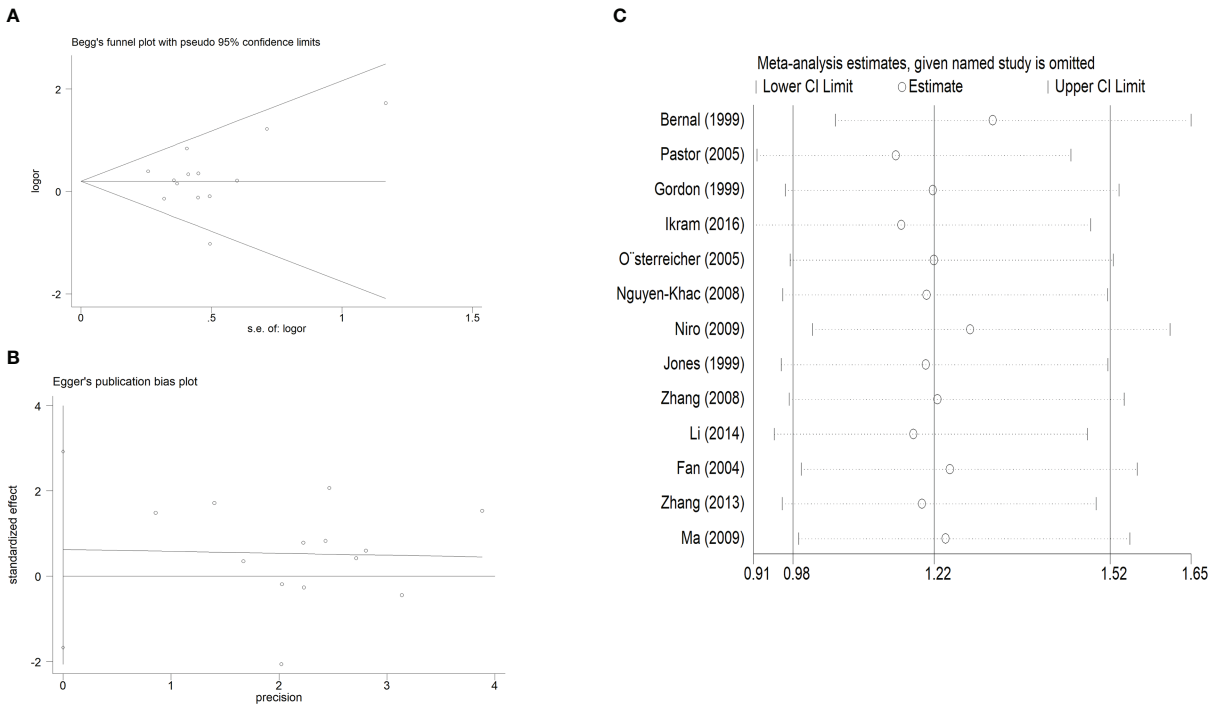

FIGURE 6
(A). Begg's funnel plot test for publication bias, with individual points corresponding to specific studies, and the mean effect size being represented by the horizontal axis. Log [OR], natural logaritm of OR. **(B)**. Egger's publication bias plot. **(C)**: Sensitivity analysis for the association between *TNF-α* rs361525 polymorphisms and LC risk.

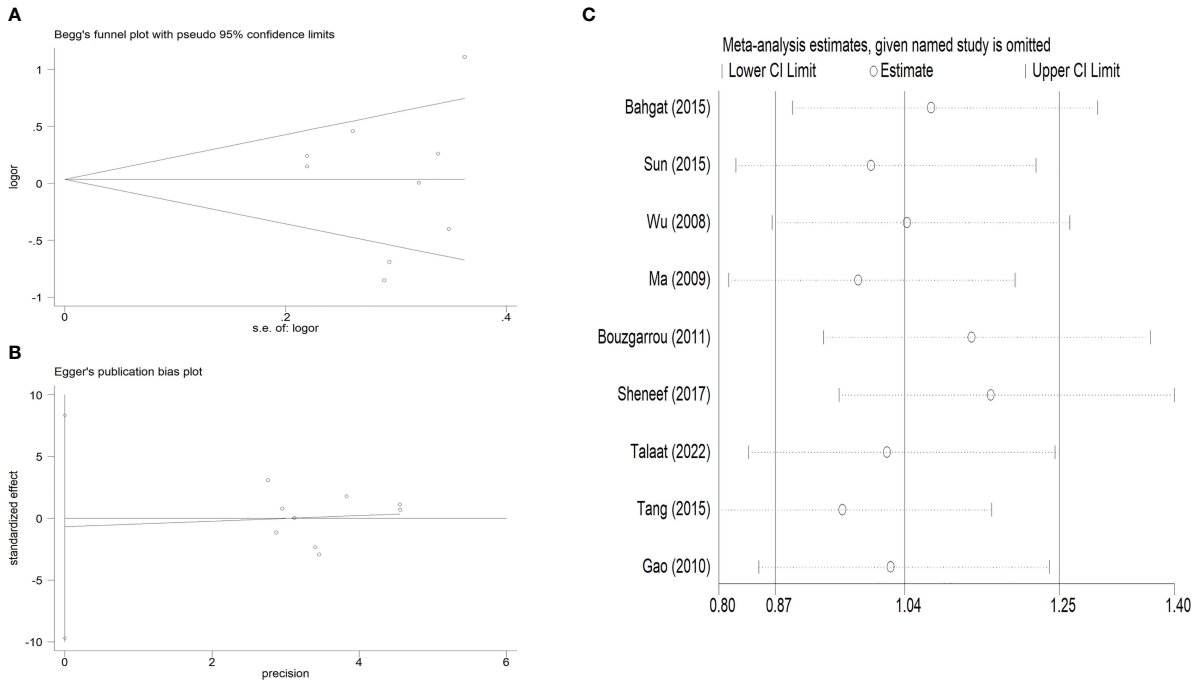

FIGURE 7
(A). Begg's funnel plot test for publication bias. **(B)**. Egger's publication bias plot. **(C)**: Sensitivity analysis for the association between *IFN-γ* rs2430561 polymorphisms and LC risk.

TABLE 3 Publication bias tests (Begg's funnel plot and Egger's test for publication bias test) for *TNF-α* and *IFN-γ* genes polymorphisms.

Egger's test						Begg's test	
Genetic type	Coefficient	Standard error	*t*	*P* value	95%CI of intercept	*z*	*P* value
rs361525							
A-allele vs. G-allele	0.622	1.045	0.6	0.564	(-1.678- 2.923)	0.18	0.855
AG vs. GG	1.239	1.083	1.14	0.277	(-1.146- 3.624)	0.67	0.502
AA+AG vs. GG	1.357	1.125	1.21	0.253	(-1.119- 3.833)	0.67	0.502
rs2430561							
A-allele vs. T-allele	0.692	3.815	-0.18	0.861	(-9.712- 8.328)	0.31	0.754
AT vs. TT	0.709	1.221	-0.58	0.58	(-3.597- 2.179)	1.36	0.175
AA+AT vs. TT	0.409	1.552	-0.26	0.8	(-4.079- 3.261)	0.94	0.348

mediated by the FasL-associated and TRIAL death domains (55). Prolonged HSC activation can contribute to LC incidence.

To date, a range of genes have been linked to the incidence of liver disease including CTLA-4, IL-18, TM6SF2, and GSTM1(10, 56, 57). Inflammation and fibrotic activity are particularly closely associated with LC risk, and various studies have accordingly highlighted possible links between *TNF-α* and *IFN-γ* SNPs and LC incidence. However, many of these studies were based on relatively small sample sizes such that their conclusions were not necessarily robust.

Pastor et al. (26) previously observed a link between the *TNF-α* rs361525 (-238) polymorphism and elevated alcoholic LC risk, while Bouzgarrou et al. (8) similarly observed a higher risk of LC incidence associated with the *IFN-γ* rs2430561 (+874) polymorphism. However, it is critical that these prior studies be combined and that analyses with larger sample sizes be performed to comprehensively examine the link between *TNF-α* and *IFN-γ* SNPs and LC risk.

In this study, in an effort to more fully solidify these relationships between *TNF-α* and *IFN-γ* SNPs and the odds of

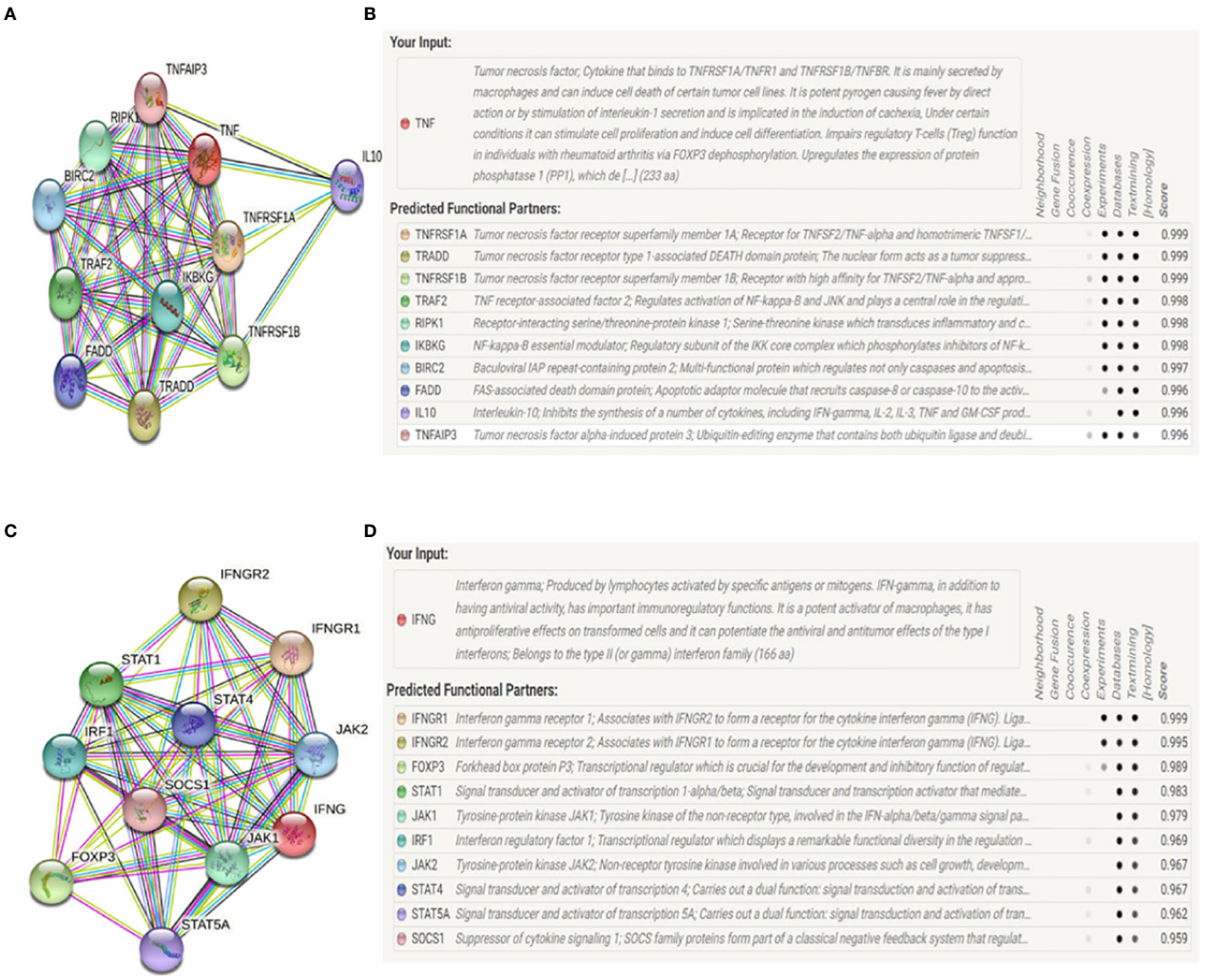

FIGURE 8
STRING network diagrams depicting interactions between human TNF-α or IFN-γ and other genes. **(A, B)**. At least 10 genes were correlated with: TNFRSF1A: tumor necrosis factor receptor superfamily member 1A; TNFRSF1B: tumor necrosis factor receptor superfamily member 1B; TRADD: tumor necrosis factor receptor type 1-associated DEATH domain protein; TRAF2: TNF receptor-associated factor 2; RIPK1: receptor-interacting serine/threonine-protein kinase 1; FADD: FAS-associated death domine protein; IKBKG: NF-kappa-B essential modulate; BIRC2: baculoviral IAP repeat-containing protein; IL-10: interleukin-10; TNFAIP3: tumor necrosis factor alpha-induced protein 3. **(C, D)**. At least 10 genes were correlated with: IFNGR1: IFN-γ receptor 1; IFNGR2: IFN-γ receptor 2; FOXP3: forkhead box protein P3; JAK1: tyrosine-protein kinase JAK1; JAK2: tyrosine-protein kinase JAK2; IRF1: interferon regulatory factor 1; STAT1: signal transducer and activator of transcription 1-alpha/beta; STAT4: signal transducer and activator of transcription 4; STAT5A: signal transducer and activator of transcription 5A; SOCS1: suppressor of cytokine signaling 1.

developing LC, a database search was conducted that ultimately identified 13 and 9 case-control studies respectively associated with the *TNF-α* rs361525 and *IFN-γ* rs2430561 polymorphisms. The pooled analysis of these results ultimately revealed that *TNF-α* rs361525 was linked to LC risk in the ALC and HBV subgroups, while *IFN-γ*rs2430561 was more strongly related to the risk of LC in HBV patients and Asian populations, potentially providing a valuable foundation for future efforts to prevent, diagnose, and treat LC in its earlier stages. This is a study that we need to pay attention to in the future, which is only aimed at determining the polymorphisms (rs361525, rs2430561) of two susceptible genes (TNF-α and IFN-γ) for LC. Based on this conclusion, physical examination of healthy people can be conducted to identify the population with gene mutations, and early intervention, observation, and follow-up can be conducted in such aspects as diet, behavioral habits, and emotional regulation to observe the risk of LC in such people, and further to verify the conclusion in this study, We wish to reduce the incidence rate and the mortality due to disease of LC through this exploration.

LC develops and progresses through a complex multi-factorial process, and the impact of any individual gene on the overall disease process may thus be limited. The STRING online database helps us to find potential interactive genes. The hub gene TNFRSF1A may offer value as a marker of PBC (58), and one GWAS study of 1,840 cases and 5,163 controls identified the TNFRSF1A rs1800693 SNP as a PBC-related risk factor (OR=1.23, 95%CI=1.14-1.33) (59). Peng et al. found TRADD expression to be associated with HBV-related LC and HCC incidence (60), while Lin et al. determined that

LC model rats exhibited increased TRAF2 protein content in skeletal muscle samples (61). Moreover, Tan et al. found that RIPK1 acts as a promoter of inflammatory activity in the setting of HCC onset and liver fibrosis (62), and Higuchi et al. reported a link between deleterious TNFAIP3 alleles and autoimmune hepatitis with cirrhosis (63). While Falleti et al. identified two IFNGR1 gene polymorphisms, they failed to find them to be significantly associated with disease (64). In contrast, two IFNGR2 polymorphisms identified by Nalpas et al. that were in strong linkage disequilibrium were found to be closely related to the incidence of liver fibrosis (65). Ramadan et al. determined that reductions in CD4/FoxP3/CD25 levels were evident in patients treated with direct-acting antivirals, contributing to better immunological outcomes in the context of LC (66). There is a strong body of evidence in support of the role of cytokine-induced JAK/STAT signaling serving as a regulator of hepatic fibrosis and regenerative activity (67). Zhu et al. found sennoside A to be capable of inhibiting SOCS1 and decreasing HSC proliferation in liver fibrosis through the inhibition of inflammation (68). Accordingly, additional work should focus on the relationships between *TNF-α*- and *IFN-γ*-associated genes, gene-gene interactions, and the pathogenesis of LC.

This study is subject to some limitations. For one, additional work focused on African and mixed populations is needed as these groups were underrepresented in studies published to date. In addition, LC is a complex multifactorial disease, and it is thus important that both gene-gene and gene-environment interactions be taken into consideration when examining the etiology of this disease. Particular lifestyle and environmental factors including sex, age, diet, smoking status, family history, history of parasite exposure, immunological factors, and viral infections may all shape the relationship between *TNF-α* or *IFN-γ* polymorphisms and LC. Further work is also necessary to assess whether LC patients face other complications including autoimmunity, hepatitis, abnormal liver function, and HCC incidence. These analyses also did not classify LC stage or pathogenic origin, highlighting important directions for additional study to more precisely guide the prevention and treatment of this disease. Furthermore, we only explored possible interacting genes through online databases, but through literature searches, we have not found that the interactions among 20 genes and the TNF-α or IFN-γ, which is also a topic for future research. Overall, this meta-analysis found some evidence for an association between *TNF-α* rs361525 and *IFN-γ* rs2430561 polymorphisms and the risk of LC suggesting that they may represent viable targets for clinical research aimed at predicting the future risk of severe liver disease. In the future research, we will also try to study a case-control study in our own cohort to confirm current conclusions.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

MZ, JL and WF: design research program, collect data, analyze statistics and write articles. LL, RD and HZ: collect data. HL: review the collected data. XL and CD: research design and paper review. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Hepatic encephalopathy increases the risk for mortality and hospital readmission in decompensated cirrhotic patients: a prospective multicenter study

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Introduction: Hepatic encephalopathy (HE) affects the survival and quality of life of patients with cirrhosis. However, longitudinal data on the clinical course after hospitalization for HE are lacking. The aim was to estimate mortality and risk for hospital readmission of cirrhotic patients hospitalized for HE.

Methods: We prospectively enrolled 112 consecutive cirrhotic patients hospitalized for HE (HE group) at 25 Italian referral centers. A cohort of 256 patients hospitalized for decompensated cirrhosis without HE served as controls (no HE group). After hospitalization for HE, patients were followed-up for 12 months until death or liver transplant (LT).

Results: During follow-up, 34 patients (30.4%) died and 15 patients (13.4%) underwent LT in the HE group, while 60 patients (23.4%) died and 50 patients (19.5%) underwent LT in the no HE group. In the whole cohort, age (HR 1.03, 95% CI 1.01–1.06), HE (HR 1.67, 95% CI 1.08–2.56), ascites (HR 2.56, 95% CI 1.55–4.23), and sodium levels (HR 0.94, 95% CI 0.90–0.99) were significant risk factors for mortality. In the HE group, ascites (HR 5.07, 95% CI 1.39–18.49) and BMI (HR 0.86, 95% CI 0.75–0.98) were risk factors for mortality, and HE recurrence was the first cause of hospital readmission.

Conclusion: In patients hospitalized for decompensated cirrhosis, HE is an independent risk factor for mortality and the most common cause of hospital readmission compared with other decompensation events. Patients hospitalized for HE should be evaluated as candidates for LT.

KEYWORDS

hepatic encephalopathy, decompensated cirrhosis, orthotopic liver transplant, hospital readmission, mortality

Introduction

Hepatic encephalopathy (HE) is a well-known relevant complication of cirrhosis, and it includes a wide spectrum of neuropsychiatric abnormalities ranging from subclinical alterations to coma (1). Although the prevalence and cumulative incidence of HE are difficult to assess, it has been estimated that 35–45% of patients with cirrhosis could experience overt HE, with an incidence of 11.6 per 100 person-years, rising to 40% by 5 years (2, 3). HE has a significant impact on the risk of hospitalization, accidental trauma, and survival, with a mortality rate of ∼60% at 1 year (4–6). HE has a significant impact not only on survival but also on the quality of life of patients and their caregivers, and it is associated with a significant financial burden for healthcare systems, increasing both direct and indirect costs (7–9). Current management strategies include the identification, prevention, and treatment of precipitating factors, the use of lactulose and rifaximin, both as prophylaxis and treatment measures, and finally liver transplantation in patients with end-stage liver disease and recurrent or persistent HE not responding to other treatments (1).

Despite its clinical, social, and economic relevance, data on the impact of HE on the clinical course of patients with decompensated cirrhosis still remain scanty. Particularly, data on the risk for HE recurrence come mainly from small retrospective studies, not specifically designed for this purpose and without a clearly defined inception point. Moreover, although it is well known that a substantial proportion of episodes of HE is related to the presence of precipitating factors, such as constipation, infections, gastrointestinal bleeding, and diuretic overuse, the relative weight of these precipitating events, as well as their prevalence, remain poorly known (10).

Therefore, prospective data collected from a well-defined inception point (i.e., hospitalization for HE with or without a history of HE) evaluating the impact of HE on mortality, the access to orthotopic liver transplant (OLT), and the risk of hospital readmission are lacking. Most of the previously published studies are affected by their retrospective design, the administrative nature of data, or the inclusion of patients with heterogeneous characteristics, in terms of severity of liver disease and characteristics of HE. Moreover, an accurate estimate of the impact of HE recurrence as a decompensation event leading to

further hospitalization is relevant, in order to assess the cost-effectiveness of secondary prophylaxis strategies.

This multicenter prospective study, including a cohort of patients with decompensated cirrhosis hospitalized for HE, aimed to estimate the mortality, the rate of access to OLT, and the risk for hospital readmission in patients with HE compared with patients with other decompensation events of cirrhosis.

Materials and methods

Study design and patients

This observational, prospective, multicenter, and investigator-driven cohort study enrolled all consecutive patients with cirrhosis hospitalized for grade ≥ 2 HE [according to the West Haven criteria (11)] at 25 Italian centers between January 2019 and November 2020 (HE group). Another cohort of patients hospitalized for decompensated cirrhosis (i.e., ascites, portal hypertensive bleeding, and jaundice), without HE at the time of hospitalization and during the previous 12 months, observed at the same centers during the same period served as the control group (no HE group).

Exclusion criteria were as follows: (a) age lower than or equal to 17 years; (b) age higher than or equal to 79 years; (c) non-cirrhotic portal hypertension; (d) acute liver failure; (e) psychiatric or neurodegenerative diseases; (f) alteration of mental status not related to HE; and (g) participation to clinical trials evaluating therapeutic interventions for HE.

Participating centers were chosen on the basis of their recognized expertise as high-volume tertiary referral centers for the management of patients with decompensated cirrhosis.

Patient data were recorded by treating clinicians from each participating center at the inception point (i.e., the index hospitalization for HE or other complications of cirrhosis in patients with or without history of liver decompensation). Data were collected in a dedicated and anonymized electronic case report form (CRF), shared by all the participating centers. Demographics, educational level, working activities, body mass index (BMI), etiology of cirrhosis, biochemistry (liver function tests, serum creatinine, sodium, and venous blood ammonia), presence and severity of ascites and HE, Child-Pugh class, model for end-stage liver disease (MELD) and MELD-Na scores, presence and severity of portal hypertension (esophageal and/or gastric varices, portal vein and spleen diameters, portosystemic shunts), portal vein thrombosis, hepatocellular carcinoma (HCC), and previous pharmacological treatments were recorded.

The diagnosis of HE was performed according to the guidelines of American Association for the Study of Liver Diseases and the European Association for the Study of the Liver (12) and by using a flow-chart for differential diagnosis between HE and other causes of neuropsychiatrist alterations, not related to HE (see Supplementary material).

The presence of esophageal and/or gastric varices was evaluated by upper endoscopy. Portal vein and spleen diameters were evaluated by abdominal ultrasound. Porto-systemic shunts and portal vein thrombosis were evaluated by abdominal contrast-enhanced computed tomography (CT), when available. The diagnosis of HCC was performed according to the European guidelines (13).

In the HE group, data on the previous history of HE, the clinical course of previous HE [episodic, recurrent, or persistent, defined according to the International Guidelines (12)], previous hospitalizations, grading of HE according to the West Haven criteria (11), animal name testing (ANT), Glasgow Coma Scale (GCS), duration of HE episode, number and types of precipitating factors, treatments received, and secondary prophylaxis were recorded. The definition of precipitating factors is presented in Supplementary material.

The study was approved by the Institutional Review Board (IRB) of the Fondazione Agostino Gemelli Hospital. Informed consent to participate in the study was acquired according to the following modalities: (a) patients with preserved state of consciousness gave their written consent to participate in the study at the time of enrollment; (b) patients with HE at the time of enrollment who were unable to express consent have been registered as potential candidates without extracting data from the medical record, and a retroactive consent has been collected from the patient at the time of the recovery of cognitive conditions, or from his legal representative in case of persistence of inability to provide consent; (c) for patients who died following the HE episode without expressing consent, an authorization from the ethics committee for the exemption from informed consent for the use of data, were obtained.

Follow-up and outcomes

After the index hospitalization, all patients were followed-up for 12 months or until death or orthotopic liver transplant (OLT). Follow-up protocol included telephone follow-up evaluations performed every month by treating clinicians and medical reviews including physical examination (including assessment of HE and its grading) and biochemistry evaluation every 3 months. During follow-up, causes and dates of hospital readmissions were recorded.

The primary outcome was mortality, with OLT considered as a competing event.

The secondary outcome was hospital readmission for further decompensation events both in the HE and no HE groups. Further decompensation events were defined as HE, ascites, infections, and portal hypertensive bleeding. Infections were defined as spontaneous bacterial peritonitis, urinary tract infections, pneumonia, and bacteremia.

Statistical analysis

Data for continuous variables are expressed as mean (standard deviation) or median (interquartile range). Data for categorical variables are expressed as frequency (percentage).

Mortality and OLT were evaluated by competing risks survival analysis and represented by cumulative incidence function (CIF) (14). Cox cause-specific model was fitted in order to estimate the effect of covariates on mortality. Covariates used for multivariable analyses were chosen based on their significance in the univariate analysis ($p < 0.10$). Covariates in the final model with a p-value of <0.05 were considered statistically significant. The results are presented as adjusted hazard ratios (HR) and their 95%

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TABLE 1 Baseline characteristics of 368 cirrhotic patients admitted to hospital for hepatic encephalopathy (HE) (HE group) or for other decompensating events (no HE group).

Variable	Overall cohort ($N = 368$)	HE group ($n = 112$)	No HE group ($n = 256$)	p-value
Age (years)	60.4 ± 10.4	62.3 ± 8.3	59.6 ± 11.1	0.03
Male sex (%)	266 (72.3)	91 (81.2)	175 (68.4)	0.01
BMI (kg/m^2)	26.9 ± 5.6	25.7 ± 4.3	27.4 ± 5.9	0.02
Education (%)				0.75
Primary school	111 (30.2)	38 (33.9)	73 (28.5)	
Middle school	137 (37.2)	37 (33.0)	100 (39.1)	
High school	93 (25.3)	28 (25.0)	65 (25.4)	
University	18 (4.9)	6 (5.4)	12 (4.7)	
Other	9 (2.4)	3 (2.7)	6 (2.3)	
Job (%)				0.31
Clerk	12 (3.3)	3 (2.7)	9 (3.5)	
Freelance	18 (4.9)	4 (3.6)	14 (5.5)	
Manager	4 (1.1)	1 (0.9)	3 (1.2)	
Workman	93 (25.3)	25 (22.3)	68 (26.6)	
Retired	133 (36.1)	50 (44.6)	83 (32.4)	
Unemployed	108 (29.3)	29 (25.9)	79 (30.9)	
Etiology of liver disease (%)				0.34
HCV	56 (15.2)	18 (16.1)	38 (14.8)	
HBV	27 (7.3)	5 (4.5)	22 (8.6)	
Alcohol	140 (38.0)	42 (37.5)	98 (38.3)	
Viral + alcohol	51 (13.9)	14 (12.5)	37 (14.5)	
Metabolic	44 (11.9)	11 (9.8)	33 (12.9)	
Autoimmune hepatitis	11 (3.00)	6 (5.4)	5 (1.9)	
Biliary diseases	13 (3.5)	5 (4.5)	8 (3.1)	
Others	26 (7.1)	11 (9.8)	15 (5.9)	
Complications and severity of cirrhosis				
Ascites absent	117 (31.8)	36 (32.1)	81 (31.6)	0.09
Grade 1–2 ascites	177 (48.1)	62 (55.4)	115 (44.9)	
Grade 3–4 ascites	74 (20.1)	14 (12.5)	60 (23.4)	
Portal thrombosis**	44 (20.6)	16 (27.1)	28 (18.2)	0.15
Presence of TIPS	16 (4.3)	11 (9.8)	5 (2.0)	<0.001
Esophageal varices absent	104 (28.2)	30 (26.7)	74 (28.9)	0.87
F1 esophageal varices	117 (31.8)	42 (37.5)	75 (29.3)	
F2 Esophageal varices	119 (32.3)	31 (27.7)	88 (34.4)	
F3 Esophageal varices	28 (7.6)	9 (8.0)	19 (7.4)	
GOV	39 (10.6)	12 (10.7)	27 (10.5)	
IGV	8 (2.2)	2 (1.7)	6 (2.3)	
Hepatocellular carcinoma**	72 (33.8)	12 (20.3)	60 (39.0)	0.01
Child-Pugh score	8.7 ± 2.1	9.9 ± 1.8	8.2 ± 2.0	<0.001

(Continued)

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TABLE 1 (Continued)

Variable	Overall cohort ($N = 368$)	HE group ($n = 112$)	No HE group ($n = 256$)	p-value
Child-Pugh class A	67 (18.2)	5 (4.5)	62 (24.2)	<0.001
Child-Pugh class B	158 (42.9)	40 (35.7)	118 (46.1)	
Child-Pugh class C	143 (38.9)	68 (60.7)	75 (29.3)	
MELD score	17.8 ± 7.6	20.2 ± 7.4	16.7 ± 7.5	<0.001
MELD-Na score	19.4 ± 7.8	21.8 ± 7.4	18.3 ± 7.7	<0.001
Biochemistry				
Hemoglobin (g/dL)	10.3 ± 1.9	10.0 ± 2.0	10.4 ± 1.9	0.05
Haematocrit (%)	31 ± 5.7	29.5 ± 5.6	31.6 ± 5.7	0.002
WBC (mmc)	5,660 ± 3,798	5,978 ± 3,234	5,521 ± 4,017	0.29
PLT (10^9/L)	93.6 ± 63.7	85.6 ± 58.1	97.0 ± 65.8	0.12
ALT (U/mL)	43 ± 51	40 ± 31	44 ± 57	0.59
AST (U/mL)	64 ± 75	60 ± 68	66 ± 78	0.5
GGT (U/mL)	109 ± 138	80 ± 76	120 ± 155	0.01
Total bilirubin (mg/dL)	4.6 ± 6.4	5.3 ± 6.3	4.2 ± 6.4	0.14
Creatinine (mg/dL)	1.05 ± 0.7	1.14 ± 0.71	1.01 ± 0.69	0.11
Albumin (g/dL)	3.1 ± 0.6	2.9 ± 0.5	3.1 ± 0.6	0.01
INR	1.6 ± 0.4	1.6 ± 0.5	1.5 ± 0.4	0.1
Sodium (mEq/L)	137 ± 4	136 ± 5	137 ± 4	0.29
Venous blood ammonia (μmol/L)	109 ± 69	130 ± 76	92 ± 59	<0.001
Non-invasive indicators of portal hypertension				
Portal vein diameter (mm)*	13.4 ± 7.8	13.3 ± 3.0	13.8 ± 2.7	0.3
Spleen diameter (cm)**	15.5 ± 3.1	15.7 ± 3.3	15.4 ± 3.0	0.59
Porto-systemic shunts**	90 (42.2)	33 (55.9)	57 (37.0)	0.02
Ongoing pharmacological treatments (%)				
Propranolol	120 (32.6)	31 (27.7)	89 (34.8)	0.41
Carvedilol	47 (12.8)	17 (15.2)	30 (11.7)	0.64
Furosemide	250 (67.9)	81 (72.3)	169 (66.0)	0.45
Spironolactone	31 (8.4)	11 (9.8)	20 (7.8)	0.8
Canrenone	85 (23.1)	25 (23.2)	60 (23.4)	0.96
Disaccharides	218 (59.2)	91 (81.3)	127 (49.6)	<0.001
Rifaximin	151 (41.0)	66 (58.9)	51 (19.9)	<0.001
PPI	224 (60.9)	75 (67.0)	149 (58.2)	0.26
Albumin	79 (21.5)	27 (24.1)	52 (20.3)	0.7
Branched-chain aminoacids	22 (6.0)	11 (9.8)	11 (4.3)	0.12

The results are presented as mean ± standard deviation for continuous variables or absolute number (percentage) for categorical variables.
HE, hepatic encephalopathy; HCV, hepatitis C virus; HBV, hepatitis B virus; BMI, body mass index; WBC, white blood cell; PLT, platelet; ALT, alanine aminotransferases; AST, aspartate aminotransferases; GGT, gamma-glutamil transferase; INR, international normalized ratio; MELD, model for end-stage liver disease; GOV, gastro-esophageal varices; IGV, isolated gastric varices; TIPS, transjugular intrahepatic porto-systemic shunt; PPI, proton pump inhibitors.
*Assessed by ultrasonography.
**Assessed by computed tomography (available in 213 patients: 59 patients with HE and 154 patients without HE).

confidence intervals (CI). Composite covariates (i.e., Child–Pugh class, MELD) were not included in the multivariable model to avoid collinearity with the individual score items. To take into account the between-center heterogeneity, a multivariable model including the center as a covariate was fitted, with centers categorized according to the number of enrolled patients (10 or less patients, between 11 and 20 patients, and more than 20 patients).

Risk factors for mortality identified by competing risks multivariable analysis were used to generate a prediction rule. The predicted probability of dying was computed for a hypothetical patient, identified by a combination of prognostic factors.

The probability of hospital readmission for further decompensating events was evaluated by competing risks analysis, represented by CIF. In this analysis, the event of interest was the first decompensation event that occurred during the follow-up, leading to hospital readmission.

Details on sample size calculation are presented in Supplementary material.

All analyses were performed in R core Team (version 4.0.3).

Results

Baseline

The study flowchart is shown in Supplementary Figure S1. The final study population included 368 patients (HE group: 112 patients; no HE group: 256 patients), and their baseline characteristics are shown in Table 1. The mean age was 60.4 ± 10.4, and 72% of patients were male. Alcohol-related liver disease was the most common etiology (38%), followed by HCV infection (15%). Most of the patients had Child–Pugh classes B and C (43 and 39%, respectively), and the mean MELD score was 17.8 ± 7.6. Esophageal varices were present in 72% of patients. CT scan was available in 213 patients, and porto-systemic shunts and portal vein thrombosis were found, respectively, in 42% and 20% of patients who underwent CT scan.

Compared with the no HE group, patients in the HE group were significantly older, more frequently male, and they had a significantly poorer nutritional status (lower BMI and albumin levels). The HE group displayed signs of more advanced liver disease, as shown by a significantly higher prevalence of Child–Pugh class C and higher MELD and MELD-Na scores. Venous blood ammonia levels were significantly higher in the HE group compared with the no HE group. Porto-systemic shunts and TIPS were significantly more frequent in the HE group.

The main clinical features and treatments of the HE group are shown in Table 2. Most of the patients (59%) had a previous history of HE, which was episodic in 13%, recurrent in 42%, and persistent in 4% of patients. Conversely, 41% of patients were at the first HE episode. At least one precipitating factor was identified in 89 patients (79.5%). The most common precipitating factors were constipation (29.5%), infections (20.5%), and diuretics overuse (17%). Most of the patients (65%) had grade 2 HE, median ANT was 6, and median GCS was 14. The most common treatments used were lactulose (oral and enema in 72% and 53% of patients, respectively) and rifaximin in 67% of patients. The median duration of the HE episode was 1.8 days. During hospitalization, HE resolved in 92.8% of patients and recurred in 4.5% of patients, while 2.7% of patients died. Most of the patients (81%) received secondary prophylaxis, including rifaximin in 73% and non-absorbable disaccharides in 61% of patients.

TABLE 2 Clinical features and treatments in 112 cirrhotic patients hospitalized for hepatic encephalopathy (HE group).

	Patients with HE ($n = 112$)
First HE episode (*n*, %)	46 (41.1)
Previous history of HE (*n*, %)	66 (58.9)
Episodic HE	15 (13.4)
Recurrent HE	47 (42.0)
Persistent HE	4 (3.6)
Number of HE episodes during last 6 months (median, range)	2 (1-6)
Precipitating events	
No precipitating events (*n*, %)	23 (20.5)
Precipitating events (*n*, %)	89 (79.5)
Constipation	33 (29.5)
Infection	23 (20.5)
Diuretics/dehydration	19 (17.0)
Gastrointestinal bleeding	14 (12.5)
HE staging	
Animal name testing (ANT) (median, range)	6 (0–19)
ANT < 10 (*n*, %)	85 (75.9)
Glasgow Coma Scale (GCS) (median, range)	14 (3–15)
HE grade (*n*, %)	
Grade 2	73 (65.2)
Grade 3	36 (32.1)
Grade 4	3 (2.7)
Treatments for HE episode (*n*, %)	
Lactulose	81 (72.3)
Rifaximin	75 (67.0)
Lactulose enema	60 (53.6)
Albumin	46 (41.1)
Branched-chain aminoacids	44 (39.3)
Systemic antibiotics	34 (30.4)
Lactitol	11 (9.8)
Fasting	8 (7.1)
Nutritional support	4 (3.6)
Low protein diet	3 (2.7)
Treatments for precipitating event (*n*, %)	
Treatments for infection	
Piperacillin/tazobactam	8 (7.1)
Ceftriaxone	7 (6.3)
Ciprofloxacin	4 (3.6)
Cefotaxime	2 (1.8)
Meropenem	2 (1.8)
Fosfomycin	2 (1.8)

(Continued)

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TABLE 2 (Continued)

	Patients with HE ($n = 112$)
Cefepime	1 (0.9)
Ceftobiprole	1 (0.9)
Tigecycline	1 (0.9)
Not specified antibiotics	4 (3.6)
Treatments for electrolytes alteration	
Sodium or potassium replacement	5 (4.5)
Treatments for gastrointestinal bleeding	
Blood transfusion	4 (3.6)
Vasoactive treatment (somatostatin or terlipressin)	4 (3.6)
Endoscopic variceal ligation	3 (2.7)
Endoscopic treatment of peptic ulcer	3 (2.7)
Endoscopic treatment of GAVE	1 (0.9)
Treatments for diuretic overuse	
Diuretic discontinuation	8 (7.1)
Diuretic dose reduction	7 (6.3)
Treatments for constipation	
Lactulose enema	36 (32.1)
Oral lactulose	23 (20.5)
Treatments for dehydration	
Intravenous fluid infusion	17 (15.2)
Outcomes of HE episode during hospitalization (*n*, %)	
Resolution	104 (92.8)
Recurrence of HE	5 (4.5)
Death	3 (2.7)
Secondary prophylaxis after HE episode (*n*, %)	88 (80.7)
Rifaximin	80 (73.3)
Non-adsorbable disaccharides	67 (61.5)
Low-protein diet	11 (10.0)
Vegetal protein diet	8 (7.3)
Branched-chain Aminoacids	2 (1.8)

HE, hepatic encephalopathy; GAVE, Gastric Antral Vascular Ectasia.

TABLE 3 Outcomes of 368 hospitalized patients with decompensated cirrhosis according to the presence or absence of hepatic encephalopathy (HE).

	Overall cohort ($N = 368$)	HE group ($n = 112$)	No HE group ($n = 256$)
Death (*n*, %)	94 (25.5)	34 (30.4)	60 (23.4)
Liver transplant (*n*, %)	65 (17.7)	15 (13.4)	50 (19.5)
At least one hospital readmission (*n*, %)	107 (29.1)	39 (34.8)	68 (26.6)
Number of hospital readmissions			
1	66 (17.9)	23 (20.5)	43 (16.8)
2	25 (6.8)	9 (8.0)	16 (6.3)
≥3	16 (4.3)	7 (6.3)	9 (3.5)
Reasons of hospital readmissions (*n*, %)			
HE	51 (13.9)	24 (21.4)	27 (10.5)
Ascites	42 (11.4)	15 (13.4)	27 (10.5)
Infections	17 (4.6)	4 (3.6)	13 (5.1)
Portal hypertensive bleeding	18 (4.9)	3 (2.7)	15 (5.9)

HE, hepatic encephalopathy.

Outcomes

Mortality

Outcomes during follow-up are shown in Table 3. In the whole cohort, 94 patients (25.5%) died and 65 patients (17.7%) underwent OLT. Cumulative probabilities of death and OLT are shown in Supplementary Figure S2. Six- and twelve-month probabilities of death were 22.4% and 29.1%, respectively, and 6- and 12-month probabilities of OLT were 14.9% and 21.2%, respectively.

In the HE group, 34 patients (30.4%) died and 15 patients (13.4%) underwent OLT. In the no HE group, 60 patients (23.4%) died and 50 patients (19.5%) underwent OLT. Figure 1 showed probabilities of death in the HE and no HE groups. Six- and twelve-month probabilities of death were higher in the HE group [34.3% (95% CI 30.4–37.5%) and 40.8% (95% CI 38.4–43.2%), respectively] than in the no HE group [18.7% (95% CI 14.8–21.3%) and 26.3% (95% CI 22.4–28.5%), respectively]. Six- and twelve-month probabilities of OLT were 10.0% (95% CI 8.8–13.4%) and 16.3% (95% CI 13.8–17.9%) in the HE group, respectively, and 16.5% (95% CI 12.4–17.8%) and 22.9% (95% CI 19.8–24.8%) in the no HE group (Supplementary Figure S3).

Univariate analysis of risk factors for death and OLT is presented in Supplementary Table S1. In the multivariable model, four variables were independently associated with mortality as follows: age (HR 1.03, 95% CI 1.01–1.06, $p = 0.018$), HE (HR 1.67, 95% CI 1.08–2.56, $p = 0.020$), sodium (HR 0.94, 95% CI 0.90–0.99, $p = 0.013$), and presence of ascites (HR 2.56, 95% CI 1.55–4.23, $p = 0.012$) (Table 4). Similar results were obtained when HE status was codified as first or episodic HE (i.e., patients at first HE episode or with a history of episodic HE. HR 1.82, 95% CI 1.02–3.27, $p = 0.043$) and recurrent or persistent HE (HR 1.80, 95% CI 1.05–3.10, $p = 0.034$), with the no HE group as reference (Table 4). Moreover, age (HR 1.03, 95% CI 1.01–1.05, $p = 0.031$), HE (HR 1.62, 95% CI 1.01–2.58, $p = 0.043$), ascites (HR 2.75, 95% CI 1.59–4.76, $p < 0.001$), and sodium (HR 0.95, 95% CI 0.91–0.99, $p = 0.019$) were confirmed as independent predictors of mortality when covariates significantly different between the HE and no HE groups, and potentially affecting survival was included in the multivariate

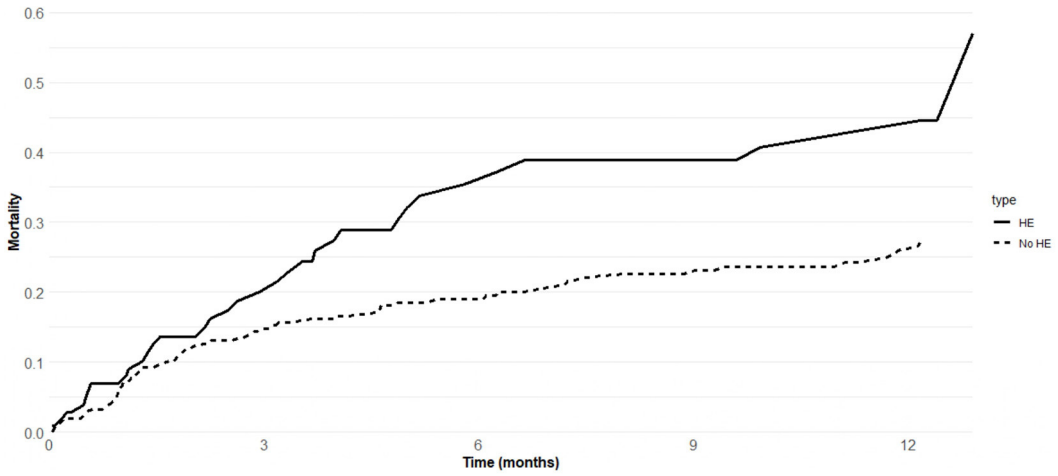

FIGURE 1
Mortality of 368 cirrhotic patients admitted to hospital for hepatic encephalopathy (HE group) or other decompensating events (no HE group).

model [i.e., male sex (HR 1.26, 95% CI 0.75–2.09, $p = 0.379$), BMI (HR 0.99, 95% CI 0.95–1.04, $p = 0.784$), HCC (HR 1.37, 95% CI 0.78–2.39, $p = 0.273$), TIPS (HR 0.67, 95% CI 0.18–2.54, $p = 0.553$), hemoglobin (HR 1.02, 95% CI 0.91–1.14, $p = 0.723$), GGT (HR 1, 95% CI 0.99–1.01, $p = 0.058$), albumin (HR 1.02, 95% CI 0.69–1.49, $p = 0.936$), and porto-systemic shunts (HR 0.93, 95% CI 0.57–1.52, $p = 0.765$)] (Supplementary Table S2).

Supplementary Figure S4 shows the predicted probabilities of death in four different patient profiles according to the presence of HE and/or ascites. In patients without HE and ascites, 6- and 12-month probabilities of death were 11.3% and 15.5%, respectively; in patients with HE and without ascites, 6- and 12-month probabilities of death were 18.8% and 25.4%, respectively; in patients without HE and with ascites, 6- and 12-month probabilities of death were 24.6% and 32.6%, respectively; in patients with HE and ascites, 6- and 12-month probabilities of death were 38.8% and 49.2%, respectively.

In the HE group, BMI (HR 0.86, 95% CI 0.75–0.98, $p = 0.027$) and ascites (HR 5.07, 95% CI 1.39–18.49, $p = 0.014$) were independently associated with mortality (Table 4).

Hospital readmissions

During follow-up, in the HE group, 39 patients (34.8%) had at least one hospital readmission, while 73 patients (65.2%) had no hospital readmission. The reasons for hospital readmissions were HE recurrence in 24 patients (21.4%), ascites in 15 patients (13.4%), infections in four patients (3.6%), and portal hypertensive bleeding in three patients (2.7%) (Table 3). Figure 2A shows the probability of hospital readmission for further decompensation events in the HE group. Six- and twelve-month rates of hospital readmission were 43.4% and 51.5%, respectively. Six- and twelve-month rates of HE recurrence were 25.1% and 27.7%, respectively. Six- and twelve-month rates of ascites recurrence were 11.6% and 14.3%, respectively. Six- and twelve-month rates of infections were 2.8% and 5.7%, respectively. Six- and twelve-month rates of bleeding were 3.8%. No significant risk factors for HE recurrence were found by univariate analysis. Probabilities of HE recurrence according to the previous history of HE are shown in Supplementary Figure S5.

In the no HE group, 68 patients (23.4%) had at least one hospital readmission, while 188 patients (73.4%) had no hospital readmission. The reasons for hospital readmissions were ascites in 27 patients (10.5%), HE in 27 patients (10.5%), portal hypertensive bleeding in 15 patients (5.9%), and infections in 13 patients (5.1%) (Table 3). Figure 2B shows the probability of hospital readmission for further decompensation events in the no HE group. Six- and twelve-month rates of hospital readmission were 26.2% and 70%, respectively. Six- and twelve-month rates of portal hypertensive bleeding were 4.4% and 37.8%, respectively. Six- and twelve-month rates of ascites were 8.9% and 12.2%, respectively. Six- and twelve-month rates of HE were 8.5% and 11.8%, respectively. Six- and twelve-month rates of infections were 4.5% and 7.1%, respectively. More details on the number and reasons for hospital readmission in the two groups are presented in Supplementary Table S3.

Discussion

In this prospective multicenter study including decompensated cirrhotic patients hospitalized for HE age, HE, ascites, and hyponatremia were independent risk factors for death by multivariable competing risks analysis. HE increased not only the risk of death but also represented the first cause of hospital readmission compared with other decompensating events.

Up to now, concerns remain about which patients with HE should be referred to a transplant center because HE is not in itself an indication of OLT unless it is associated with advanced liver failure as assessed by the MELD score. However, it has been recently shown that the MELD score suffers from poor accuracy and calibration for predicting short-term mortality (15), and that the severity of liver disease may be underestimated by MELD alone in patients with HE (16). To the best of our knowledge, we demonstrated for the first time that not only patients with

TABLE 4 Risk factors for mortality and liver transplant in the whole cohort of cirrhotic patients ($n = 368$) hospitalized for decompensating events and in 112 patients hospitalized for hepatic encephalopathy (HE group) by multivariable competing risks analysis.

Whole cohort*						
	Death			Liver transplant		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age (years)	1.03	1.01–1.05	0.025	0.96	0.94–0.99	0.002
HE (present vs. absent)	1.69	1.09–2.62	0.018	1.27	0.70–2.30	0.437
Sodium (mEq/L)	0.94	0.90–0.98	0.009	0.97	0.91–1.03	0.261
Ascites (present vs. absent)	2.50	1.49–4.18	<0.001	0.87	0.51–1.49	0.613
HE cohort**						
	Death			Liver transplant		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
BMI (kg/m^2)	0.86	(0.75–0.98)	0.027	1	(0.85–1.17)	0.960
Ascites (present vs. absent)	5.07	(1.39–18.49)	0.014	0.39	(0.09–1.66)	0.203

In the whole cohort, similar results were obtained when HE status was codified as first or episodic HE (i.e., patients at first HE episode or with history of episodic HE. HR 1.82, 95% CI 1.02–3.27, $p = 0.043$) and recurrent or persistent HE (HR 1.80, 95% CI 1.05–3.10, $p = 0.034$), with No HE group as reference.
HE, hepatic encephalopathy; BMI, body mass index; HR, hazard ratio; CI, confidence interval.
*The results are adjusted for center effect.
**The results are adjusted for infections as precipitating events and for the presence of hepatocellular carcinoma.

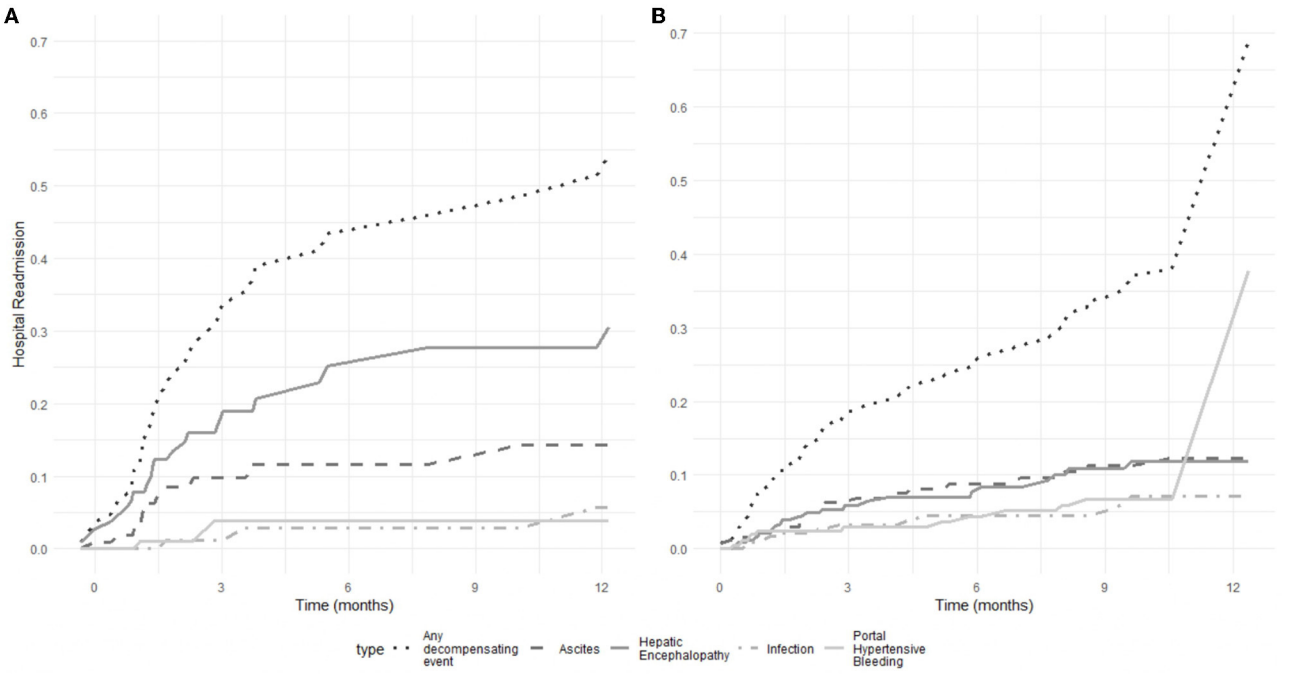

FIGURE 2
Probability of hospital readmission for further decompensating events (hepatic encephalopathy, ascites, infections, and portal hypertensive bleeding) in 112 patients with cirrhosis hospitalized for hepatic encephalopathy **(A)** and 256 patients hospitalized for decompensated cirrhosis without hepatic encephalopathy **(B)**.

recurrent or persistent HE but also with a single episode of HE had a significantly worse survival by multivariable competing risks analysis; thus, one of the main implications for clinical practice of our findings is that patients with a single HE episode requiring hospitalization should be promptly considered for OLT. These findings could potentially contribute to future changes in clinical guidelines.

As expected, ascites was confirmed as an independent risk factor for death both in the whole cohort and in the HE group. Notably, the negative impact of HE on the survival of patients with cirrhosis was independent of other complications of advanced chronic liver disease, including ascites and hyponatremia. While the development of ascites mainly depends on the severity of portal hypertension, HE is related not only to portal hypertension,

which leads to the opening of spontaneous porto-systemic shunts, but also reflects other pathophysiological mechanisms, such as impaired nutritional status, sarcopenia, and frailty (17, 18). It is not surprising that in our study, patients with HE had not only a significantly higher prevalence of spontaneous porto-systemic shunts assessed by CT but also significantly lower albumin levels, lower BMI, and higher MELD score compared with patients with decompensated cirrhosis without HE. The incorporation of novel variables related to nutritional status and chronic inflammation could potentially improve the allocation systems for OLT (19, 20).

Body mass index (BMI) was significantly lower in the HE group compared with the no HE group, and it was independently associated with worse survival in the HE group. This last finding suggests the relevance of nutritional status as a determinant of clinical outcomes in patients with cirrhosis. Decompensated cirrhosis represents a hyper-metabolic state, resulting in accelerated protein catabolism and higher energy consumption (21). Although BMI is not a widely accepted marker of nutritional status, particularly in patients with ascites, previous studies have shown that lower BMI is related to sarcopenia and decreased muscle mass in patients with cirrhosis (22), and that sarcopenia is a significant predictor of death independently from the degree of liver dysfunction (23). The role of BMI appears particularly relevant since our cohort had a relatively low prevalence of metabolic cirrhosis. Future research should investigate the role of lumbar muscle cross-sectional area measured by CT or other measures of muscle strength as surrogate biomarkers of sarcopenia in predicting the risk of death in patients with cirrhosis and HE. Moreover, further studies are needed to assess if combining standard medical treatment with nutritional interventions, physical exercise, and hormone-substituting therapies aiming to improve sarcopenia and muscle strength may be able to reduce the risk of death and HE recurrence.

The prospective design of our study allowed us to accurately estimate the risk of hospital readmission for HE recurrence. It is important to underline that the 12-month risk of HE recurrence was high, and that HE recurrence was the first leading cause of hospital readmission compared with other further decompensation events, including ascites, infections, and portal hypertensive bleeding. These findings underline the significant burden of HE on healthcare systems, in terms of repeated hospital admissions and related direct and indirect costs (8, 9, 24). Particularly, the results of our prospective study confirmed those of previously published retrospective studies, showing that HE is a strong predictor of early hospital readmission in patients with cirrhosis (25–27). Notably, the risk of HE recurrence was high, regardless of the characteristics of previous HE episodes (episodic or recurrent), suggesting that secondary prophylaxis should be recommended in clinical practice after the first HE episode. The prospective design of our study and the accurate assessment of received pharmacological treatments and outcomes during the follow-up can be useful in order to design future pharmacoeconomic studies. These latter are needed to evaluate the cost-effectiveness of both secondary prophylaxis and treatment strategies, including rifaximin, lactulose, or branched-chain amino acids.

Our study was also able to provide updated data on the prevalence of precipitating events. Approximately 80% of HE patients showed a precipitating factor, the most prevalent being constipation, infections, diuretic overuse, and gastrointestinal bleeding. These results could be useful in order to plan effective primary prophylaxis strategies. Infections are now considered complications of decompensated cirrhosis (28), and they could accelerate the course of the disease, precipitating acute-on-chronic liver failure (29). However, effective strategies to prevent infections in decompensated cirrhosis are lacking. Although long-term therapy with albumin has showed to significantly reduce the risk of infections and grade III-IV HE improving survival in a randomized controlled trial (RCT) including outpatients with cirrhosis and uncomplicated ascites (30), this beneficial effect was not confirmed in hospitalized patients with more advanced disease treated with a short course of albumin (31). An RCT including Child–Pugh C patients showed that long-term norfloxacin significantly decreased the incidence of any gram-negative bacterial infections, but it failed to improve 6-month mortality (32). The use of rifaximin for the prevention of infections is not accepted since evidence from clinical trials is still weak (33). Fecal microbiota transplant (FMT) has shown to be safe and potentially efficacious for the treatment of HE (34), and its potential role in preventing infections is currently under evaluation (35). About gastrointestinal bleeding as precipitating factor of HE, the last Baveno VII recommendations suggested the rapid removal of blood from the gastrointestinal tract by using lactulose oral or enemas in order to prevent HE (33). Although it has been shown that PPI are associated with overt HE and increased mortality (36), it is noteworthy that a high prevalence of PPI use was observed among decompensated cirrhotic patients, suggesting that more stringent criteria for PPI prescription should be used in this setting.

Our study suffers from some limitations. First, the enrollment of patients by 25 referral centers may have affected our results, since discrepancies in the HE grading between observers, treatment and prophylaxis strategies, and policy of OLT allocation may differ among centers. However, according to recently published EASL guidelines, our study used a standardized flow-chart for differential diagnosis, and only patients with the diagnosis of at least grade 2 overt HE as a reason for hospitalization were included. Second, treatments and prophylaxis strategies for HE were highly heterogeneous among centers, and no firm conclusions regarding the efficacy and safety of treatments, including lactulose, rifaximin, albumin, and beta-blockers, could be derived. However, the results of our multivariable competing risks model were confirmed after adjusting for the center effect as a covariate. Third, our sample size was relatively small. Fourth, our study only included hospitalized patients with HE; therefore, our findings could not be generalized to outpatients with HE. Fifth, even if multicenter, our study included patients who were enrolled only in Italian centers; therefore our results need to be validated in other settings, with different prevalences of etiologies of liver disease or metabolic co-factors affecting survival. Finally, although all patients in the HE group were consecutively included during the enrollment period, the same did not apply to the no HE group, potentially introducing a selection bias in the control group.

In conclusion, in decompensated cirrhotic patients hospitalized for HE as an inception point, HE is an independent risk factor for mortality, and it is the most common cause of hospital readmission

compared with other decompensation events. In patients with HE, ascites and BMI were independent risk factors for death, suggesting that improvement of sarcopenia represents an urgent unsolved medical need in this setting.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board (IRB) of the Fondazione Agostino Gemelli Hospital, Rome, Italy. The patients/participants provided their written informed consent to participate in this study.

Author contributions

OR, CCe, VC, MMe, PC, SM, VM, MMi, GSa, GR, AB, PB, RS, MPe, FS, EV, AC, SF, MPi, MB, FA, GSo, MR, FM, SL, AF, AP, GD, SN, LR, AG, and CCa were responsible for the project and writing of the manuscript. All authors have seen and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1184860/full#supplementary-material

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Risk factors for hepatic hydrothorax in patients with cirrhosis: a clinical retrospective study

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Aims and background: Hepatic hydrothorax, which presents as an unexplained pleural effusion, is one of the important complications in patients with end-stage cirrhosis. It has a significant correlation with prognosis and mortality. The aim of this clinical study was to detect the risk factors for hepatic hydrothorax in patients with cirrhosis and to better understand potentially life-threatening complications.

Methods: Retrospectively, 978 cirrhotic patients who were hospitalized at the Shandong Public Health Clinical Center from 2013 to 2021 were involved in this study. They were divided into the observation group and the control group based on the presence of hepatic hydrothorax. The epidemiological, clinical, laboratory, and radiological characteristics of the patients were collected and analyzed. ROC curves were used to evaluate the forecasting ability of the candidate model. Furthermore, 487 cases in the experimental group were divided into left, right, and bilateral groups, and the data were analyzed.

Results: The patients in the observation group had a higher proportion of upper gastrointestinal bleeding (UGIB), a history of spleen surgery, and a higher model for end-stage liver disease (MELD) scores compared with the control group. The width of the portal vein (PVW) ($P = 0.022$), prothrombin activity (PTA) ($P = 0.012$), D-dimer ($P = 0.010$), immunoglobulin G (IgG) ($P = 0.007$), high-density lipoprotein cholesterol (HDL) ($P = 0.022$), and the MELD score were significantly associated with the occurrence of the hepatic hydrothorax. The AUC of the candidate model was 0.805 ($P < 0.001$, 95% CI = 0.758–0.851). Portal vein thrombosis was more common in bilateral pleural effusion compared with the left and right sides ($P = 0.018$).

Conclusion: The occurrence of hepatic hydrothorax has a close relationship with lower HDL, PTA, and higher PVW, D-dimer, IgG, and MELD scores. Portal vein thrombosis is more common in cirrhotic patients with bilateral pleural effusion compared to those with unilateral pleural effusion.

KEYWORDS

liver cirrhosis, hepatic hydrothorax, risk factors, MELD scores, portal vein

Introduction

Hepatic hydrothorax (HH) is an important complication of decompensated cirrhosis and portal hypertension. It is a pleural effusion, typically of more than 500 mL, in patients with liver cirrhosis that do not present with coexisting underlying cardiac or pulmonary diseases (1, 2). The occurrence of HH has been reported in 4–16% of patients with cirrhosis. Most patients with HH have ascites in combination, of which 59–85% are right-sided, 12–17% are left-sided, and 2–29% are bilateral (1, 3–7). There were very few reported cases of HH without ascites (8, 9). HH was first reported by Morrow et al. (10) while describing a rapid accumulation of massive right pleural effusion; however, the pathogenesis is still unclear. One of the well-accepted theories is that a "positive" intra-abdominal pressure and a "negative" intrathoracic pressure of the pleural cavity cause ascites to enter the thoracic cavity through a physiological defect in the diaphragm (11–14). HH has a close relationship with poor prognosis (15). Once HH appears, liver transplantation is considered to be the most specific and effective treatment for those with refractory disease. In addition to diuretics, sodium restriction, and therapeutic thoracentesis (15–18), the use of trans-jugular intrahepatic portosystemic shunts (TIPSs) and surgical repair of diaphragmatic defects relieve the symptoms of HH but are usually limited in clinical practice due to economic factors (19, 20).

Previous studies have suggested some factors that are related to the occurrence of HH. Hou et al. claimed HH was positively associated with moderate-large ascites, Child–Pugh class B–C, lower albumin (Alb), higher prothrombin time (PT), and international normalized ratio (INR) (21). Deleuran et al. and Matei et al. indicated that bilirubin, diabetes, and non-use of non-selective beta-blockers were risk factors for hepatic hydrothorax (7, 22). Based on the abovementioned studies, we retrospectively analyzed the epidemiological characteristics, clinical manifestations, and laboratory indicators of cirrhotic patients with pleural effusion and tried to explore more risk factors of HH, in order to better understand these complications of cirrhosis.

Materials and methods

Patients and setting

By using electronic medical records, a total of 3,336 patients with cirrhosis, including 1,183 cases with pleural effusion and 2,153 cases without pleural effusion, who were hospitalized at the Shandong Public Health Clinical Center from 2013 to 2021 were involved in this study. All patients matched the International Classification of Diseases, 10th revision (ICD-10) codes for liver cirrhosis (ICD-10: K74.1–K74.6/K70.300) and ascites (R18). For patients with repeated hospitalizations during 2013–2021, only their first hospitalization, which satisfied the above criteria, was entered. Of these, 1,183 patients had confirmed pleural effusion by relevant imaging examinations (chest X-ray, ultrasonography, or CT). Finally, a total of 487 patients with HH were enrolled in our study as the observation group. Similarly, 491 patients were randomly selected from the remaining 2,153 samples without pleural effusion as the control group by applying the random number generator in SPSS (Figure 1). The patients in the two met all the criteria and the exclusion criteria were as follows: (1) cardiopulmonary dysfunction (ICD-10: I00-I99, J00-J89, J95-J97), (2) tuberculosis (ICD-10: A15-A16), (3) malignancies (ICD-10: C00-C97), (4) pleural disease (ICD-10: J90-J94), and (5) patients with TIPS (ICD-9-CM: 39.1). Among the 487 cases with HH, there were 99 cases with left pleural effusions, 217 cases with right pleural effusions, and 171 cases with bilateral pleural effusions.

Abbreviations: HH, hepatic hydrothorax; MELD, models for end-stage liver disease; CTP score, Child–Turcotte–Pugh score; PVW, the width of the portal vein; PTA, prothrombin activity.

Data collection

We analyzed the epidemiological characteristics, clinical manifestations, and laboratory findings of patients from each group, including demographic characteristics, etiology, complications or combination diseases, operation history, blood transfusion history, and laboratory tests. Demographic characteristics comprised age and sex. Etiology involved hepatitis B cirrhosis, hepatitis C cirrhosis, alcoholic cirrhosis, autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and cirrhosis caused by unknown factors. Complications or combination diseases involved upper gastrointestinal bleeding, esophageal and gastric fundal varices, hepatic encephalopathy, bacterial peritonitis, portal vein thrombosis, hypertension, and diabetes. Operation history included splenectomy or splenic embolization. Laboratory findings included alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), albumin (Alb), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), creatinine (Cr), white blood cell count (WBC), hemoglobin (Hb), platelet count (PLT), prothrombin activity (PTA), D-dimer, immunoglobulin G (IgG), interleukin-6 (IL-6), C reactive protein (CRP), and serum amyloid A (SAA). The width of the portal vein (PVW) by ultrasonic examination was also taken into account in the study. The examinations for diagnosis of portal vein thrombosis were enhanced by CT or magnetic resonance imaging. Liver disease severity was estimated using the Child–Pugh stage and the model of end-stage liver disease (MELD) score. The abovementioned data were the first lab test results of the patients after the diagnosis of HH during this hospitalization.

Statistics analysis

Clinical parameters were evaluated using the chi-square test for discrete variables and the *t*-test or Mann–Whitney *U*-test for continuous variables. Multiple logistic regression analyses were performed to identify the factors associated with the occurrence of HH. For the regression results, 30% of the total sample was randomly selected as the validation group to verify the statistical results. The Kruskal–Wallis test was used for comparison among the three groups of left, right, and bilateral pleural effusions. A *P*-value of <0.05 was considered statistically significant. All data

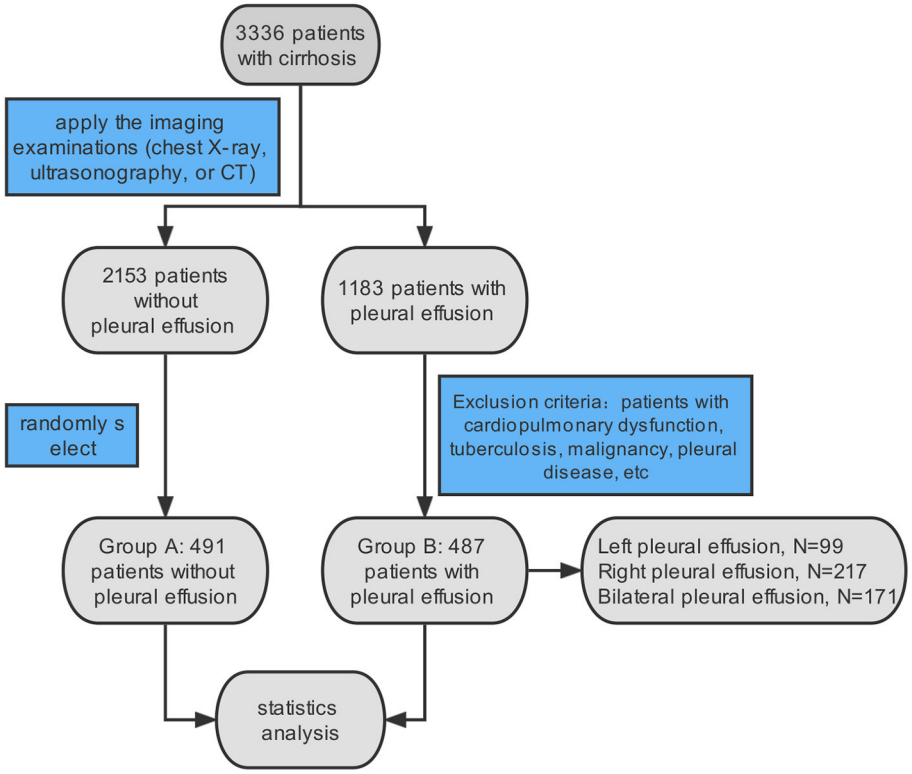

FIGURE 1
Flow chart of selecting patients. A total of 3,336 patients with cirrhosis and ascites were identified according to the ICD 10 diagnosis. In total of 487 patients were included in our study, meeting the imaging examination and exclusion criteria.

analyses were performed using SPSS v. 25.0 (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics in the HH group compared with the control group

A total of 988 cirrhotic inpatients were enrolled in this study, including 487 cases with HH as the observation group and 491 cases without HH as the control group. There were 358 male subjects in the observation group and 339 male subjects in the control group ($P = 0.107$). There was no difference between the two groups in the proportion of those aged between 40 and 60 years (58.3 vs. 55.1%, $P = 0.472$). The predominant etiology in both groups was HBV infection (73.5 vs. 75.0%, $P = 0.756$). Complications or combination diseases, including hypertension, diabetes, esophagogastric varices (EGV), hepatic encephalopathy (HE), and portal vein thrombosis showed no difference in the two groups. However, more individuals in the HH group had a history of spleen surgery (splenectomy or splenic embolization) compared with the control group (10.9 vs. 4.9%, $P = 0.000$). MELD scores and Child–Pugh scores were also assessed in both groups, and the results showed that the proportion of high MELD scores was higher in the observation group compared with the control group ($P = 0.038$) (Table 1).

Lab test results in the two groups

The comparison of many indicators between the two groups had statistical significance. Details are as follows: the proportion of patients in the HH group who had low TC (31.0 vs. 22.4%, $P = 0.002$), HDL (58.5 vs. 41.5%, $P = 0.000$), Hb (63.2 vs. 52.7%, $P = 0.001$), PLT (58.3 vs. 34.2%, $P = 0.018$), and PTA (71.0 vs. 60.1%, $P = 0.000$) was higher than in the control group. Compared with individuals without HH, there were more patients with lower IgG (64.9 vs. 72.3%, $P = 0.012$), and higher D-dimer (88.1 vs. 75.4%, $P = 0.000$), CRP (59.5 vs. 48.7%, $P = 0.001$), and PVW (29.2 vs. 21.4%, $P = 0.005$) (Table 2).

PVW, MELD scores, and CTP scores were the risk factors for HH

As shown in Table 3, PVW ($P = 0.013$, OR = 1.195, 95% CI = 1.038–1.376), PTA ($P = 0.009$, OR = 0.392, 95% CI = 0.194–0.792), D-dimer ($P = 0.009$, OR = 3.822, 95% CI = 1.398–10.450), IgG ($P = 0.009$, OR = 2.127, 95% CI = 1.212–3.735), HDL ($P = 0.003$, OR = 0.384, 95% CI = 0.206–0.716), and MELD scores were significantly associated with the occurrence of HH. Taking the MELD score <15 as the control group, the OR for the occurrence of HH in patients with MELD scores of 15–18 was 4.184, and the OR in the patients with a MELD score of >18 was 4.066. An equation was derived from binary logistic regression in this study as follows:

TABLE 1 Baseline characteristics in the HH group compared with the control group.

Variables	Without HH N = 491	HH N = 487	Z/χ2	*P*
Sex (male/female) n	339/153	358/129	2.599	0.107
Age *n* (%)			0.015	0.472
<40	41 (8.3%)	43 (8.8%)		
40–60	271 (55.1%)	284 (58.3%)		
>60	180 (36.6%)	160 (32.9%)		
Etiology *n* (%)			4.205	0.756
HBV	357 (75.0%)	349 (73.5%)		
HCV	10 (2.1%)	8 (1.7%)		
PBC	18 (3.8%)	20 (4.2%)		
AIH	7 (1.5%)	6 (1.3%)		
Alcoholic liver	49 (10.3%)	51 (10.7%)		
Unknown reason	50 (10.4%)	53 (10.9%)		
Complication/combination disease *n* (%)				
Hypertension	89 (18.1%)	108 (22.2%)	2.544	0.111
Diabetes	84 (17.1%)	79 (16.2%)	0.128	0.721
UGIB	35 (7.1%)	54 (11.1%)	4.678	**0.031**
EGV	144 (29.3%)	165 (33.9%)	2.149	0.542
HE	35 (7.1%)	38 (7.8%)	0.168	0.682
Bacterial peritonitis	237 (48.2%)	229 (47.0%)	0.014	0.565
Portal vein thrombosis	62 (12.8%)	79 (16.7%)	0.037	0.155
Another clinical character *n* (%)				
History of spleen surgery	24 (4.9%)	53 (10.9%)	0.122	**0.000**
MELD Score *n* (%)			6.529	**0.038**
<15	291 (59.4%)	258 (53.1%)		
15–18	72 (14.7%)	100 (20.6%)		
>18	127 (25.9%)	128 (26.3%)		
CTP score M (IQR)	9.00 (8.00, 10.00)	9.00 (8.00, 11.00)	−1.806	0.071
Child-Pugh score *n* (%)			3.233	0.199
A	30 (6.1%)	26 (5.3%)		
B	264 (53.9)	238 (49.0%)		
C	196 (40.0)	222 (45.7%)		

HH, hepatic hydrothorax; *n* (%), the frequency with percentage for categorical variables; M, median; IQR, Interquartile range; UGIB, Upper gastrointestinal bleeding; EGV, Esophageal and gastric fundal varices; HE, Hepatic encephalopathy; History of spleen surgery, splenectomy or splenic embolization. The bold values indicate a statistically significant *p*-value for their analysis of that variable ($p < 0.05$).

Logit $P = \ln[P/(1\text{-}P)] = -24.509 - 0.957^*(\text{HDL}) + 0.755^*(\text{IgG}) - 0.937^*(\text{PTA}) + 1.341^*(\text{D-dimer}) + 0.178^*(\text{PVW}) + 1.431^*(\text{MELD } 15\text{–}18) + 1.403^*(\text{MELD} > 18)$. Comparing the candidate model with the MELD score (AUC = 0.514, 95% CI = 0.452–0.576, $P = 0.655$) and CTP (AUC = 0.511, 95% CI = 0.448–0.573, $P = 0.736$), we found that the AUCs of the candidate risk models were 0.805 (95% CI = 0.758–0.851, $P < 0.001$) (Table 4, Figure 2), and the model's AUCs in the validation group were 0.826 (95% CI = 0.752–0.901, $P < 0.001$) (Table 5, Figure 3).

The incidence of portal vein thrombosis was higher in bilateral pleural effusion compared with left and right pleural effusions

Among 487 cases with HH, there were 99 cases with left pleural effusions (20.4%), 217 cases with right (44.5%) pleural effusions, and 171 cases with bilateral pleural effusions (35.1%). Clinical characteristics and lab test results shown in Tables 6, 7

TABLE 2 Laboratory test index between the two groups.

Indicators	Normal range	Without HH ($N = 491$)	HH ($N = 487$)	$\chi 2$	P
		N (%)	N (%)		
ALT (U/L)	0–40			0.783	0.376
≤40		264 (53.8%)	276 (56.7%)		
>40		227 (46.2%)	211 (43.3%)		
AST (U/L)	0–40			2.544	0.111
≤40		157 (32.0%)	180 (37.0%)		
>40		334 (68.0%)	307 (63.0%)		
GGT (U/L)	12–64			2.738	0.098
≤64		258 (52.5%)	281 (57.7%)		
>64		233 (47.5%)	307 (42.3%)		
ALB (g/L)	35–55			0.058	0.809
≥35		86 (17.5%)	88 (18.1%)		
<35		405 (82.5%)	399 (81.9%)		
TG (mmol/L)	0.56–1.70			1.535	0.215
≥0.56		415 (84.5%)	399 (81.9%)		
<0.56		76 (15.5%)	88 (18.1%)		
TC (mmol/L)	2.60–6.19			10.015	**0.002**
≥2.60		381 (77.6%)	336 (69.0%)		
<2.60		110 (22.4%)	151 (31.0%)		
HDL (mmol/L)	1.03–1.78			27.811	**<0.001**
≥1.03		287 (58.5%)	202 (41.5%)		
<1.03		204 (41.5%)	285 (58.5%)		
Cr (mmol/L)	50.4–98.1			1.378	0.240
≤98.1		462 (94.1%)	449 (92.2%)		
>98.1		29 (5.9%)	38 (7.8%)		
WBC×10^9/L	4–10			1.378	0.240
≤10		462 (94.1%)	449 (92.2%)		
>10		29 (5.9%)	38 (7.8%)		
N×10^9/L	0.8–4			2.276	0.131
≤4		382 (77.8%)	359 (73.7%)		
>4		109 (22.2%)	128 (26.3%)		
Hb (g/L)	F:110–150 M:120–160			11.053	**0.001**
≥110 (F)/120 (M)		232 (47.3%)	179 (36.8%)		
<110 (F)/120 (M)		259 (52.7%)	308 (63.2%)		
PLT×10^9/L	100–300			5.588	**0.018**
≥100		323 (65.8%)	203 (41.7%)		
<100		168 (34.2%)	284 (58.3%)		
PTA (%)	70–130			13.311	**<0.001**
≥70		196 (39.9%)	141 (29.0%)		
<70		295 (60.1%)	346 (71.0%)		
D-dimer (mg/L)	0–0.5			26.764	**0.000**

(Continued)

TABLE 2 (Continued)

Indicators	Normal range	Without HH ($N = 491$)	HH ($N = 487$)	$\chi 2$	P
		N (%)	*N* (%)		
≤0.5		121 (24.6%)	58 (11.9%)		
>0.5		370 (75.4%)	429 (88.1%)		
IgG (g/L)	7–16			6.346	**0.012**
≤16		136 (27.7%)	171 (35.1%)		
>16		355 (72.3%)	316 (64.9%)		
IL-6 (pg/mL)	0–7			1.857	0.173
≤7		27 (5.5%)	18 (3.7%)		
>7		464 (94.5%)	469 (96.5%)		
SAA (mg/L)	0–10			2.896	0.089
≤10		197 (40.1%)	170 (34.9%)		
>10		294 (59.9%)	317 (65.1%)		
CRP (ng/L)	0.068–8.2			11.860	**0.001**
≤8.2		252 (51.3%)	197 (40.5%)		
>8.2		239 (48.7%)	290 (59.5%)		
PVW (mm)	6–10			7.927	**0.005**
≤13		386 (78.6%)	345 (70.8%)		
>13		105 (21.4%)	142 (29.2%)		

HH, hepatic hydrothorax; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; ALB, albumin; TG, triglyceride, TC, total cholesterol; HDL, high-density lipoprotein cholesterol, Cr, creatinine, WBC, white blood cell count; N, neutrophil; Hb, hemoglobin; PLT, platelet count, PTA, prothrombin activity; IgG, immunoglobin G; IL-6, interleukin-6; CRP, C reactive protein; SAA, serum amyloid A; PVW, the width of the portal vein. The bold values indicate a statistically significant *p*-value for their analysis of that variable ($p < 0.05$).

TABLE 3 Binary logistic regression analysis to identify risk factors for hepatic hydrothorax.

Indicators	Univariate analysis		Multivariable analysis	
	OR (95%CI)	*p*	OR (95%CI)	*p*
UGIB	0.614 (0.393–0.958)	**0.032**	0.506 (0.191–1.337)	0.170
History of spleen surgery MELD score	0.420 (0.255–0.692)	**0.001**	1.214 (0.418–3.528)	0.721
<15	1.00		1.00	
15–18	1.567 (1.108–2.215)	**0.011**	4.184 (1.465–11.952)	**0.008**
>18	1.137 (0.844–1.530)	0.398	4.066 (1.557–10.621)	**0.004**
TC (mmol/L)	1.590 (0.393–0.653)	**0.002**	0.965 (0.525–1.775)	0.910
HDL (mmol/L)	0.506 (1.532–2.547)	**0.000**	0.384 (0.206–0.716)	**0.003**
Hb (g/L)	1.541 (1.194–1.990)	**0.001**	0.955 (0.447–2.040)	0.906
PLT × 10^9/L	1.366 (1.054–1.770)	**0.018**	1.918 (0.997–3.692)	0.051
PTA (%)	1.639 (1.256–2.139)	**0.000**	2.552 (1.262–5.161)	**0.009**
D-dimer (mg/L)	2.441 (1.729–3.445)	**0.000**	3.822 (1.398–10.450)	**0.009**
IgG (g/L)	1.417 (1.080–1.858)	**0.012**	2.127 (1.212–3.735)	**0.009**
CRP (ng/L)	1.627 (1.245–2.126)	**0.000**	1.046 (0.542–2.020)	0.893
PVW (mm)	1.079 (1.012–1.151)	**0.02**	1.195 (1.038–1.376)	**0.013**

B, beta, regression coefficient; OR, Odds ratio; 95%CI, Confidence interval; PVW, the width of the portal vein. The bold values indicate a statistically significant *p*-value for their analysis of that variable ($p < 0.05$).

were analyzed to explore differences between the three groups. The results showed that the incidence of portal vein thrombosis was higher in bilateral pleural effusion compared with left and right pleural effusions ($P = 0.018$).

TABLE 4 Receiver operating characteristic analysis for MELD scores, CTP scores, and the candidate risk model in all selected patients.

Indicators	AUC	SE	*P*	95%CI	
				Lower	Upper
MELD Score	0.514	0.032	0.655	0.452	0.576
CTP Score	0.511	0.032	0.736	0.448	0.573
Candidate risk model	0.805	0.024	0.000	0.758	0.851

AUC, Area under curve; 95%CI, Confidence interval; SE, Standard error.

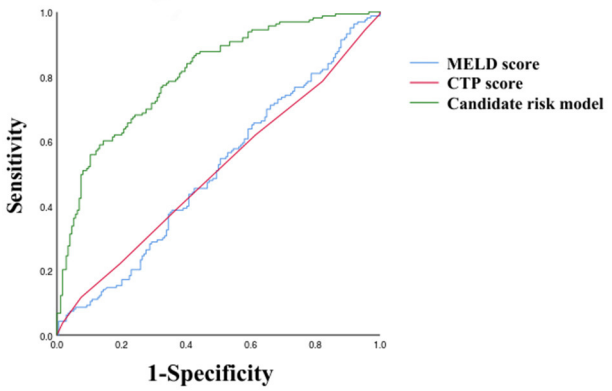

FIGURE 2
Receiver operating characteristic curves of MELD score, CTP score, and Candidate risk model.

TABLE 5 Receiver operating characteristic analysis for the candidate risk model in the internal validation group.

Indicators	AUC	SE	*P*	95%CI	
				Lower	Upper
Candidate risk model	0.826	0.038	0.000	0.752	0.901

AUC, Area under curve; 95%CI, Confidence interval; SE, Standard error.

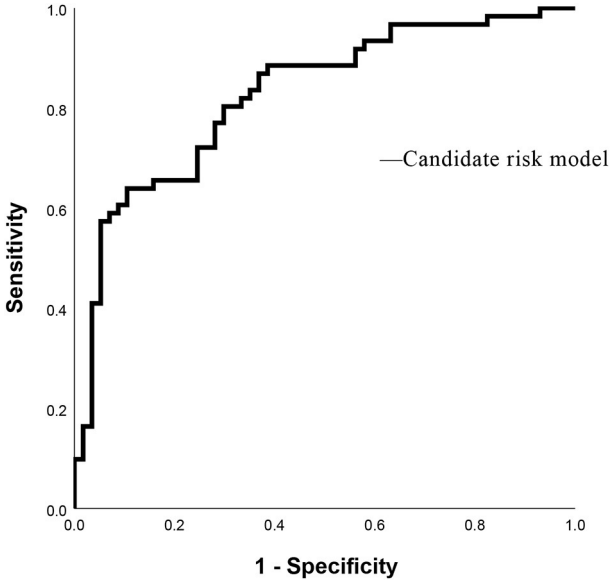

FIGURE 3
Receiver operating characteristic curves of Candidate risk model in validation group.

However, it is important to note that the abovementioned results are not meant to be used for the minority of patients who develop HH without developing ascites.

Discussion

The patients who developed severe complications of cirrhosis usually presented clinical manifestations of chest tightness, shortness of breath, cough, nausea, and, in a few patients, severe dyspnea and tachycardia. There was no significant difference in presentation from the diseases in the exclusion criteria, thus, the abovementioned exclusion criteria needed to be added to the diagnosis to exclude the relevant patients who did not meet the requirements of this study. Other than that, HH has a close relationship with a poor prognosis (23, 24). Therefore, our research aimed to explore the risk factors of hepatic hydrothorax for cirrhotic patients, which can help clinicians to develop secondary prevention strategies and improve the patient's prognosis. The MELD score is an exact predictive model suitable for patients with liver cirrhosis at the end stage and is extensively applied to assess liver disease severity and mortality (17, 18). The result of the univariate analysis showed that patients had a significant difference in MELD scores between the two groups. Combined with the findings of Hou et al., it is possible that the incidence of HH is strongly associated with the severity degree of cirrhosis (21). Similarly, the result of the logistics regression also found that a higher MELD score is a risk factor for HH.

The association of the MELD score and HH supports other findings of the binary logistic regression. For patients with liver cirrhosis, lower HDL and PTA and higher PVW, D-dimer, and IgG also correlate with the development of HH. The explanations are as follows: (1) HDL, IgG, and PTA: These three can indicate the degree of liver function impairment. First, HDL production decreased because of the liver's decreased synthesis of apolipoproteins A and B (25, 26). Some studies suggested that HDL-cholesterol is a reliable predictor of the liver's function and of disease progression in chronic liver failure (27–29). IgG is an antibody produced by plasma cells that are transformed from B lymphocytes when stimulated by antigens from bacteria, viruses, and toxins and can specifically bind to antigens (30). In patients with chronic hepatitis, humoral immunity is activated, resulting in elevated IgG in the body. Therefore, a higher IgG also means more severe hepatitis and cirrhosis (31, 32). PTA calculated by PT reflects the activity of blood coagulation factorsI, II, V, VII, and X. Both the prolongation of PT and the decrease of PTA are due to liver impairment and reduced synthesis of coagulation factors. Hence, PTA is also thought to be a key predictor of liver function (33, 34).

Therefore, we conclude that because the three factors mentioned above can indicate the severity of cirrhosis and the impairment of the liver's synthetic function, they have a strong association with the occurrence of HH, which is in line with the findings of Mouelhi et al. (35). (2) D-dimer: The D-dimer of

TABLE 6 Clinical characteristics among left, right, and bilateral pleural effusions.

Variables	Left	Right	Bilateral	H/χ2	*P*
	N = 99	*N* = 217	*N* = 171		
Sex (male/female) n	72/27	154/63	130/41	0.055	0.973
Age *n* (%)				1.963	0.742
<40	11 (11.1%)	17 (7.8%)	18 (10.5%)		
40–60	57 (57.6%)	121 (55.8%)	89 (52%)		
>60	31 (31.3%)	79 (36.4%)	64 (37.4%)		
Etiology n (%)				15.015	0.241
HBV	70 (73.7%)	170 (78.3%)	109 (63.7%)		
HCV	1 (1.1%)	4 (1.8%)	3 (1.8%)		
PBC	3 (3.2%)	8 (3.7%)	9 (5.3%)		
AIH	1 (1.1%)	1 (0.5%)	4 (2.3%)		
Alcoholic Liver	8 (8.4%)	20 (9.2%)	23 (13.5%)		
Unknown Reason	16 (16.2%)	14 (6.4%)	17 (10.0%)		
Complication/combination disease n (%)					
Hypertension	29 (29.6%)	47 (21.7%)	32 (18.7%)	4.337	0.114
Diabetes	19 (19.4%)	28 (12.9%)	32 (18.7%)	3.257	0.196
UGIB	12 (12.2%)	16 (7.4%)	25 (14.6%)	5.396	0.067
EGV	35 (35.7%)	73 (33.6%)	57 (33.3%)	0.286	0.867
HE	6 (6.1%)	16 (7.4%)	16 (9.4%)	4.939	0.552
Bacterial peritonitis	51 (52.0%)	95 (43.8%)	82 (48%)	1.965	0.374
Portal vein thrombosis	9 (9.4%)	32 (15.1%)	37 (22.4%)	8.048	**0.018**
Liver cancer	17 (17.3%)	48 (22.2%)	20.80%	5.826	0.667
Another clinical character n (%)					
History of spleen surgery	16 (16.3%)	19 (8.8%)	18 (10.5%)	4.022	0.134
MELD Score *n* (%)				2.868	0.580
<15	72 (73.5%)	164 (75.6%)	120 (70.1%)		
15–18	14 (14.3%)	27 (12.4%)	20 (11.7%)		
>18	12 (12.2%)	26 (12.0%)	31 (18.2%)		
CTP score M (IQR)	9.00 (8.00, 11.00)	9.00 (8.00, 11.00)	9.50 (8.00, 11.00)	2.036	0.361
Child-Pugh score				8.658	0.070
A	6 (6.1%)	13 (6.0%)	7 (4.1%)		
B	50 (50.5%)	113 (52.3%)	68 (39.8%)		
C	43 (43.4%)	90 (41.7%)	96 (56.1%)		

HH, hepatic hydrothorax; *n* (%), the frequency with percentage for categorical variables; M, median; IQR, Interquartile range; UGIB, Upper gastrointestinal bleeding; EGV, Esophageal and gastric fundal varices; HE, Hepatic encephalopathy; History of spleen surgery, splenectomy or splenic embolization. The bold values indicate a statistically significant *p*-value for their analysis of that variable ($p < 0.05$).

the patients with HH was significantly higher than those in the control group. Since D-dimer has a positive correlation with both portal hypertension and the MELD score (36–39), the odds of HH increase with a higher D-dimer. (3) PVW: An increased width of the portal vein means elevated portal vein pressure. Some studies suggested that it may result in the high pressure of vena azygos, and then the lymph and other body fluids will leak into the pleural cavity (40, 41).

However, in terms of risk factors for HH, our study differed slightly from the conclusions of previous studies. Hou et al. stated that HH was positively associated with moderate-large ascites, Child–Pugh class B–C, lower albumin, higher prothrombin time, and the international normalized ratio (21). Deleuran et al. and Matei et al. indicated that bilirubin, diabetes, and the non-use of non-selective beta-blockers were risk factors for hepatic hydrothorax (7, 22). These differences probably relate to the

TABLE 7 Laboratory test index among left, right, and bilateral pleural effusions.

Variables	Normal range	Left ($N = 99$)	Right ($N = 217$)	Bilateral ($N = 171$)	H	*P*
		Median (IQR)	Median (IQR)	Median (IQR)		
ALT (U/L)	0–40	34.00 (21.00, 59.00)	34.50 (20.00, 64.50)	34.50 (19.75, 82.00)	0.055	0.973
AST (U/L)	0–40	49.00 (30.00, 83.00)	54.00 (33.00, 91.00)	51.00 (29.00, 122.00)	0.977	0.614
GGT (U/L)	12–64	47.00 (24.00, 104.00)	51.00 (23.00, 108.50)	57.00 (27.00, 130.00)	1.350	0.509
ALB (g/L)	35–55	28.20 (24.60, 33.60)	28.30 (24.95, 32.90)	28.50 (24.60, 32.40)	0.037	0.982
TG (mmol/L)	0.56–1.70	0, 875 (0.658, 1.175)	0.780 (0.610, 1.015)	0.825 (0.620, 1.150)	5.886	0.053
TC (mmol/L)	2.60–6.19	3.01 (2.373, 3.84)	3.27 (2.34, 3.93)	3.06 (2.45, 3.90)	1.234	0.539
HDL (mmol/L)	1.03–1.78	0.89 (0.65, 1.18)	0.97 (0.64, 1.32)	0.90 (0.55, 1.22)	3.766	0.152
Cr (umol/L)	50.4–98.1	63.60 (54.30, 74.00)	62.10 (52.80, 75.00)	63.20 (54.50, 73.40)	1.143	0.565
WBC$\times 10^9$/L	4–10	4.12 (2.86, 6.54)	3.97 (2.89, 5.76)	4.38 (3.09, 6.11)	2.083	0.353
N$\times 10^9$/L	0.8–4	2.70 (1.60, 4.43)	2.43 (1.60, 3.80)	2.76 (1.88, 4.60)	2.791	0.248
Hb (g/L)	110–150	109.00 (87.00, 125.00)	110.00 (90.50, 125.00)	109.00 (85.00, 126.00)	0.257	0.879
PLT$\times$10?/L	100–300	86.00 (50.00, 147.00)	87.00 (53.50, 132.00)	87.00 (56.00, 137.00)	0.328	0.849
PTA (%)	70–130	60.00 (49.00, 73.00)	61.00 (49.00, 70.50)	62.00 (50.75, 73.00)	0.520	0.771
IgG (g/L)	7–16	14.07 (10.62, 18.87)	16.63 (13.08, 20.29)	15.33 (11.54, 19.83)	5.967	0.051
IL-6 (pg/mL)	0–7	27.78 (15.59, 64.80)	30.79 (13.95, 71.34)	33.90 (5.46, 1, 519)	0.647	0.724
SAA (mg/L)	0–10	13.60 (5.13, 77.35)	14.00 (4.23, 53.65)	12.90 (4.33, 55.15)	0.450	0.798
CRP (ng/L)	0.068–8.2	10.89 (3.82, 36.26)	10.29 (3.35, 24.96)	12.36 (4.88, 35.17)	3.821	0.148
PVW (mm)	6–10	12.00 (12.00, 15.00)	12.00 (12.00, 14.00)	13.00 (12.00, 14.00)	1.041	0.594

PVW, the width of the portal vein; IQR, Interquartile range.

following points: (1) the patients in our study were all from Asia, and the etiology of cirrhosis was mostly HBV, which was different from the context of other studies and (2) different types of studies may cause diverse results.

By comparing lab data among left, right, and bilateral pleural effusions, the right side is a more common distribution, which is similar to the conclusion of the previous studies (4, 42, 43). In addition to the hypothesis of the physiological defect in the diaphragm, Huang et al. suggested that it is likely due to the close anatomical relationship of bare areas of the liver with the diaphragm and the fact that the left side of the diaphragm is thicker and more muscular than the right side (44). In addition to the aforementioned finding, we have another conclusion: The severity of liver cirrhosis has no relationship with the distribution of pleural effusion, and portal vein thrombosis is more common in cirrhotic patients with bilateral pleural effusion compared to those with unilateral pleural effusion, but the mechanism is still unknown. The fact that portal vein thrombosis further aggravates portal hypertension, resulting in increased parietal pleural pressure in the umbilical and hemiazygos veins, may be the main factor.

Limitations of the study are as follows: (1) In choosing samples, retrospective studies are more prone to selection bias, but the inclusion of a larger sample size in our study mitigated this effect. (2) We have only studied the risk factors for the development of HH in cirrhosis patients with ascites. Whether our conclusion applies to the entire cirrhotic population, the answer is unknown. (3) In fact, we collected data on the depth of ascites as a criterion to assess the severity of ascites that were reported in the patient's abdominal imaging findings. However, the univariate analysis of this variable was not statistically significant, which may be related to the fact that this value only reflected the depth of ascites in some anatomical planes of the patient's abdomen, and it could not accurately reflect the severity of the patient's ascites. Therefore, we did not take it as a variable.

Conclusion

It was found that lower HDL and PTA and higher PVW, D-dimer, IgG, and MELD scores have a strong correlation with the development of hepatic hydrothorax. Moreover, portal vein thrombosis is more common in cirrhotic patients with bilateral pleural effusion compared to those with unilateral pleural effusion.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

This study was approved by the Ethics Committee of Shandong Public Health Clinical Center (SPHCC-2021-16). The

patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

WD: study concept and design and critical revision of the manuscript for important intellectual content. XB and XL: acquisition of data. XB and XL: analysis and interpretation of data and statistical analysis. XB: drafting of the manuscript. YS and WL: administrative, technical, or material support. QL: study supervision. All authors read and approved the final manuscript.

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Conflict of interest

WL was employed by ICON Plc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Neutrophil-to-lymphocyte ratio predicts 30-, 90-, and 180-day readmissions of patients with hepatic encephalopathy

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Introduction: Hepatic encephalopathy (HE) is a significant complication of cirrhosis, known to be associated with hospital readmission. However, few new serological indicators associated with readmission in HE patients have been identified and reported. The objective of our study was to identify simple and effective predictors reated to readmission in HE patients.

Materials and methods: We conducted a retrospective study at a single center on adult patients admitted with HE from January 2018 to December 2022. The primary endpoint was the first liver-related readmission within 30, 90, and 180 days, and we collected electronic medical records from our hospital for sociodemographic, clinical, and hospitalization characteristics. We utilized logistic regression analysis and multiple linear regression analysis to determine the predictors that were associated with the readmission rate and the length of the first hospitalization.

Results: A total of 424 patients were included in the study, among whom 24 (5.7%), 63 (14.8%), and 92 (21.7%) were readmitted within 30, 90, and 180 days, respectively. Logistic regression analysis showed that insurance status, alcoholic liver disease (ALD), ascites, the model for end-stage liver disease (MELD) score, and neutrophil-to-lymphocyte ratio (NLR) were significantly associated with 30-, 90-, and 180-day readmissions. Age and hepatocellular carcinoma (HCC) were predictors of 90- and 180-day readmissions. ALD was identified as a unique predictor of readmission in men, while hypertension was a predictor of 180-day readmission in women. Variceal bleeding, chronic kidney disease, and MELD score were associated with the length of the first hospitalization.

Conclusions: NLR at discharge was identified as a significant predictor of 30-, 90- and 180-day readmissions in patients with HE. Our findings suggest that incorporating NLR into routine clinical assessments could improve the evaluation of the prognosis of liver cirrhosis.

KEYWORDS

hepatic encephalopathy, decompensated cirrhosis, neutrophil-to-lymphocyte ratio, readmission, hospitalization

Introduction

Hepatic encephalopathy (HE) is a significant complication of advanced liver disease that is associated with poor outcomes. The incidence of HE in patients with cirrhosis can reach 11.6/100 person-years (1). Once HE develops, the 1- and 3-year cumulative survival rates are only 50 and 25% (2). HE is characterized by changes in personality, consciousness, cognition, and motor function and is associated with a range of clinical complications (3). Additionally, HE has a negative impact on the health-related quality of life of affected patients (4).

According to reports, the readmission rates of patients with cirrhosis were 20.7 and 30.1% within 30 and 90 days, respectively, with HE being the most common reason for readmission (5). Furthermore, HE was also the leading cause of readmission for patients with decompensated cirrhosis within 180 days (45.4%), with a mortality rate of 35.0% within 180 days ($p = 0.001$) (6). Readmission not only predicts poor outcomes for cirrhotic and HE patients, but it also represents a significant financial burden. A national study conducted in America found that the total healthcare cost for cirrhotic patients with readmission was significantly higher than for those without readmission within 30 days ($64,795 vs. $31,017, $p < 0.001$) (7).

Several factors have been reported as predictors of readmission in HE patients, including insurance status, alcoholic liver disease (ALD), the presence of portal hypertension, international normalized ratio (INR), the model for end-stage liver disease (MELD) score, ascites, receiving paracentesis, and acute kidney injury (1, 7–9). Previous studies reported that volume status, including ascites, receiving paracentesis, and acute kidney injury, were associated with early readmission, as HE patients with refractory ascites and/or recurrent acute kidney injury often require repeated paracentesis (7, 10). Uninsured patients were found to have higher alcohol-related admissions and lower inpatient and 90-day transplant rates, even after controlling for age, gender, race, ethnicity, comorbid conditions, and cirrhosis status (8). INR was found to independently predict early readmission in patients with HE, and MELD score was the only predictor when HE was considered the only cause of readmission (9). Furthermore, portal hypertension was associated with HE, as patients with severe liver disease manifested by portal hypertension had an incidence rate of 27.11 (95% CI, 26.84, 27.38) hospitalizations compared to 4.25 (95% CI, 4.18, 4.31) hospitalizations per person-years for those without, giving an incidence rate ratio (IRR) of 6.38 (95% CI, 6.27, 6.51) (1).

Although the neutrophil-to-lymphocyte ratio (NLR) has been reported to be associated with diseases, such as cardiac and chronic obstructive pulmonary disease (11, 12), as well as hepatopathy-related diseases, such as alcoholic hepatitis, hepatocellular carcinoma (HCC), and others (13–16), its correlation with readmission in HE patients has not been clearly researched. To clarify whether NLR is one of the predictors of readmission in HE patients, we conducted this retrospective study.

Materials and methods

Data source

We conducted a retrospective study at a single center, including adult patients admitted with HE between January 2018 and December 2022. The patients were identified through the electronic medical record of our hospital using admission diagnoses of HE with ICD-9 codes 570.X, 572.2, 348.3, 348.31, 348.39, and 291.2. Patients with incomplete information and those who were lost to follow-up were excluded. The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) and was approved by the Ethics Committee of Peking University People's Hospital (No. 2022PHB251-01). Informed consent was obtained from all patients included in the study.

Baseline characteristics

We collected sociodemographic, clinical, and hospitalization characteristics, including gender, age, insurance (including Chinese medical insurance for residents and Chinese worker medical insurance), baseline liver disease [alcohol or non-alcohol (viral hepatitis, non-alcoholic steatohepatitis, drug-induced liver injury, and autoimmune liver disease)], comorbidities [hypertension, diabetes, and chronic kidney disease (CKD)], and complications [HCC, ascites, variceal bleeding, and spontaneous bacterial peritonitis (SBP)]. Laboratory results at the first discharge, including ammonia, MELD score, and ALD diagnosed as alcohol intake $\geq$40 g per day in men and $\geq$20 g per day in women, lasting $\geq$5 years, were also collected (17). Baseline liver disease (hepatitis B and C, autoimmune liver disease, and drug-induced liver injury), with the exception of alcoholic liver disease, were extracted from admission and discharge diagnoses, and only one occurrence of each diagnosis was recorded. Laboratory data were obtained from the hospital's clinical data center. The MELD score was calculated using total bilirubin, creatinine, INR, and a history of cholestatic liver disease. The NLR was calculated as the ratio of absolute neutrophil count to absolute lymphocyte count.

Study outcomes

We collected information on readmissions through the electronic medical record of our hospital, which includes medical history, physical examinations, daily notes, laboratory results, and discharge summaries. To capture episodes of hospitalization at other hospitals, we contacted patients who had liver-related readmissions to any hospital since the first hospitalization. Hospitalization characteristics recorded included complications of cirrhosis at the first admission (volume-related complications such as ascites, edema, or SBP; other complications or more than one complication such as HCC, variceal bleeding, and hepatic hydrothorax), cause of readmission (HE, volume-related, other, or more than one complication), and the length of stay for the first hospitalization (calculated from admission to discharge).

We defined the primary endpoint as the first liver-related readmission occurring within 30, 90, and 180 days following the initial hospitalization. Liver-related readmission was defined as readmission due to any of the following: HE, volume-related complications (ascites, edema, and SBP), HCC, variceal bleeding, and hepatic hydrothorax. HE was diagnosed according to the West Haven criteria and patients' clinical manifestations and ammonia levels. Ascites were diagnosed by imaging examination or clinical percussion revealing fluid in the peritoneal cavity. HCC was diagnosed based on pathology or imaging examination. Variceal bleeding was diagnosed based on clinical features and endoscopy. SBP was diagnosed when the ascitic fluid neutrophil count exceeded 250/mm^3. Edema was diagnosed based on patient complaints and pitting edema of both lower limbs. Hepatic hydrothorax was diagnosed based on an imaging examination. Patients could have more than one liver-related reason for admission, and all other readmissions were excluded from the analysis.

Statistical analysis

Continuous variables were presented as mean ± standard deviations (SD) and categorical variables as count with percentage. Differences between groups were analyzed using Student's *t*-test for age, length of stay, MELD, NLR, and ammonia at discharge, and chi-square test for insurance, baseline liver disease, comorbidities, complications, treatment, and cause of readmission. Predictors associated with readmission and the length of the first hospitalization were analyzed using logistic regression analysis and multiple linear regression analysis, respectively. Statistical significance was considered at $p < 0.05$. Data analyses were performed using SPSS version 23.0 (IBM Corp, Armonk, NY, USA).

Results

Baseline sociodemographic and clinical characteristics

We included 424 patients admitted with HE, and their baseline characteristics are presented in Table 1. Within 30, 90, and 180 days, 24 (5.7%), 63 (14.8%), and 92 (21.7%) patients were readmitted, respectively. Among the patients, 283 (66.7%) were male patients, and the mean age was 59.9 ± 11.5 years; 120 (28.3%) patients had alcoholic cirrhosis. There were 280 patients classified as grade 0, 66 were of grade 1, 42 were of grade 2, 33 were of grade 3, and 1 was of grade 4. At baseline, 40 (9.4%), 246 (58.0%), 67 (15.8%), and 20 (4.7%) patients had HCC, ascites, variceal bleeding, and SBP, respectively. Patients who were readmitted within 180 days were older (62.0 ± 9.6 vs. 59.3 ± 12.0, $p = 0.025$) and had a higher proportion of no insurance [25 (27.2%) vs. 52 (15.7%), $p = 0.010$], ALD [34 (37.0%) vs. 86 (25.9%), $p = 0.027$], CKD [21 (22.8%) vs. 39 (11.7%), $p = 0.007$], HCC [15 (16.3%) vs. 25 (7.5%), $p = 0.012$], and ascites [65 (70.7%) vs. 181 (54.5%), $p = 0.004$] than those who were not readmitted within 180 days.

Hospitalization characteristics

Patients were admitted for liver-related reasons other than HE, including volume-related (247, 58.3) and other complications (177, 41.7%). The mean length of hospitalization for all patients at the time of admission was 17.9 ± 12.1 days. HE was the most common reason for readmission, with 15 (62.5%), 30 (47.6%), and 48 (52.1%) patients being readmitted for HE within 30, 90, and 180 days, respectively. Furthermore, patients who were readmitted had a higher NLR level at their first discharge (8.17 ± 12.1 vs. 3.95 ± 4.96; 7.17 ± 9.72 vs. 3.67 ± 4.44; 6.80 ± 8.62 vs. 3.47 ± 4.28, $p < 0.001$) compared to those without readmission in 30, 90, or 180 days (see Table 2). Patients who were readmitted within 30 days had the longest length of stay during their first hospitalization, which was significantly higher than those without readmission in 30 days (24.8 ± 19.2 vs. 17.7 ± 11.90, $p < 0.001$).

Predictors of 30-, 90-, and 180-day readmissions

Table 3 displays the results of logistic regression analysis for predictors of readmission within 30, 90, and 180 days. Significant predictors of readmission included no insurance, ALD, ascites, MELD score, and NLR at first discharge for all three time periods. Age and HCC were also significant predictors of readmission at 90 and 180 days.

However, gender differences were observed in the predictors of readmission. In men (Table 4), in addition to HCC, ascites, MELD score, and NLR at discharge, ALD was a significant predictor of 30-, 90-, and 180-day readmissions. Interestingly, ALD was not a significant predictor of readmission in women, while hypertension was significantly associated with 180-day readmission in women (Table 5).

Predictors of the length of hospitalization at the first admission

Table 6 shows that variceal bleeding ($p = 0.006$), CKD ($p = 0.003$), and MELD score at discharge ($p = 0.024$) were significant predictors of the length of hospitalization for patients at their first admission.

Discussion

Patients who are readmitted for HE are at an increased risk of morbidity and mortality, making readmissions costly for both patients and healthcare systems. Our study found that NLR at discharge was a significant predictor of 30-, 90-, and 180-day readmissions for patients with HE. This new predictor offers valuable insights that can inform clinical decision-making and improve the evaluation of the prognosis of liver cirrhosis. Additionally, our study showed that CKD, one of the most important comorbidities, was a significant factor affecting the length of hospitalization at the first admission for patients with HE.

TABLE 1 Baseline characteristics of included patients.

Characteristics	All ($n = 424$)	30-day readmission ($n = 24$)	90-day readmission ($n = 63$)	180-day readmission ($n = 92$)
Age (years), mean ± SD	59.9 ± 11.5	62.8 ± 11.0	61.9 ± 9.9	62.0 ± 9.6*
Male, *n* (%)	283 (66.7)	15 (62.5)	43 (68.3)	63 (68.5)
Insurance, *n* (%)				
Any insurance	347 (81.8)	17 (70.8)	47 (74.6)	67 (72.8)
No Insurance	77 (18.2)	7 (29.2)	16 (25.4)	25 (27.2)*
Baseline liver disease, *n* (%)				
Alcohol	120 (28.3)	10 (41.7)	23 (36.5)	34 (37.0)*
Non-alcohol	304 (71.7)	14 (58.3)	40 (63.5)	58 (63.0)
Comorbidities, *n* (%)				
Hypertension	130 (30.6)	11 (45.8)	21 (33.3)	28 (30.4)
Diabetes	123 (29.0)	6 (25.0)	22 (34.9)	33 (35.9)
Chronic kidney disease	60 (14.2)	8 (33.3)*	16 (25.4)*	21 (22.8)*
Complications, *n* (%)				
Hepatocellular carcinoma	40 (9.4)	3 (12.5)	12 (19.0)*	15 (16.3)*
Ascites	246 (58.0)	20 (83.3)*	45 (71.4)*	65 (70.7)*
Variceal bleeding	67 (15.8)	5(20.8)	16 (25.4)*	20 (21.7)
Spontaneous bacterial peritonitis	20 (4.7)	2 (8.3)	2 (3.2)	4 (4.3)
Treatment, *n* (%)				
Lactulose alone	250 (58.9)	13 (54.2)	40 (63.5)	57 (62.0)
Lactulose + rifaximin	17 (4.0)	1 (4.2)	3 (4.8)	3 (3.3)
Rifaximin alone	81 (19.1)	9 (37.5)	17 (27.0)	22 (23.9)
Other	76 (17.9)	1 (4.2)	3 (4.8)	10 (10.9)

Non-alcoholic liver disease: viral hepatitis, non-alcoholic steatohepatitis, drug-induced liver injury, or autoimmune liver disease.
*Comparisons between patients readmitted with and without 30, 90, and 180 days, $p < 0.05$.

Therefore, managing comorbidities in addition to treating HE can help reduce the length of hospital stay for these patients.

We found that NLR at the first discharge was a crucial predictor of 30-, 90-, and 180-day readmissions. As neutrophils play an important role in many types of liver disease and inappropriate activation and homing of neutrophils to the microvasculature can induce immune-mediated liver injury, NLR is a serological marker that reflects the level of inflammation and is superior to simple white blood cell counts in cirrhotic patients (18). It was reported that NLR could correctly identify these patients who had a high risk of mortality despite low MELD scores and a high risk of mortality (11), and NLR was positively correlated with Child–Turcotte–Pugh (CTP) score. In patients with Child–Pugh class C, NLR is an independent predictor of poor 1-month survival (13). A high NLR reflects the severity and progression of cirrhosis and has been associated with an increased risk of mortality in patients with alcoholic hepatitis and HE (14, 15). It has been reported that for every 1-unit increase in NLR at admission, the increased risk of death in patients with alcoholic hepatitis was 20 and 9% in 30 days and 12 months, respectively (16). Another study reported that elevated NLR was significantly associated with an increased risk of 30-day mortality in HE patients (14). As NLR reflects the level of inflammation, it may aggravate the symptoms of HE by enhancing ammonia-induced neurotoxicity through the blood–brain barrier (19). Elevated plasma levels of inflammatory markers in patients with HE are correlated with the severity of HE and are not determined by the severity of underlying liver disease or ammonia levels (20). Additionally, the neutrophil count is higher in decompensated cirrhotic patients than in compensated cirrhotic patients, while the lymphocyte count is lower in decompensated cirrhosis (21). This suggests that a higher level of NLR reflects worse patient status in decompensated cirrhosis. In patients with HCC, NLR has been identified as an independent factor for worse survival and predicts the response to treatment (18). Low NLR was significantly associated with a better survival rate and recurrence-free or disease-free survival (22). A review of the association between NLR and SBP concluded that NLR was a valid biomarker that can be readily integrated into clinical settings to prevent and predict SBP in cirrhotic patients (23). NLR has also been found to be a predictor of survival and readmission in other diseases such as cardiac and chronic obstructive pulmonary disease (11, 12). To the best of our knowledge, our study is the first to report the predictive role of NLR in the readmission of patients with HE. The correlation between NLR and readmission suggests

TABLE 2 Hospitalization characteristics.

Index hospitalization	All	30-day readmission			90-day readmission			180-day readmission		
		Not readmitted	Readmitted	*p*-value	Not readmitted	Readmitted	*p*-value	Not readmitted	Readmitted	*p*-value
	$n = 424$	$n = 400$	$n = 24$		$n = 361$	$n = 63$		$n = 332$	$n = 92$	
Other complications of cirrhosis at the first admission, *n* (%)										
Volume-related	247 (58.3)	227 (56.8)	20 (83.3)	0.010	202 (56.0)	45 (71.4)	0.026	182 (54.8)	65 (70.7)	0.008
Other or > 1 complication	177 (41.7)	173 (43.2)	4 (16.7)		159 (44.0)	18 (28.6)		150 (45.2)	27 (29.3)	
The length of stay (days), mean ± SD	17.9 ± 12.1	17.5 ± 11.4	24.8 ± 19.2	0.004	17.7 ± 11.8	19.1 ± 13.6	0.389	17.6 ± 11.9	18.9 ± 12.6	0.347
MELD score at discharge, mean ± SD	13.5 ± 8.9	13.0 ± 8.8	21.4 ± 6.3	<0.001	12.6 ± 8.8	19.0 ± 7.2	<0.001	12.4 ± 8.9	17.7 ± 7.4	0.060
NLR at discharge, mean ± SD	4.19 ± 5.68	3.95 ± 4.96	8.17 ± 12.1	<0.001	3.67 ± 4.44	7.17 ± 9.72	<0.001	3.47 ± 4.28	6.80 ± 8.62	<0.001
Ammonia at discharge (μmol/L), mean ± SD	71.2 ± 33.7	71.2 ± 34.0	71.8 ± 30.7	0.934	71.8 ± 34.2	68.1 ± 30.5	0.429	70.6 ± 31.3	73.5 ± 41.2	0.466
The cause of readmission, *n* (%)										
Hepatic encephalopathy		—	15 (62.5)		—	30 (47.6)		—	48 (52.1)	
Volume-related		—	5 (20.8)		—	22 (34.9)		—	20 (21.7)	
Other or > 1 complication		—	4 (16.7)		—	11 (17.4)		—	24 (26.1)	

Volume-related: ascites, edema, or spontaneous peritonitis. Other or >1 complication: hepatocellular carcinoma, variceal bleeding, hepatic hydrothorax, acute kidney injury, and electrolyte abnormalities or more than one complication.
MELD, model for end-stage liver disease; NLR, neutrophil-to-lymphocyte ratio.

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TABLE 3 Predictors of 30-, 90-, and 180-day readmissions.

Characteristics	β	*p*-value	OR	95% CI
30-day readmission				
No insurance	1.155	0.047	3.173	1.015, 9.915
Alcoholic liver disease	1.302	0.037	3.675	1.081, 12.491
Ascites	2.019	0.006	7.529	1.780, 31.855
MELD score at discharge	0.191	<0.001	1.210	1.125, 1.302
NLR at discharge	0.058	0.044	1.060	1.002, 1.122
90-day readmission				
Age	0.036	0.024	1.036	1.005, 1.069
No insurance	0.770	0.048	2.159	1.006, 4.633
Alcoholic liver disease	0.821	0.035	2.274	1.061, 4.873
Hepatocellular carcinoma	1.044	0.022	2.841	1.162, 6.945
Ascites	0.772	0.036	2.164	1.051, 4.456
MELD score at discharge	0.165	<0.001	1.180	1.122, 1.240
NLR at discharge	0.066	0.008	1.068	1.017, 1.112
180-day readmission				
Age	0.040	0.003	1.041	1.013, 1.069
No insurance	1.003	0.003	2.727	1.415, 5.256
Alcoholic liver disease	0.932	0.006	2.539	1.311, 4.915
Hepatocellular carcinoma	0.891	0.031	2.438	1.085, 5.476
Ascites	0.701	0.022	2.016	1.105, 3.679
MELD score at discharge	0.141	<0.001	1.151	1.102, 1.203
NLR at discharge	0.077	0.002	1.080	1.030, 1.133

MELD, model for end-stage liver disease; NLR, neutrophil-to-lymphocyte ratio.

TABLE 4 Predictors of 30-, 90-, and 180-day readmissions in men.

Characteristics	β	*P*	OR	95% CI
30-day readmission				
Alcoholic liver disease	1.423	0.043	4.150	1.043, 16.521
Ascites	2.710	0.027	15.031	1.372, 164.729
MELD score at discharge	0.218	<0.001	1.244	1.128, 1.372
90-day readmission				
Alcoholic liver disease	0.915	0.028	2.496	1.106, 5.635
Hepatocellular carcinoma	1.171	0.028	3.224	1.134, 9.170
MELD score at discharge	0.184	<0.001	1.202	1.128, 1.280
180-day readmission				
Alcoholic liver disease	0.966	0.008	2.627	1.291, 5.348
Ascites	1.292	0.003	3.640	1.561, 8.466
MELD score at discharge	0.162	<0.001	1.176	1.112, 1.244
NLR at discharge	0.072	0.022	1.075	1.010, 1.144

MELD, model for end-stage liver disease; NLR, neutrophil-to-lymphocyte ratio.

that controlling inflammation in HE patients in clinical practice can help reduce the liver disease-related readmission rate in HE patients.

TABLE 5 Predictors of 30-, 90-, and 180-day readmissions in women.

Characteristics	β	*P*	OR	95% CI
30-day readmission				
No insurance	3.006	0.013	20.209	1.861, 219.444
MELD score at discharge	0.264	0.005	1.302	1.082, 1.567
90-day readmission				
Age	0.075	0.023	1.077	1.010, 1.149
MELD score at discharge	0.135	0.006	1.144	1.039, 1.260
180-day readmission				
Age	0.085	0.002	1.089	1.031, 1.149
No insurance	1.745	0.007	5.725	1.609, 20.376
Hypertension	1.500	0.017	4.481	1.309, 15.331
MELD score at discharge	0.124	0.007	1.131	1.035, 1.237
NLR at discharge	0.109	0.039	1.115	1.006, 1.237

MELD, model for end-stage liver disease; NLR, neutrophil-to-lymphocyte ratio.

TABLE 6 Predictors of the length of hospitalization at the first admission.

Characteristics	B	SE	β estimate	*p*	95.0% CI for β
Constant	16.046	3.917		0.000	8.346, 23.746
Age	−0.065	0.053	−0.063	0.219	−0.169, 0.039
Men	0.460	1.319	0.018	0.727	−2.133, 3.052
No insurance	−1.838	1.465	−0.060	0.210	−4.717, 1.042
Alcoholic liver disease	−2.577	1.374	−0.099	0.061	−5.279, 0.124
Hepatocellular carcinoma	0.467	1.919	0.012	0.808	−3.305, 4.239
Ascites	1.741	1.168	0.073	0.137	−0.555 ± 4.038
Spontaneous bacterial peritonitis	4.264	2.765	0.076	0.124	−1.172, 9.699
Variceal bleeding	4.402	1.593	0.135	0.006	1.269, 7.534
Chronic kidney disease	4.973	1.658	0.149	0.003	1.713, 8.232
Diabetes	−0.422	1.242	−0.016	0.734	−2.864, 2.019
Hypertension	1.789	1.272	0.070	0.160	−0.711, 4.289
Ammonia at discharge	0.020	0.017	0.056	0.249	−0.014, 0.053
MELD score at discharge	0.204	0.090	0.111	0.024	0.026, 0.381
N/L at discharge	0.064	0.102	0.031	0.530	−0.136, 0.264

MELD, model for end-stage liver disease; NLR, neutrophil-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio.

In our study, demographic variables, except for age and insurance, were not found to be significant predictors of readmission. It is worth noting that in China, not all individuals have access to medical insurance. Lack of medical insurance can hinder access to timely treatment and routine examinations, which may lead to a worsened condition and an increased risk of

readmission. This is consistent with previous research by Andrew, who identified age and medical insurance as social-demographic predictors of 30-day readmission in patients with HE (7). Our study further found that insurance status was a significant predictor of readmission within 180 days. Therefore, access to medical insurance is crucial for patients with HE to receive timely and appropriate medical care and reduce the risk of readmission.

Regarding patients' complications and comorbidities, our study found that HCC and ascites were significantly related to HE patients' readmission. Ascites is a common complication of decompensated cirrhosis, and it directly increases the risk of further complications such as SBP, umbilical hernias, and respiratory compromise, all of which may require readmission (24). A previous study concluded that ascites were associated with early readmission in patients with cirrhosis (25). Ascites production is related to portal hypertension and splanchnic vasodilation, and it can also lead to renal dysfunction and hepatorenal syndrome (26). Previous research has suggested that ascites and renal dysfunction played important roles in the outcomes of patients with cirrhosis, and the use of diuretics and renal failure may impact HE as well (19, 26). Meanwhile, HCC is difficult to treat and associated with high mortality rates and surgical morbidity, increasing the risk of readmission (27). Hepatic resection and transplantation of HCC patients are still associated with a high risk of mortality and postoperative readmission rates. Furthermore, these procedures subject patients to higher medical and surgical morbidities compared to those encountered in the general surgery population (28). These complications and comorbidities can undoubtedly aggravate the condition of HE patients. In view of gender differences, ALD was a significant predictor of readmission in men. ALD has been found to be an important predictor of readmission in patients with cirrhosis and HE (29), and alcohol abuse is a common reason for readmission in cirrhotic patients (30). A study reported that in patients with alcoholic liver cirrhosis, there was a steady rise in the risk-adjusted 30-day all-cause readmission rate as well as alcoholic liver cirrhosis-specific readmission rate and readmission proportion (31). Furthermore, the increasing rate of readmission in ALD patients also increases the total hospital cost and the total days of hospital stay. Effective alcohol use disorder interventions can help reduce costs related to inpatient cirrhosis management (32). Managing HCC and ALD patients well in clinical practice can reduce the risk of readmission for patients with HE.

MELD score was used to identify prognosis and survival in patients with cirrhosis (33). Previous studies found that MELD score, INR, and hemoglobin were predictors of early readmission in cirrhotic patients after the resolution of HE (9). We did not include INR as a single factor, as the MELD score at discharge could represent the HE patients' status.

In our study, we observed gender differences in the predictors of readmission for patients with HE. In addition to ascites, MELD score, and NLR at the first discharge, ALD was significantly associated with readmission in men. Interestingly, hypertension, instead of ALD, predicted long-term readmission in women. Gender differences have been observed in many studies on chronic liver disease and HE. For example, women may experience a more favorable clinical course than men in early chronic liver disease, with sex hormones believed to have a protective effect on fibrosis progression (34, 35). Women with HE exhibited better cognitive performance than men. In a study on gut microbial composition in HE patients, it was observed that certain pathways and microbiota, such as Lactobacillaceae, androstenedione degradation, and cell wall synthesis, remained different between the sexes. These differences may contribute to the disparity in cognition between men and women with cirrhosis and ultimately affect the morbidity of HE in each gender (36). It should be noted that only 141 (33.3%) participants were women, and 9, 20, and 29 female patients were readmitted in 30, 90, and 180 days, so the smaller proportion of female patients may also be a contributing factor to the observed results. Therefore, large-scale studies are needed to further confirm the gender differences in the readmission of HE that we have observed.

Our study has identified NLR as a novel predictor of readmission in HE patients, improving the diagnosis of HE. The management of comorbidities, such as hypertension and CKD, is also crucial to the healthcare of patients with HE. However, our study has some limitations. First, although we took measures to ensure the accuracy of our data by conducting follow-ups with patients, as a retrospective single-center study, to confirm our results, multicenter studies should be carried out in the future. Moreover, we did not explore the mechanisms underlying the relationship between NLR and readmission or potential interventions to reduce readmission rates. Additionally, our study did not include other treatments, such as anti-infection and diuresis, which may become confounding factors for HE readmission. Anti-infection treatment may also affect NLR, and the lack of data on other inflammation indicators, such as CRP and IL-6, is another limitation. Furthermore, we did not collect information on HE patients' comorbidities and complications, such as dehydration, diuretic usage, human albumin infusion, and malnutrition, which may have influenced our findings. Therefore, future studies should aim to address these limitations and better understand these aspects.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Peking University People's Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

RH designed the work and reviewed the article. LZ and WZ interpreted the data and drafted the

manuscript. LZ, WZ, JW, QJ, and DM collected the data. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Insulin-like growth factor 1 predicts decompensation and long-term prognosis in patients with compensated cirrhosis

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Aim: Insulin-like growth factor 1 (IGF-1), which is primarily produced in hepatocytes and is associated with liver functional reserve, plays a crucial role in the pathological condition of cirrhosis. This study aimed to investigate the usefulness of serum IGF-1 levels for predicting the long-term prognosis and decompensation development in patients with cirrhosis.

Methods: We retrospectively evaluated 148 patients with cirrhosis and divided them into three groups according to baseline IGF-1 levels: low (L)-, intermediate (I)-, and high (H)-IGF-1 groups. The cumulative survival rates were compared among these groups in compensated and decompensated cirrhosis, respectively. Significant and independent factors associated with mortality and decompensation development were identified using Cox proportional hazards regression analysis.

Results: The median observation period was 57.1 (41.7–63.2) months. Thirty (20.3%) patients died of liver disease-related events and 21 (22.3%) patients with compensated cirrhosis developed decompensation. Multivariate analysis identified low serum IGF-1 levels as a significant and independent factor associated with mortality (all patients: hazard ratio [HR], 0.967; $p = 0.004$; patients with compensated cirrhosis: HR, 0.927; $p = 0.002$). The cumulative survival rates were significantly lower in the L-IGF-1 group than in the H-IGF-1 and I-IGF-1 groups (all patients: $p < 0.001$ and = 0.009; patients with compensated cirrhosis: $p = 0.012$ and 0.003, respectively). However, in decompensated cirrhosis, the cumulative survival rates demonstrated no significant differences among the three groups. The cumulative decompensation incidence rates were significantly higher in the L-IGF-1 group than in the H-IGF-1 and I-IGF-1 groups ($p < 0.001$ and = 0.009, respectively). Low serum IGF-1 levels were significantly and independently associated with decompensation development (HR, 0.939; $p < 0.001$).

Conclusion: Low serum IGF-1 levels were significantly and independently associated with decompensation development and poor long-term prognosis in patients with compensated cirrhosis. Therefore, IGF-1 may be useful for predicting decompensation-related events and should be regularly monitored in the management of compensated phase.

KEYWORDS

cirrhosis, insulin-like growth factor 1, liver functional reserve, prognosis, decompensation

1. Introduction

Cirrhosis is the end stage of chronic liver disease (CLD) with different etiology, and it is a major cause of morbidity and mortality, leading to over 1.32 million (2.4%) global deaths (1, 2). It progresses from a compensated phase (in which most cases are asymptomatic) to a decompensated phase (in which variceal hemorrhage, ascites, encephalopathy, and jaundice can occur) (3). A better prognosis was observed in patients with compensated than those with decompensated cirrhosis, with median survival times of >12 years and <2 years, respectively (3). Therefore, the management of patients with compensated cirrhosis is crucial to inhibit decompensated cirrhosis progression and thereby reduce the mortality rate. The Child–Pugh (CP) classification, as determined based on serum albumin, total bilirubin, and prothrombin time (PT) levels, ascites degree, and encephalopathy grade, is widely used to evaluate liver functional reserve (4). This classification can reflect clinical features and estimate patients' prognoses. A better prognosis was observed in patients with CP class A (nearly corresponding to compensated cirrhosis) than those with CP class B/C (nearly corresponding to decompensated cirrhosis) (3). However, this scoring system includes subjective components, i.e., ascites and encephalopathy, and therefore may reduce clinical assessment reliability and survival prediction accuracy (5). Furthermore, it can hardly assess prognosis in patients with compensated cirrhosis (6).

Insulin-like growth factor 1 (IGF-1), which is primarily produced in hepatocytes and involved in mediating growth and metabolism, plays a crucial role in the pathological condition of CLD (7–9). Its circulating levels have been reported to correlate with liver functional reserve. They are decreased with advanced disease stage and malnutrition status, thereby exacerbating or causing insulin resistance, reactive oxygen species, inflammation, mitochondrial dysfunction, liver fibrosis, osteoporosis, and sarcopenia (7–12). A previous study revealed significantly lower 2 years survival rates in inpatients with cirrhosis with low plasma IGF-1 levels than those with high plasma IGF-1 levels (13). Furthermore, combined serum IGF-1 levels and CP scores (IGF-CP score) more accurately predict 1 year mortality than CP or model for end-stage liver disease (MELD) scores alone in patients with decompensated cirrhosis (5). Thus, circulating IGF-1 levels may be useful for estimating liver functional reserve or pathological condition and predicting prognosis in patients with cirrhosis. However, previous studies were limited to decompensated cirrhosis or short-term research duration and have not yet reported the association of IGF-1 with long-term prognosis in patients with compensated and decompensated cirrhosis, respectively.

This study aimed to investigate the usefulness of serum IGF-1 levels for predicting the long-term prognosis of patients with cirrhosis and the difference in the predictive performance of IGF-1 between patients with compensated and decompensated cirrhosis. Furthermore, we evaluated the usefulness of serum IGF-1 levels for predicting decompensation development in patients with compensated cirrhosis.

2. Materials and methods

2.1. Study participants

This retrospective study included 148 consecutive patients with cirrhosis who presented to the Jikei University School of Medicine (Tokyo, Japan) and Fuji City General Hospital (Shizuoka, Japan) between 2017 and 2020. Inclusion criteria were (i) diagnosis of cirrhosis caused by any etiology and (ii) available baseline serum IGF-1 measurements. Exclusion criteria were (i) preexisting malignancies, including hepatocellular carcinoma (HCC); (ii) liver transplantation history; and (iii) acute liver failure. Cirrhosis diagnosis was based on laboratory tests and imaging/endoscopic findings, such as surface nodularity, liver deformity with right lobe shrinkage and left lobe enlargement, ascites, splenomegaly, portosystemic collateral, and esophageal/gastric varices (14). Decompensated cirrhosis was diagnosed by the development of variceal hemorrhage, ascites, encephalopathy, and jaundice (15). Liver functional reserve was evaluated using the MELD score and CP classification (4, 16). The endpoint of this study was death from liver disease-related events. Patients who underwent liver transplantation for liver failure were considered as death and those who died from non-liver-related causes were considered censored cases. This study complied with the 2013 Declaration of Helsinki and was approved by the ethics committees of the Jikei University School of Medicine (approval number: 34-021) and Fuji City General Hospital (approval number: 279).

2.2. Laboratory assessments

Serum albumin, total bilirubin, creatinine, sodium, Mac-2 binding protein glycosylation isomer (M2BPGi, a hepatic fibrosis marker), and PT were measured using standard laboratory methods. Serum IGF-1 levels were evaluated using an immunoradiometric assay (Fujirebio, Tokyo, Japan).

2.3. Patient classification based on baseline serum IGF-1 levels

The median baseline IGF-1 levels of all patients and those with compensated and decompensated cirrhosis were 54 (interquartile range, 41–74), 61 (47–78), and 46 (32–61) ng/mL, respectively (Supplementary Figure S1). Patients were classified into three groups according to the quartiles: low (L)-IGF1$_{all}$ group (≤41 ng/mL), intermediate (I)-IGF1$_{all}$ group (41–74 ng/mL), and high (H)-IGF1$_{all}$ group (≥74 ng/mL) for all patients; L-IGF1$_{com}$ group (≤47 ng/mL), I-IGF1$_{com}$ group (47–78 ng/mL), and H-IGF1$_{com}$ group (≥78 ng/mL) for those with compensated cirrhosis; and L-IGF1$_{deco}$ group (≤32 ng/mL), I-IGF1$_{deco}$ group (32–61 ng/mL), and H-IGF1$_{deco}$ group (≥61 ng/mL) for those with decompensated cirrhosis.

2.4. Statistical analysis

Between-group differences were compared using the Mann–Whitney *U* test or the Kruskal–Wallis test followed by the Steel–Dwass *post hoc* test, as appropriate, for continuous variables that were presented as medians (interquartile ranges), and the chi-squared test for categorical variables that were presented as numbers (percentages). Correlations between IGF-1 and continuous variables were analyzed using Spearman's rank correlation test. The cumulative survival rates were estimated using the Kaplan–Meier method, and the between-group differences were compared using the log-rank test and Bonferroni multiple-comparison method. Significant and independent

factors associated with mortality were identified using univariate and multivariate Cox proportional hazards models. SPSS Statistics version 27 (IBM Japan, Tokyo, Japan) was used for all statistical analyses. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Patient characteristics

Table 1 summarizes the baseline clinical characteristics of 148 included patients. The median age was 69 (57.0–76.0) years, and 96 (64.9%) patients were men. The median MELD score was 8.0 (7.0–11.0). The prevalence of decompensated cirrhosis was 36.5% (54/148). Serum IGF-1 levels in patients with decompensated cirrhosis were significantly lower than in those with compensated cirrhosis (median, 46 ng/mL vs. 61 ng/mL; $p < 0.001$; Supplementary Figure S1). Additionally, they significantly differed among CP classes ($p < 0.001$; Supplementary Figure S2) and were significantly higher in patients with the CP class A (median, 62 ng/mL) than in those with the CP class B (median, 43 ng/mL; $p < 0.001$) and class C (median, 34 ng/mL; $p = 0.005$) in the *post hoc* analysis. However, when the patients were divided into three groups according to age (<65, 65–74, and ≥75 years), serum IGF-1 levels did not significantly differ among the three groups ($p = 0.738$; Supplementary Figure S3).

3.2. Clinical characteristics of patients according to baseline serum IGF-1 levels

The L-IGF1$_{all}$, I-IGF1$_{all}$, and H-IGF1$_{all}$ group distributions were 27.7% (41/148), 47.3% (70/148), and 25.0% (37/148), respectively (Table 1). Etiology, decompensated cirrhosis prevalence, M2BPGi, and liver functional reserve-related factors (CP score, MELD score, total bilirubin, albumin, and PT) significantly differed among the three groups.

3.3. Correlations between IGF-1 and liver functional reserve-related factors and fibrosis marker

The correlations between IGF-1 and liver functional reserve-related factors or M2BPGi were investigated using Spearman's rank correlation test (Figures 1A–F). Serum IGF-1 levels significantly correlated with total bilirubin ($r = -0.280$), albumin ($r = 0.387$), PT ($r = 0.481$), and M2BPGi ($r = -0.481$) levels, CP score ($r = -0.422$), and MELD score ($r = -0.319$) ($p < 0.001$ for all).

3.4. Comparison of cumulative survival rates according to baseline serum IGF-1 levels

The median observation period was 57.1 (41.7–63.2) months. During the follow-up period, 30 (20.3%) patients died of liver disease-related events, including liver failure ($n = 21$), rupture of esophageal varices ($n = 6$), liver transplantation ($n = 2$), and hepatocellular carcinoma ($n = 1$). The 1-, 3-, and 5 years cumulative survival rates were 95.1, 73.9, and 56.2% in the L-IGH1$_{all}$ group; 98.6, 89.5, and 81.4% in the I-IGH1$_{all}$ group; and 100.0, 94.3, and 94.3% in the H-IGH1$_{all}$ group (Figure 2). The cumulative survival rates were significantly lower in the L-IGF1$_{all}$ group than in the H-IGF1$_{all}$ and I-IGF1$_{all}$ groups ($p < 0.001$ and $= 0.009$, respectively). The

TABLE 1 Characteristics of the three groups based on serum IGF-1 levels.

Variable	ALL	L-IGF1$_{all}$	I-IGF1$_{all}$	H-IGF1$_{all}$	*p*-value
Patients, *n* (%)	148	41 (27.7)	70 (47.3)	37 (25.0)	
Man, *n* (%)	96 (64.9)	27 (65.9)	43 (61.4)	26 (70.3)	0.652
Age (years)	69.0 (57.0–76.0)	66.0 (53.5–76.0)	73.0 (59.0–76.5)	64.0 (55.0–73.5)	0.085
BMI (kg/m^2)	23.6 (21.5–26.1)	22.5 (20.4–25.7)	23.6 (21.3–26.0)	24.8 (22.4–26.8)	0.083
Etiology					
HBV/HCV/alcohol/other, *n*	14/38/58/38	2/12/21/6	6/17/21/26	6/9/16/6	0.042
Decompensated cirrhosis, *n* (%)	54 (36.5)	23 (56.1)	25 (35.7)	6 (16.2)	0.001
Child–Pugh score	6 (5–7)	7 (5–8)	6 (5–7)	5 (5–6)	<0.001
MELD score	8.0 (7.0–11.0)	10.0 (8.0–13.0)	8.0 (7.0–10.0)	7.0 (6.0–10.0)	<0.001
Total bilirubin (mg/dL)	0.8 (0.6–1.3)	1.0 (0.7–2.0)	0.8 (0.5–1.2)	0.7 (0.6–1.1)	0.014
Albumin (g/dL)	3.8 (3.3–4.2)	3.4 (3.2–3.9)	3.9 (3.3–4.1)	4.2 (3.7–4.4)	<0.001
Creatinine (mg/dL)	0.9 (0.7–1.1)	0.8 (0.7–1.1)	0.9 (0.7–1.1)	0.9 (0.8–1.2)	0.148
Sodium (mEq/L)	140 (138–141)	139 (137–140)	140 (138–142)	140 (139–142)	0.061
Prothrombin time (%)	81 (68–100)	63 (50–81)	83 (71–99)	91 (79–100)	<0.001
IGF-1 (ng/mL)	54 (41–74)	32 (26–39)	55 (49–63)	87 (78–102)	<0.001
M2BPGi (C.O.I.)	2.79 (1.36–5.56)	4.47 (2.88–8.21)	2.66 (1.63–4.74)	1.19 (0.86–3.25)	<0.001

Continuous variables are shown as median (interquartile range). Statistical analysis was performed using the chi-squared test or the Kruskal–Wallis test, as appropriate. BMI, body mass index; C.O.I., cut-off index; HBV, hepatitis B virus; HCV, hepatitis C virus; IGF-1, insulin-like growth factor 1; M2BPGi, Mac-2 binding protein glycosylation isomer; MELD, model for end-stage liver disease; L-IGF1$_{all}$, low-IGF-1 group; I-IGF1$_{all}$, intermediate-IGF-1 group; H-IGF1$_{all}$, high-IGF-1 group.

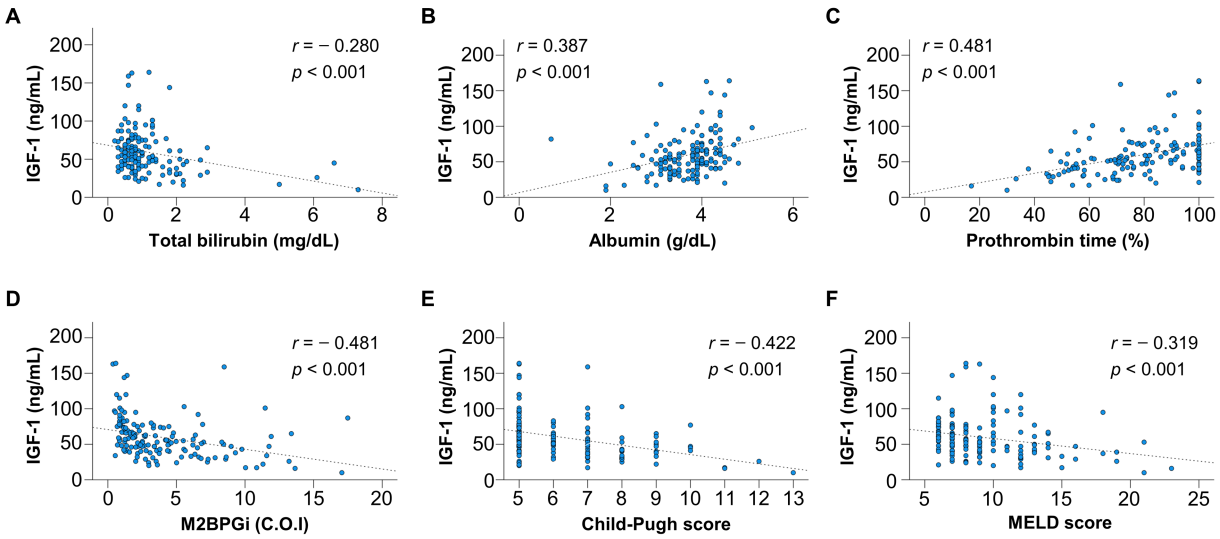


FIGURE 1
Correlation of insulin-like growth factor 1 (IGF-1) with liver functional reserve-related parameters and fibrosis marker. IGF-1 significantly correlated with **(A)** total bilirubin, **(B)** albumin, **(C)** prothrombin time, **(D)** Mac-2 binding protein glycosylation isomer (M2BPGi), **(E)** Child–Pugh score, and **(F)** model for end-stage liver disease (MELD) score ($p < 0.001$ for all).

L-IGF1$_{all}$ group had significantly lower cumulative survival rates than the H-IGF1$_{all}$ and I-IGF1$_{all}$ groups when nine patients who died from non-liver-related causes (L-IGF1$_{all}$, $n = 6$; I-IGF1$_{all}$, $n = 2$; H-IGF1$_{all}$, $n = 1$) were considered the same as liver-related deaths ($p < 0.001$ for both; Supplementary Figure S4).

We divided the patients into the compensated and decompensated cirrhosis groups and compared the cumulative survival rates among the three IGF1 groups in each cirrhosis group (Figure 3). The 1-, 3-, and 5 years cumulative survival rates in the compensated cirrhosis group were 100.0, 86.9, and 72.4% in the L-IGF1$_{com}$ group; 100.0, 100, and 96.3% in the I-IGF1$_{com}$ group; and 100.0, 100.0, and 100.0% in the H-IGF1$_{com}$ group (Figure 3A). The L-IGF1$_{com}$ group had significantly lower cumulative survival rates than the H-IGF1$_{com}$ and I-IGF1$_{com}$ groups ($p = 0.012$ and 0.003, respectively). Meanwhile, the 1-, 3-, and 5 years cumulative survival rates in the decompensated cirrhosis group were 92.9, 68.1, and 34.0% in the L-IGF1$_{deco}$ group; 92.3, 59.1, and 47.1% in the I-IGF1$_{deco}$ group; and 100.0, 84.6, and 76.9% in the H-IGF1$_{deco}$ group (Figure 3B). These rates demonstrated no significant differences among the three groups ($p = 0.177$). However, the redivision from 3 to 2 groups (H-IGF1$_{deco}$ versus combined I-IGF1$_{deco}$ and L-IGF1$_{deco}$) demonstrated a marginally significant difference in the cumulative survival rates between the two groups ($p = 0.064$; Supplementary Figure S5).

3.5. Prognostic factors in patients with cirrhosis

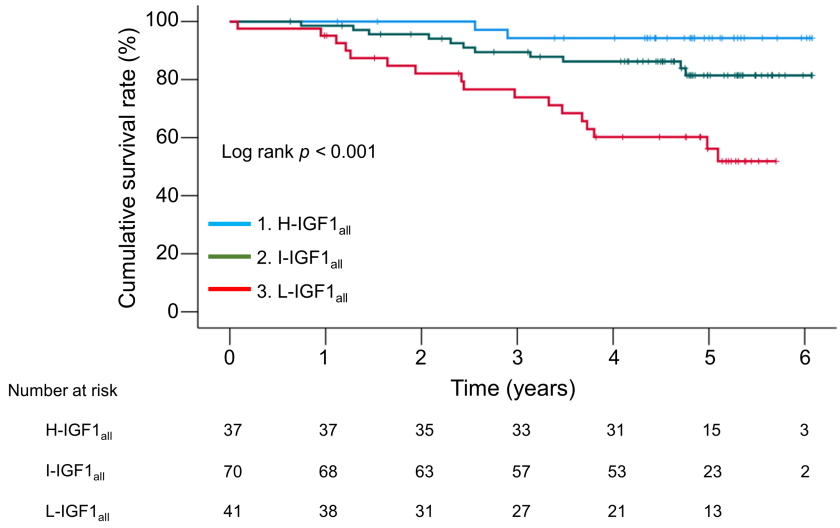
Univariate analysis revealed that the following variables were significantly associated with mortality: decompensated cirrhosis, CP score, MELD score, total bilirubin, albumin, sodium, PT, IGF-1, and M2BPGi in all patients (Supplementary Table S1); PT, IGF-1, and M2BPGi in those with compensated cirrhosis (Supplementary Table S2); and CP score, MELD score, total bilirubin, albumin, IGF-1, and M2BPGi in those with decompensated cirrhosis (Supplementary Table S3). Cox proportional hazards regression analysis identified the following variables as significant and independent prognostic factors: high CP score [hazard ratio (HR), 1.248; 95% confidence interval (CI), 1.014–1.538; $p = 0.037$], low albumin levels (HR, 0.346; 95% CI, 0.204–0.589; $p < 0.001$), and low IGF-1 levels (HR, 0.967; 95% CI, 0.945–0.989; $p = 0.004$) in all patients (Table 2); low IGF-1 levels (HR, 0.927; 95% CI, 0.884–0.972; $p = 0.002$) in those with compensated cirrhosis (Table 3); and low albumin levels (HR, 0.097; 95% CI, 0.038–0.245; $p < 0.001$) in those with decompensated cirrhosis (Table 4).

3.6. Comparison of cumulative incidence of decompensation according to baseline serum IGF-1 levels

During the follow-up period, 21 (22.3%) patients with compensated cirrhosis developed decompensation. The 1-, 3-, and 5 years cumulative incidence rates of decompensation were 20.8, 38.0, and 52.8% in the L-IGH1$_{com}$ group; 0.0, 4.5, and 22.4% in the I-IGH1$_{com}$ group; and 0.0, 0.0, and 0.0% in the H-IGH1$_{com}$ group (Figure 4). The cumulative decompensation incidence rates were significantly higher in the L-IGH1$_{com}$ group than in the H-IGH1$_{com}$ and I-IGH1$_{com}$ groups ($p < 0.001$ and $= 0.009$, respectively).

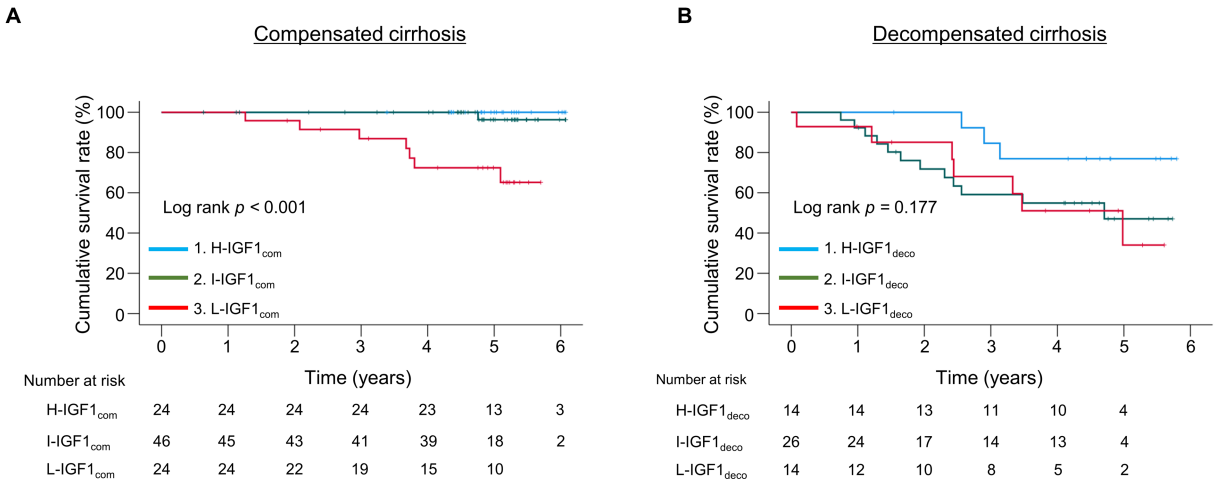
3.7. Factors associated with decompensation development

In the univariate analysis, CP score, total bilirubin, albumin, PT, IGF-1, and M2BPGi were significantly associated with decompensation development in patients with compensated cirrhosis (Supplementary Table S4). Cox proportional hazards regression analysis identified low albumin levels (HR, 0.447; 95% CI, 0.223–0.896; $p = 0.023$) and low IGF-1 levels (HR, 0.939; 95% CI,



Groups	*p*-value
1 vs. 2	0.339
vs. 3	< 0.001
2 vs. 3	0.009

FIGURE 2
Comparison of the cumulative survival rates among the three groups stratified following the baseline serum levels of insulin-like growth factor 1 (IGF-1) levels. The cumulative survival rates were significantly lower in the low-IGF-1 (L-IGF1$_{all}$) group than in the high-IGF-1(H-IGF1$_{all}$) and intermediate-IGF-1(I-IGF1$_{all}$) groups ($p < 0.001$ and = 0.009, respectively).



Groups	*p*-value
1 vs. 2	1.000
vs. 3	0.012
2 vs. 3	0.003

FIGURE 3
Comparison of the cumulative survival rates among the three groups stratified following the baseline serum levels of insulin-like growth factor 1 (IGF-1) levels in compensated or decompensated cirrhosis. **(A)** In compensated cirrhosis, the cumulative survival rates were significantly lower in the low-IGF-1 (L-IGF1$_{com}$) group than in the high-IGF-1 (H-IGF1$_{com}$) and intermediate-IGF-1 (I-IGF1$_{com}$) groups ($p = 0.012$ and 0.003, respectively). **(B)** In decompensated cirrhosis, the cumulative survival rates did not significantly differ among the three groups ($p = 0.177$).

0.913–0.966; $p < 0.001$) as significant and independent factors associated with decompensation development (Table 5).

4. Discussion

Most patients with compensated cirrhosis are asymptomatic and have a better prognosis compared to those with decompensated cirrhosis (3). However, various liver disease-related events develop and mortality increases evidently during liver disease progression from the compensated phase to the decompensated phase (15). Therefore, early decompensation prediction and medical countermeasures against complications are crucial in patients with compensated cirrhosis. IGF-1 can inhibit or improve hepatic inflammation and fibrosis, and therefore, may be deeply involved in the pathological CLD condition (7–9). Reportedly, circulating IGF-1

TABLE 2 Significant factors associated with mortality in all patients.

Variable	Univariate		Multivariate	
	HR (95%CI)	*p*-value	HR (95%CI)	*p*-value
Decompensated cirrhosis	6.664 (2.955–15.029)	<0.001		
Child–Pugh score	1.695 (1.454–1.977)	<0.001	1.248 (1.014–1.538)	0.037
MELD score	1.202 (1.106–1.305)	<0.001		
Total bilirubin (mg/dL)	1.606 (1.296–1.991)	<0.001		
Albumin (g/dL)	0.376 (0.274–0.515)	<0.001	0.346 (0.204–0.589)	< 0.001
Sodium (mEq/L)	0.796 (0.688–0.920)	0.002		
Prothrombin time (%)	0.969 (0.951–0.987)	<0.001		
IGF-1 (ng/mL)	0.952 (0.932–0.973)	<0.001	0.967 (0.945–0.989)	0.004
M2BPGi (C.O.I.)	1.208 (1.125–1.297)	<0.001		

CI, confidence interval; C.O.I., cut-off index; HR, hazard ratio; IGF-1, insulin-like growth factor 1; M2BPGi, Mac-2 binding protein glycosylation isomer; MELD, model for end-stage liver disease.

TABLE 3 Significant factors associated with mortality in patients with compensated cirrhosis.

Variable	Univariate		Multivariate	
	HR (95%CI)	*p*-value	HR (95%CI)	*p*-value
Prothrombin time (%)	0.957 (0.915–1.001)	0.054		
IGF-1 (ng/mL)	0.927 (0.884–0.972)	0.002	0.927 (0.884–0.972)	0.002
M2BPGi (C.O.I.)	1.418 (1.008–1.996)	0.045		

CI, confidence interval; C.O.I., cut-off index; HR, hazard ratio; IGF-1, insulin-like growth factor 1; M2BPGi, Mac-2 binding protein glycosylation isomer.

TABLE 4 Significant factors associated with mortality in patients with decompensated cirrhosis.

Variable	Univariate		Multivariate	
	HR (95%CI)	*p*-value	HR (95%CI)	*p*-value
Child–Pugh score	1.548 (1.218–1.968)	<0.001		
MELD score	1.113 (1.005–1.233)	0.039		
Total bilirubin (mg/dL)	1.286 (1.002–1.651)	0.048		
Albumin (g/dL)	0.104 (0.041–0.265)	<0.001	0.097 (0.038–0.245)	<0.001
IGF-1 (ng/mL)	0.974 (0.951–0.998)	0.034		
M2BPGi (C.O.I.)	1.086 (0.985–1.196)	0.097		

CI, confidence interval; C.O.I., cut-off index; HR, hazard ratio; IGF-1, insulin-like growth factor 1; M2BPGi, Mac-2 binding protein glycosylation isomer; MELD, model for end-stage liver disease.

levels decrease with disease progression, and the decreased levels are associated with poor short-term prognosis in patients with cirrhosis, especially decompensated cirrhosis (5, 13). The present study is the first to report the association between serum IGF-1 levels and long-term prognosis in patients with compensated and decompensated cirrhosis, respectively. Intriguingly, serum IGF-1 levels were the only significant prognostic factor in patients with compensated cirrhosis, whereas serum albumin (but not IGF-1) levels in those with decompensated cirrhosis. Notably, deaths were not observed, as well as cases with decompensation development in the H-IGF1$_{com}$ group when limited to patients with compensated cirrhosis. Meanwhile, the H-IGF1$_{deco}$ group in patients with decompensated cirrhosis also had the highest survival rates, although marginally significant, and all H-IGF1$_{deco}$ patients survived during the first 2 years.

One study of patients with cirrhosis (without HCC: $n = 64$; CP class B/C, 73.4%) revealed that plasma IGF-1 levels (mean: 80.0, 62.7, and 32.6 ng/mL for CP class A, B, and C, respectively) were inversely correlated with the CP score, and the L-IGF1 group had lower 2 years survival rates than the H-IGF1 group (13). Another study of patients with decompensated cirrhosis (without HCC) revealed that serum IGF-1 levels (median: 70.1, 40.5, and 32.4 ng/mL for CP class A, B, and C, respectively) and IGF-CP scores were associated with 1 year mortality (5). This IGF-CP scoring system more accurately predicted 1 year mortality than the CP score alone. Another study of patients with CP class A and advanced HCC receiving antiangiogenic therapy revealed that low serum IGF-1 levels (<63.6 ng/mL) reduced the progression-free and overall survival rates (17). Our study revealed that the median IGF-1 levels were 62, 43, and 34 ng/mL for CP class A, B, and C, respectively, and 61 and 46 ng/mL for compensated and decompensated cirrhosis, respectively. The IGF-1 predictive value of poor prognosis in patients with cirrhosis (without HCC) may be approximately 40 ng/mL,

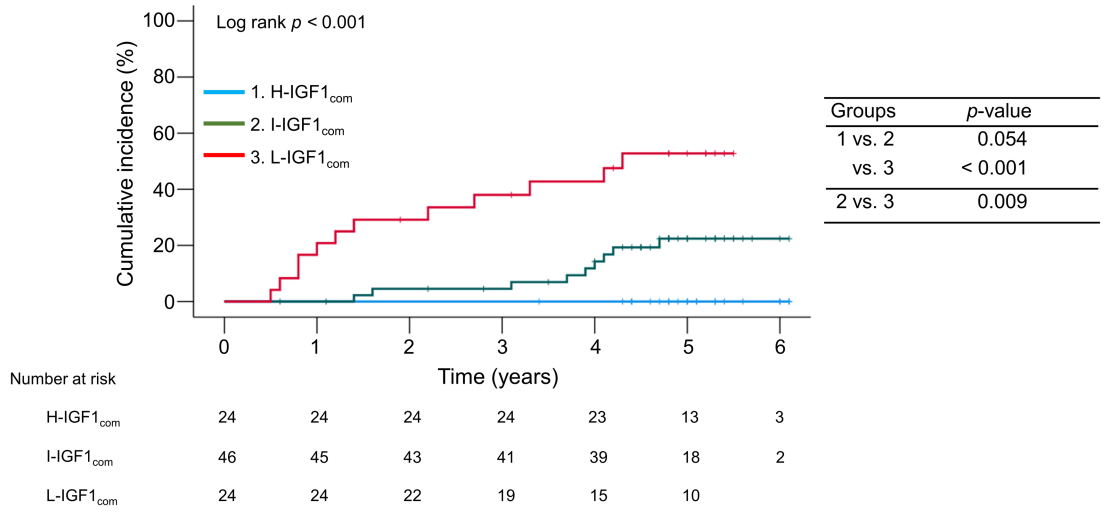


FIGURE 4
Comparison of the cumulative incidence rates of decompensation among the three groups stratified following the baseline serum insulin-like growth factor 1 (IGF-1) levels in compensated cirrhosis. The cumulative incidence rates of decompensation were significantly higher in the low-IGF-1 (L-IGF1$_{com}$) group than in the high-IGF-1 (H-IGF1$_{com}$) and intermediate-IGF-1 (I-IGF1$_{com}$) groups ($p < 0.001$ and = 0.009, respectively).

TABLE 5 Significant factors associated with development of decompensation.

Variable	Univariate		Multivariate	
	HR (95%CI)	*p*-value	HR (95%CI)	*p*-value
Child–Pugh score	2.932 (1.623–5.295)	<0.001		
Total bilirubin (mg/dL)	3.065 (1.178–7.975)	0.022		
Albumin (g/dL)	0.603 (0.373–0.974)	0.039	0.447 (0.223–0.896)	0.023
Prothrombin time (%)	0.958 (0.932–0.985)	0.003		
IGF-1 (ng/mL)	0.942 (0.917–0.968)	<0.001	0.939 (0.913–0.966)	<0.001
M2BPGi (C.O.I.)	1.348 (1.097–1.655)	0.004		

CI, confidence interval; C.O.I., cut-off index; HR, hazard ratio; IGF-1, insulin-like growth factor 1; M2BPGi, Mac-2 binding protein glycosylation isomer.

considering that the cutoff IGF-1 values for the L-IGF1 groups were 41, 47, and 32 ng/mL in all patients and those with compensated and decompensated cirrhosis, respectively.

The MELD and CP scores provide prognostic information for patients with decompensated cirrhosis (18). The MELD score predicts short-term mortality in end-stage liver disease and is useful for determining organ allocation to liver transplantation candidates on transplant waiting lists (19, 20). Meanwhile, the CP score predicts differences in prognosis between patients with CP classes A and B/C (3). However, these scoring systems can hardly identify patients with poor prognoses when limited to those with compensated cirrhosis (6, 18). Therefore, circulating IGF-1 levels may be a useful predictor of long-term prognosis in the compensated phase.

The present study revealed that serum IGF-1 levels were significantly correlated with liver functional reserve-related factors, which was consistent with previous studies (10, 13, 21, 22). Serum IGF-1 levels correlated positively with albumin and PT levels and negatively with total bilirubin level, CP score, and MELD score. Reportedly, serum IGF-1 levels are substantially low in a large percentage of patients with compensated cirrhosis despite normal albumin and prealbumin levels; hence, they may be a more sensitive and earlier indicator of impaired liver function than other conventional parameters (13). As described above, patients with compensated cirrhosis must inhibit the progression to decompensation. The MELD and CP scores are widely used to estimate liver functional reserve; however, these provide little information on the risk of developing decompensation (18). The present study identified serum IGF-1 levels as the only significant factor associated with decompensation development. The L-IGF1$_{com}$ group had significantly higher cumulative incidence rates of decompensation than the H-IGF1$_{com}$ and I-IGF1$_{com}$ groups. Intriguingly, the H-IGF1$_{com}$ group demonstrated no decompensation. Therefore, serum IGF-1 levels may be a simple and useful indicator of liver functional reserve and the risk of developing decompensation.

Hepatic stellate cells (HSCs), which are activated by oxidative stress, proinflammatory cytokines, and autophagy, participate in liver regeneration and fibrosis (23). Meanwhile, cellular senescence of activated HSCs suppresses liver fibrogenesis (24). IGF-1 administration in mouse models with methionine-choline-deficient diet-induced nonalcoholic steatohepatitis and dimethylnitrosamine-induced cirrhosis ameliorated hepatic steatosis, inflammation, and fibrosis (25). Additionally, IGF-1 induced HSCs into cellular senescence *in vitro and in vivo* and inhibited fibrogenesis in a p53-dependent manner. Similarly, IGF-1 administration improved

liver function (increased albumin, total protein, and coagulation factor levels) and reduced oxidative liver damage and fibrosis in rat models with CCl_4-induced cirrhosis (26). Preoperative serum IGF-1 and IGF-binding protein-3 levels in the cirrhosis group who underwent liver transplantation were lower than those in the control group, but their postoperative levels recovered to normal (27). A pilot study of patients with cirrhosis revealed that IGF-1 administration for 4 months increased serum albumin levels and improved energy metabolism, as assessed by resting energy expenditure (28). These basic and clinical research findings indicate that IGF-1 which is largely produced in hepatocytes is closely involved in hepatic inflammation and fibrosis regulation; hence, advanced disease stage and impaired liver functional reserve reduce IGF-1 levels, which may in turn exacerbate the disease conditions and cause poor prognosis.

This study has some limitations. First, this was a retrospective, small-scale study; therefore, prospective, large-scale studies are needed to confirm our findings. Second, we were unable to assess the IGF-binding protein levels, which regulate the biological activity of IGF-1 and may be associated with liver functional reserve (8, 9, 21). Finally, serum IGF-1 levels were measured only at baseline, whereas longitudinal changes in serum IGF-1 levels were not investigated; thus, the differences in the longitudinal changes between patients with and without decompensation development or between patients with good and poor prognoses remain unclear.

5. Conclusion

In conclusion, low serum IGF-1 levels were associated with decompensation development and poor long-term prognosis in patients with compensated cirrhosis. Therefore, IGF-1 may be useful for predicting decompensation-related events and should be regularly monitored in the management of compensated cirrhosis.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by The ethics committees of the Jikei University School of Medicine (approval number: 34-021) and Fuji City General Hospital (approval number: 279). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

CS participated in the conception and design of the study. CS, TK, KU, MN, TO, and YT acquired, analyzed, and interpreted the data. CS and AT drafted the manuscript. MS and AT interpreted the data and revised the manuscript. AT substantively revised and completed the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1233928/full#supplementary-material

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Hepatic venous pressure gradient and rebleeding risk of patients with nonalcoholic steatohepatitis cirrhosis after variceal bleeding

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Background and aims: Hepatic venous pressure gradient (HVPG) has a strong predictive value for variceal rebleeding in cirrhotic patients, but the accuracy of HVPG may be compromised in nonalcoholic steatohepatitis (NASH) cirrhosis. This study aimed to evaluate the accuracy of HVPG and portal pressure gradient (PPG) for predicting rebleeding in NASH cirrhosis after acute variceal bleeding.

Patients and methods: Thirty-eight NASH cirrhosis patients and 82 hepatitis B virus (HBV) cirrhosis patients with acute variceal bleeding were included in this study. All patients recived transjugular intrahepatic portalsystemic shunt (TIPS). The prognostic value of HVPG and PPG for variceal rebleeding was evaluated.

Results: Compared with HBV cirrhosis, NASH cirrhosis demonstrated a lower HVPG (15.3 ± 3.8 vs. 18.0 ± 4.8; $p = 0.003$) and lower PPG (18.0 ± 3.7 vs. 20.0 ± 3.4; $p = 0.005$). HVPG (AUC = 0.82; $p = 0.002$) and PPG (AUC = 0.72; $p = 0.027$) had promising prognostic value among NASH cirrhosis patients. The optimal threshold of HVPG and PPG for predicting rebleeding in NASH cirrhosis was 17 mmHg and 20 mmHg. At multivariate analysis, HVPG ≥17 mmHg was a significant predictor of variceal rebleeding (HR 9.40; 95% CI 1.85–47.70; $p = 0.007$).

Conclusion: In the patients with cirrhosis and vairceal bleeding, the levels of HVPG and PPG were found to be low in NASH cirrhosis than HBV cirrhosis. However, the prevalence of rebleeding was similar between two groups. HVPG measurement is still an accurate way to assess the risk of variceal rebleeding in NASH cirrhosis.

KEYWORDS

nonalcoholic steatohepatitis (NASH), hepatitis B virus (HBV), transjugular intrahepatic portosystemic shunt (TIPS), variceal bleeding, hepatic venous pressure gradient (HVPG)

Introduction

Nonalcoholic fatty liver disease (NAFLD), also known as metabolic dysfunction-associated fatty liver disease, has become an important public health concern and has a global prevalence of 25% (1). Nonalcoholic steatohepatitis (NASH) (a severe form of NAFLD typically characterized by lobular inflammation, ballooning degeneration and fibrosis) can progress to end-stage liver disease, such as cirrhosis and hepatocellular carcinoma (HCC), and eventually

liver-related mortality (2–4). Approximately 20% of patients with NASH will progress to cirrhosis and encounter cirrhosis-associated decompensation outcomes (e.g., variceal bleeding, hepatic encephalopathy, hepatorenal syndrome, and ascites) (5, 6). Therefore, early identification of patients at high risk for cirrhosis-related complications is beneficial for prognosis in NASH cirrhosis.

Hepatic venous pressure gradient (HVPG) is considered the surrogate marker of portal pressure gradient (PPG) and represents standard reference for staging cirrhotic portal hypertensio (7–9). HVPG value >5 mmHg indicates portal hypertension, and a value >10 mmHg indicates clinically significant portal hypertension (CSPH), while HVPG value of 20 mmHg or higher predicts a high incidence of acute variceal hemorrhage at endoscopy and a high mortality (10, 11). Several studies have evaluated the capacity of HVPG to correspond to liver-related complications, especially in viral and alcoholic cirrhosis, but few studies have focused on HVPG measurement in clinically decompensated NASH cirrhosis and its correlation with variceal rebleeding. The predictive value of HVPG in previous investigations was controversial, and NASH patients had similar portal hypertensive complications at lower HVPG compared with other liver disease etiologies (7, 12). Under normal conditions, HVPG greater than 10 mmHg predisposes patients to esophageal variceal bleeding and other portal hypertension-related complications. However, HVPG of no more than 10 mmHg in NASH may lead to the above-described complications (13, 14). On the other hand, a reduction in HVPG in each stage of NASH fibrosis was observed compared to hepatitis C virus (HCV) disease (15), which raises the concern of whether HVPG is accurate in predicting evaluating portal hypertensive complications in NASH cirrhosis.

Variceal bleeding is a life-threatening complication with a high rebleeding rate and mortality among portal hypertension-related events. Even if variceal bleeding is controlled, stricter means are needed to monitor and prevent rebleeding. Within the first days following an initial hemorrhage episode, the mortality rate reaches 20%, and the rebleeding rate is as high as 30–50% (16, 17). Therefore, we applied this study to compare portal and hepatic venous pressure among patients with NASH cirrhosis and HBV cirrhosis and to evaluate the accuracy of HVPG for predicting variceal rebleeding and other clinical decompensation events.

Patients and methods

Patients

Forty-six NASH cirrhosis patients and 146 HBV cirrhosis patients underwent transjugular intrahepatic portosystemic shunt (TIPS) due to acute variceal bleeding in three tertiary medical centers (Yuzhong Hospital of the Second Affiliated Hospital of Chongqing Medical University, Jiangnan Hospital of the Second Affiliated Hospital of Chongqing Medical University and Chongqing Fuling Central Hospital of Chongqing University) from February 2017 to March 2021 were enrolled. All patients were followed up until September 2021. All patients signed informed consent forms. The research was approved by the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University. NASH cirrhosis was diagnosed in patients with fatty liver who developed cirrhotic signs confirmed *via* histological and imaging evidence and at least one metabolic risk factor without a history of alcohol abuse and other known causes of chronic liver disease. The metabolic risk factors included being overweight or obese (body mass index (BMI) $\geq$25 kg/m^2), hypertension, diabetes mellitus and hyperlipidemia. All HBV patients had evidence of HBV infection (HBV surface antigen and HBV DNA positive).

The inclusion criteria were as follows: (1) decompensated NASH cirrhosis or HBV cirrhosis (histological or/and radiological criteria), (2) clinical manifestations of hematemesis and/or melena, (3) acute variceal bleeding confirmed by endoscopy according to Baveno II criteria (18), (4) age > 18 years and < 80 years; (5) absence of liver transplantation, and (6) no significant alcohol abuse.

The exclusion criteria were as follows: (1) advanced hepatocellular carcinoma according to Milan criteria, (2) absence of hemodynamic measurement, (3) previous treatment of portal hypertension and its complications, such as TIPS placement and endoscopic treatment for variceal bleeding, (4) Child–Pugh score > 13, (5) complete portal vein thrombosis, (6) bleeding from ectopic varices, and (7) comorbidities and medications that may affect portal hypertension and gastrointestinal bleeding, such as heart failure, peptic ulcer, beta-blocker, and antithrombotic therapy.

Interventions

After admission, clinical history, physical examination, laboratory tests, and radiological imaging (hepatic portal vein computed tomography angiography) were performed. All patients admitted for variceal bleeding were first treated with proton pump inhibitors and vasoactive drugs (terlipressin and octreotide). Blood and glucose-electrolyte solutions were transfused to maintain hemodynamic stability. They received early endoscopic treatment within 24 h after admission. Endoscopic treatment for esophageal and gastric varices included endoscopic variceal ligation (multiband ligation device [Wilson-Cook Medical]) and histoacryl injection. TIPS placement was performed, and portal hypertension was evaluated during the first 48 h after bleeding when patients were under a stable hemodynamic condition.

TIPS placement

The measurement of HVPG was performed during the TIPS procedure and adherence to standard operating procedures. Strict quality control standards were established to ensure the reliability of pressure measurement during the procedures. All procedures were performed under conscious sedation and local anesthesia. Using the transjugular approach, a transjugular liver access set (Cook, Bloomington, IN, United States) was guided into the inferior vena cave, right hepatic vein and portal vein. Viatorr® PTFE-covered stents (Gore, Flagstaff, AZ, United States) were implanted following balloon dilation. Embolization of the gastric coronary vein was considered

Abbreviations: HVPG, hepatic venous pressure gradient; NASH, nonalcoholic steatohepatitis; HCV, hepatitis C virus; HBV, hepatitis B virus; TIPS, transjugular intrahepatic portosystemic shunt; PPG, portosystem pressure gradient; BMI, body mass index; HCC, hepatocellular carcinoma; FHVP, free hepatic venous pressure; WHVP, wedged hepatic venous pressure; ALT, alanine aminotransferase; INR, international normalized ratio; GGT, gamma-glutamyltransferase; HR, hazard ratio; CI, confidence interval; CSPH, clinically significant portal hypertension.

when portography clearly showed dilatation of the gastric coronary vein. The preoperative HVPG, preoperative PPG and the postoperative PPG were measured. The HVPG was obtained by calculating the difference between the wedged hepatic venous pressure (WHVP) and the free hepatic venous pressure (FHVP). The PPG was obtained by calculating the difference between the portal pressure (PP) and the inferior vena cava pressure.

Outcomes and follow-up

The primary endpoint was variceal rebleeding, defined as hematemesis and/or melena according to the Baveno Consensus (19). Variceal rebleeding was diagnosed using endoscopy when varices were bleeding, or signs of recent bleeding were observed, and varices were the only potential source of bleeding. The secondary endpoints were: shunt dysfunction defined as a maximum intrastent flow velocity less than 50 cm/s or higher than 200 cm/s, hepatic encephalopathy, new or recurrent ascites, liver failure, hepatocellular carcinoma and overall survival.

Patients were followed up using endoscopy, biochemical assessment and Doppler ultrasonography every 1, 3 and 6 months after TIPS and every year thereafter. Shunt patency and blood flow velocity was assessed by Doppler Ultrasound. Survival was calculated from the date after surgery to mortality or the latest follow-up. Patients were encouraged to quit smoking and alcohol and maintain a low-fat and low-carbohydrate diet during the follow-ups.

Statistical analysis

SPSS version 26.0 was used for statistical analysis. Continuous variables are expressed as the mean and standard deviation, and an unpaired Student's t test or the Mann–Whitney test was used to compare groups. Count variables are expressed as constituent ratios or rates, and Pearson's χ^2 or Fisher's exact test was used to comparing groups. Correlation was calculated by Pearson correlation. Cumulative probabilities of clinical outcomes were analyzed using the competing risk model. Survival was assessed by Kaplan–Meier and log-rank test. Both univariate and multivariate analyzes were used to assess the risk factors associated with variceal rebleeding using the Cox proportional hazard regression model. Discrimination of predictive variables for rebleeding was performed using logistic regression models. Moreover, we identified the optimal cutoff values using logistic regression by calculating the area under the receiver operating characteristic curve (AUC). $p \leq 0.05$ was considered statistically significant.

Results

Baseline characteristics of patients

Between February 2017 and March 2021, 192 patients with NASH cirrhosis or HBV cirrhosis with acute variceal bleeding were enrolled. A total of 38 patients were excluded due to incomplete information, 10 due to portal vein thrombosis, 10 due to previous TIPS treatment, 8 due to hepatocellular carcinoma, 4 due to obviously impaired liver function with a Child–Pugh score > 13, and 2 due to ectopic variceal bleeding. Among the 120 included patients, 38 (31.7%) had NASH cirrhosis, and 82 (68.3%) had HBV cirrhosis.

TABLE 1 Baseline demographics and characteristics of patients.

	NASH (n = 38)	HBV (n = 82)	*p* value
Age	56.7 ± 8.8	49.2 ± 9.4	<0.001
Female	21 (55.3)	17 (20.7)	<0.001
BMI (kg/m^2)	24.7 ± 4.1	21.7 ± 2.6	0.032
Overweight/Obese	18 (47.4)	9 (11.0)	<0.001
Ascites			0.319
Mild	15 (39.5)	33 (40.2)	
Moderate/Excessive	4 (10.5)	17 (20.7)	
Metabolic syndrome	19 (50.0)	6 (7.3)	<0.001
Esophageal varices	34 (89.5)	77 (93.9)	0.392
Gastric varices	30 (78.9)	66 (80.5)	0.844
Hypertension	8 (21.0)	5 (6.1)	0.014
Diabetes	28 (73.7)	27 (32.9)	<0.001
Hypertriglyceridemia	10 (26.3)	0	<0.001
Child–Pugh score	6.7 ± 1.5	7.2 ± 1.7	0.097
Child–Pugh class			0.124
Child class A	22 (57.9)	33 (40.2)	
Child class B	15 (39.5)	40 (48.9)	
Child class C	1 (2.6)	9 (11.0)	
MELD	10.5 ± 2.4	11.8 ± 3.2	0.034
Platelets (×10^9/L)	83.5 ± 41.2	67.0 ± 49.7	0.003
Albumin (g/dL)	3.4 ± 0.6	3.3 ± 0.6	0.520
Bilirubin (mg/dL)	1.4 ± 0.9	1.6 ± 1.3	0.254
ALT (U/L)	27.4 ± 17.8	42.3 ± 55.8	0.031
AST (U/L)	38.1 ± 27.9	42.5 ± 41.1	0.492
GGT (U/L)	64.2 ± 71.4	43.4 ± 35.4	0.034
AP (U/L)	92.7 ± 56.6	80.5 ± 28.3	0.118
INR	1.3 ± 0.2	1.4 ± 0.3	0.004
Serum creatinine (mg/dL)	0.8 ± 0.2	0.7 ± 0.2	0.602
Serum sodium (mmol/L)	138.9 ± 3.6	138.0 ± 4.3	0.247
Portal vein diameter (mm)	15.6 ± 3.1	16.8 ± 3.3	0.030

BMI, body mass index; HCC, hepatocellular carcinoma; MELD, model of end-stage liver disease score; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; AP, alkaline phosphatase; INR, international normalized ratio.

The baseline characteristics of all patients with NASH cirrhosis and HBV cirrhosis are shown in Table 1. The mean age of NASH cirrhosis was 56.7 (interquartile range [IQR], 50–65) years, the mean follow-up time was 27.6 months, and that of HBV cirrhosis was 49.2 years (IQR 44–56) and 24.7 months, respectively. Among patients with NASH cirrhosis, the proportion of females was 55.3%, which was significantly higher than that of patients with HBV cirrhosis (17%, $p < 0.001$). Nineteen patients (50%) with NASH cirrhosis had metabolic syndrome, 18 patients (47.4%) were overweight or obese,

28 patients (73.7%) had diabetes mellitus, 10 patients (26.3%) had hypertriglyceridemia, and 8 patients (21%) had hypertension.

Biochemical analysis of liver function showed that NASH cirrhosis patients had better liver function results and significantly lower Model for End-Stage Liver Disease (MELD) scores ($p = 0.034$). Alanine aminotransferase ($p = 0.031$) and the international normalized ratio ($p = 0.004$) were significantly higher in the HBV group, while the level of gamma-glutamyltransferase (GGT) was significantly higher in the NASH group ($p = 0.034$). In addition, the platelet count was higher in NASH cirrhosis ($p = 0.003$).

HVPG/PPG measurement

Patients with NASH cirrhosis had a lower portal pressure (26.3 ± 6.1 vs. 30.1 ± 4.7; $p < 0.001$), lower WHVP (24.1 ± 5.3 vs. 27.6 ± 5.5; $p = 0.001$), lower HVPG (15.3 ± 3.8 vs. 18.0 ± 4.8; $p = 0.003$) and lower PPG (18.0 ± 3.7 vs. 20.0 ± 3.4; $p = 0.007$) than those with HBV cirrhosis (Table 2). Remarkable correlation between the HVPG and PPG was determined by a Pearson correlation coefficient of 0.78. The R-squared value was 0.608. High HVPG levels were more frequently found in HBV cirrhosis. The HVPG level in 3 (7.9%) NASH patients versus 25 (30.5%) HBV patients was greater than or equal to 20 mmHg ($p = 0.006$). Low HVPG (<10 mmHg) levels were observed in 3 (7.9%) NASH patients and 3 (3.7%) HBV patients. After successful TIPS treatment, the PPG significantly decreased from 18.0 ± 3.7 mmHg vs. 20.0 ± 3.4 mmHg to 7.6 ± 4.1 mmHg vs. 9.2 ± 3.8 mmHg (NASH cirrhosis vs. HBV cirrhosis,). The PPG level after TIPS treatment of NASH cirrhosis was significantly lower than that after TIPS treatment of HBV cirrhosis ($p = 0.04$). Compared to the baseline, an PPG value, a mean reduction of 10.4 mmHg was observed in NASH cirrhosis and 10.8 mmHg in HBV cirrhosis. No significant difference was found between them. After TIPS treatment, the PPG effectively decreased to a level of <12 mmHg in 104 (86.7%) patients, but all patients achieved a sufficient reduction of PPG of more than 20%. The 1-year shunt dysfunction rate was 5.9% vs. 8.1% and 2-year shunt dysfunction rate was 18% vs. 24.6% (NASH group vs. HBV group).

Rebleeding

During the follow-up, a total of 38 patients (11 NASH patients and 27 HBV patients) had at least one rebleeding. Analyzed by competing risk analysis, there were no significant differences in the cumulative incidence of rebleeding at 6 months (5.3% vs. 12.2%), 1 year (14.6% vs. 18.6%) or 2 years (22.7% vs. 32.9%) between the two groups. Furthermore, Kaplan–Meier survival curves indicated no significant difference in the overall rebleeding rate between NASH and HBV cirrhosis. Variceal rebleeding patients had higher baseline HVPG and PPG levels than nonrebleeding patients in both groups. Patients with PPG greater than 12 mmHg after TIPS placement were at higher risk for rebleeding than those without (71.2% vs. 50.0%, $p = 0.059$). Patients with a higher HVPG level of ≥20 mmHg had a significantly higher variceal rebleeding rate than those with an HVPG of <20 mmHg (64.3% vs. 21.7%, $p < 0.001$). According to competing risk model, the observed cumulative probability of variceal rebleeding was significantly higher in those with an HVPG ≥20 mmHg than in those with an HVPG <20 mmHg at the 6-month (25.0% vs. 5.4%), 1-year (36.2% vs. 11.4%) and 2-year (49.9% vs. 23.0%) follow-ups. In logistic regression, HVPG ≥20 mmHg was associated with an increased risk of variceal rebleeding (HR 6.48; 95% CI 2.59–16.23; $p < 0.001$) compared with an HVPG <20 mmHg. The effect with the PPG after TIPS did not reach significance. The c-statistic for baseline HVPG and PPG for predicting variceal rebleeding were 0.82 (95% CI 0.66–0.97; $p = 0.002$) and 0.72 (95% CI 0.53–0.92; $p = 0.027$) in NASH patients, and the optimal threshold for baseline HVPG and PPG were ≥ 17.0 mmHg (specificity 85.2%, sensitivity 72.7%) and ≥ 20.9 mmHg [specificity 96.3%, sensitivity 54.5% (Figure 1A)]. The ROC curve of HVPG greater than 17 mmHg was shown in Figure 1C. In the HBV group, the c-statistic for baseline HVPG and PPG for predicting variceal rebleeding were 0.75 (95% CI 0.36–0.86; $p < 0.001$) and 0.71 (95% CI 0.59–0.83; $p = 0.002$), and the optimal threshold for baseline HVPG and PPG were ≥ 21.6 mmHg (specificity 48.1%, sensitivity 92.7%) and ≥ 20.2 mmHg [specificity 74.1%, sensitivity 63.6% (Figure 1B)]. The ROC cruves of PPG after TIPS placement were not statistically different. Elevation of the baseline HVPG level per 1 mmHg increased the rebleeding risk by 1.50 in NASH cirrhosis (95% CI 1.11–2.03; $p = 0.008$) and 1.23 in HBV cirrhosis (95% CI 1.09–1.40; $p = 0.001$). The survival curves of variceal rebleeding in the NASH and HBV groups according to HVPG are depicted in Figure 2. The incidence of rebleeding was significantly higher in patients with an HVPG ≥17 mmHg in the NASH group (HR 7.06; 95% CI 1.88–26.56; $p = 0.001$). Multivariate analysis showed that HVPG ≥17 mmHg (HR 9.40; 95% CI 1.85–47.70; $p = 0.007$), lower albumin (HR 1.25; 95% CI 1.06–1.48; $p = 0.007$), and higher GGT (HR 1.02; 95% CI 1.01–1.03; $p = 0.002$) were independent predictors of variceal rebleeding in the NASH cirrhosis group (Table 3). The PPG

TABLE 2 Portal hemodynamics of patients before and after the treatment.

	NASH (n = 38)	HBV (n = 82)	p value
PP before TIPS (mmHg)	26.3 ± 6.1	30.1 ± 4.7	<0.001
WHVP before TIPS (mmHg)	24.1 ± 5.3	27.6 ± 5.5	0.001
FHVP before TIPS (mmHg)	8.8 ± 3.0	9.7 ± 3.4	0.186
HVPG before TIPS (mmHg)	15.3 ± 3.8	18.0 ± 4.8	0.003
PPG before TIPS (mmHg)	18.0 ± 3.7	20.0 ± 3.4	0.005
PPG after TIPS (mmHg)	7.6 ± 4.1	9.2 ± 3.8	0.040
Portal vein velocity after TIPS (cm/s)	38.6 ± 16.3	38.8 ± 12.7	0.756

PP, portal pressure; WHVP, wedged hepatic venous pressure; FHVP, free hepatic venous pressure; PPG, portosystem pressure gradient; HVPG, hepatic venous pressure gradient; TIPS, transjugular intrahepatic portosystemic shunt.

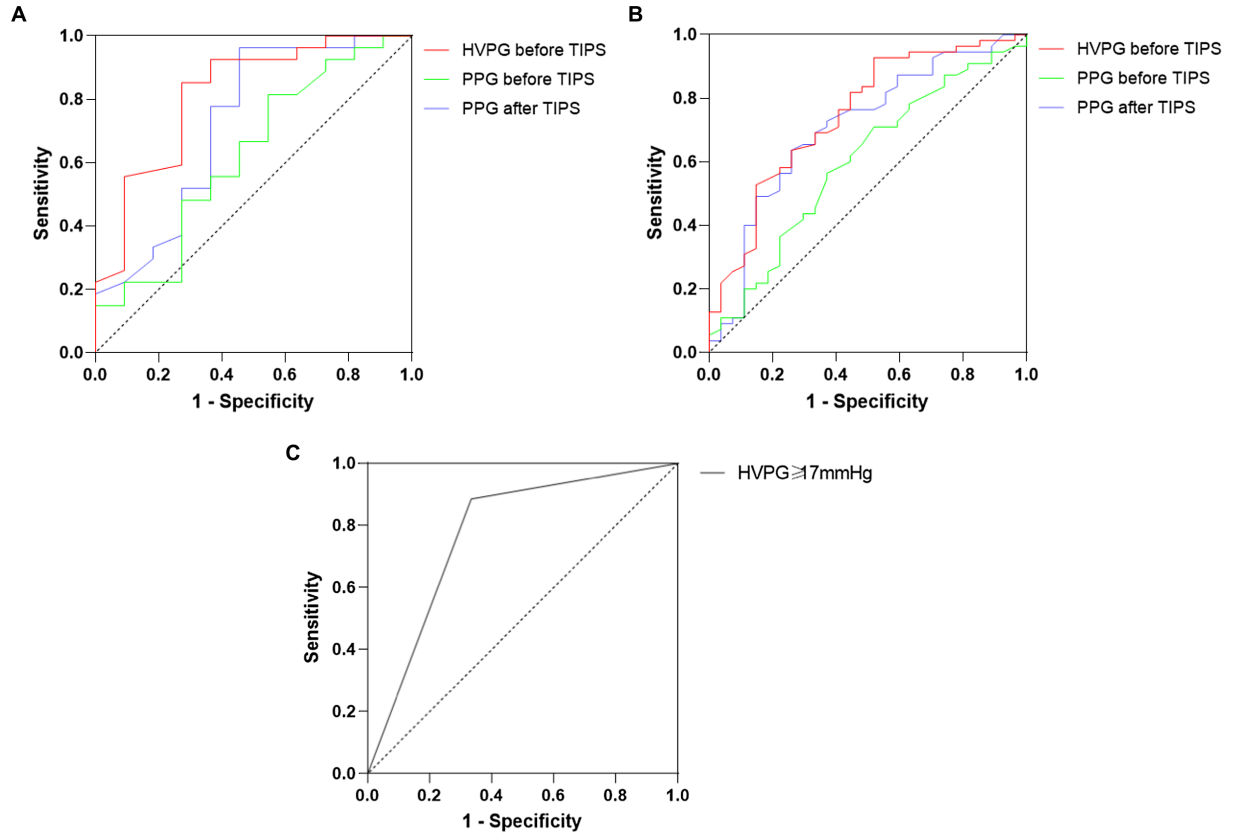

FIGURE 1
(A) ROC curve for the value of HVPG and PPG before and after TIPS in predicting variceal rebleeding in patients with NASH cirrhosis. **(B)** ROC curve for the value of HVPG and PPG before and after TIPS in predicting variceal rebleeding in patients with HBV cirrhosis. **(C)** ROC curve for the value of HVPG ≥17.0 mmHg after TIPS in predicting variceal rebleeding in patients with NASH cirrhosis.

before and after TIPS had no significance in the multivariate Cox regression model. The median survival time was 22 months for NASH patients with an HVPG ≥17 mmHg and 26 months for HBV patients with an HVPG ≥17 mmHg, although no significant difference was found between the two groups.

Other complications of cirrhosis

At enrollment, 19 (50.0%) NASH patients had ascites, 3 (7.9%) had encephalopathy, and 2 (5.3%) had acute-on-chronic liver failure. During a mean follow-up of 27.6 months, 13 (34.2%) patients with NASH cirrhosis developed cirrhotic complications other than variceal bleeding, including encephalopathy (7 patients), HCC (1 patient), acute-on-chronic liver failure (7 patients), and ascites (4 patients). Fifty (61.0%) HBV cirrhosis patients had ascites, 10 (12.2%) had encephalopathy, 7 (8.5%) had liver failure, and 4 (4.9%) had HCC at the time of the first visit of the study. Compared with patients with NASH cirrhosis, 42 (51.2%) patients with HBV cirrhosis developed cirrhotic complications other than variceal bleeding (22 patients with encephalopathy, 10 patients with HCC, 7 patients with liver failure, 8 patients with ascites) during 2 years of follow-up. None of patient received liver transplants. Although the NASH group showed a lower total incidence of cirrhotic complication outcomes, the occurrence rates of hepatic encephalopathy (26.3% vs. 39%), acute-on-chronic liver failure (23.7% vs. 34.1%), and ascites (60.5% vs. 70.7%) were similar between two groups. According to the Kaplan–Meier analysis, the incidence of HCC was significantly higher in HBV cirrhosis (17.1% vs. 2.6%, $p = 0.008$). In the NASH group, the HVPG level was significantly higher in patients with cirrhotic complications than in those without complications (16.2 ± 3.9 vs. 13.4 ± 2.8; $p = 0.026$). The prevalence of cirrhotic complications increased with the HVPG level. Each 1 mmHg elevation in HVPG was associated with a 27.8% increase in the risk of clinical events ($p = 0.035$). The c-statistic of HVPG for the predictive value of cirrhotic complications was 0.75 in NASH patients (95% CI 0.59–0.90; $p = 0.014$).

During follow-up, 1 patient with NASH died due to liver failure, and 2 patients with HBV died due to HCC and lethal variceal bleeding. Furthermore, there were no differences in survival between the groups.

Discussion

HVPG measurement is a reliable method to assess portal hypertension. Nevertheless, the correlation between HVPG and cirrhotic decompensation has not yet been well documented in NASH cirrhosis. HVPG has been verified to contribute to the progression of cirrhotic decompensation in other etiologies. It is suggested that the threshold value of HVPG for risk stratification is likely to be different

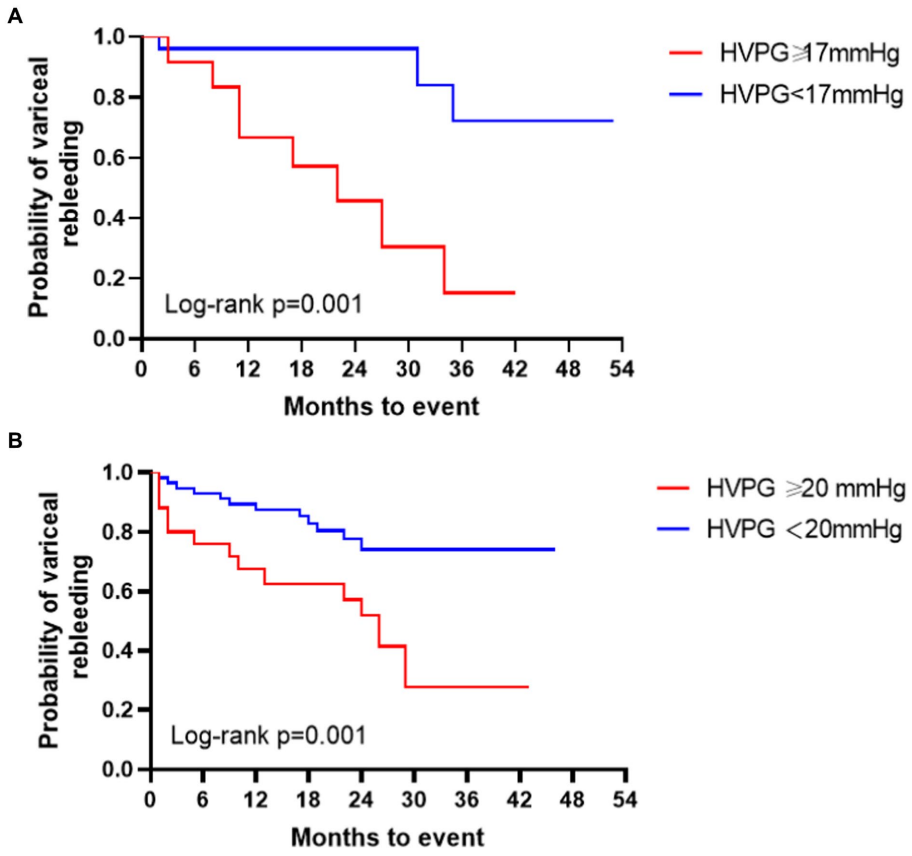

FIGURE 2
(A) Survival curves of the probability of variceal rebleeding in patients with NASH cirrhosis based on HVPG level. **(B)** Survival curves of the probability of variceal rebleeding in patients with HBV cirrhosis based on HVPG level.

TABLE 3 Univariate and multivariate analysis for predictors of variceal rebleeding in NASH cirrhosis.

	Univariate analysis		Multivariate analysis	
Variable	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Overweight/Obese	3.73 (0.98–14.28)	0.054		
HVPG ≥17 mmHg	7.57 (1.96–29.23)	0.003	9.40 (1.85–47.70)	0.007
PPG	1.20 (1.04–1.38)	0.014		
Albumin	0.88 (0.78–1.00)	0.055	0.80 (0.68–0.94)	0.007
GGT	1.01 (1.00–1.02)	0.014	1.02 (1.01–1.03)	0.002
AP	1.01 (1.00–1.02)	0.005		

CI, Confidence interval; HR, Hazard ratio; HVPG, hepatic venous pressure gradient; PPG, portosystem pressure gradient; GGT, gamma-glutamyl transpeptidase; AP: alkaline phosphatase.

in NASH cirrhosis (7, 12). In this study, we evaluated the correlation between HVPG levels and cirrhotic complications in NASH cirrhosis.

Our results showed lower PP (26.3 ± 6.1 vs. 30.1 ± 4.7; $p < 0.001$), lower WHVP (24.1 ± 5.3 vs. 27.6 ± 5.5; $p = 0.001$), lower HVPG (15.3 ± 3.8 vs. 18.0 ± 4.8; $p = 0.003$) and lower PPG (18.0 ± 3.7 vs. 20.0 ± 3.4; $p = 0.005$) in NASH cirrhosis with variceal hemorrhage than in HBV cirrhosis. Futhermore, this study confirmed a high agreement between HVPG and PPG in NASH cirrhosis. In the current study, lower HVPG and lower WHVP were found in NASH disease than in HCV disease, and decreases in pressure measurements were observed in different stages of fibrosis, particularly in the lower stage of fibrosis (stage ≤3) (15). Compared with the other etiologies of cirrhosis, these decreased pressure variables of NASH cirrhosis were identical to those in a previous study regarding a similar degree of liver dysfunction (20, 21). Our study indicated that low HVPG levels may lead to variceal bleeding in NASH cirrhosis. The low level of portal pressure in NAFLD has recently attracted much attention, raising the question of whether HVPG measurements may probably be underestimated in NASH cirrhosis. HVPG is is an indirect measure of portal pressure, and its accuracy may be questioned for pre-sinus or post-sinus portal hypertension. Previous studies hypothesized that the potential special vasoreactivity mechanism in NAFLD reduces the effect of fibrosis on

portal pressure (12). NASH pathogenesis is correlated with lobular inflammation and portal fibrosis. Portal inflammatory infiltrate leads to a ductular reaction, resulting in progressive fibrosis and thus an increase in portal vascular resistance (22, 23). It has also been postulated that increased perisinusoidal pressure caused by biliary injury may influence the accuracy of portal pressure in NASH (24, 25). Moreover, these studies have raised concerns about whether portal hypertension in NASH can be perfectly distinguished by HVPG measurement. Decreased HVPG values for staging fibrosis in NASH have been verified. The measurement of HVPG in NASH and HCV etiology shared the same and strong correlation with the stage of fibrosis (15). HVPG has been clinically significant in the prognosis of cirrhotic complications in compensated and decompensated cirrhosis in NASH cirrhosis, as in other etiologies (14, 26).

In this study, the results suggested that the measurement of HVPG was an accurate predictor of portal hypertension in NASH cirrhosis, such as recurrent variceal bleeding. Although the optimal baseline HVPG threshold for predicting rebleeding of NASH cirrhosis was lower than 20 mmHg, univariate and multivariate analyzes revealed that HVPG ≥17 mmHg was an independent predictor for variceal rebleeding. The median survival time was shorter in NASH patients than in HBV patients when the HVPG was greater than 17 mmHg, although no survival difference was observed. Likewise, our study showed a strong correlation between rebleeding episodes and HVPG elevation. The relationship between high HVPG and complications in NASH cirrhosis has been demonstrated in our study, which can help us corroborate the specific predictive value of HVPG for predicting the development of variceal bleeding. Previous reports on portal hemodynamics indicated that HVPG of ≥20 mmHg had been shown to significantly correlate with a high incidence of cirrhotic complications (27–29). As mentioned above, the role of the HVPG in predicting the occurrence of cirrhotic complications in decompensated NASH cirrhosis is controversial. It is difficult to identify NASH patients at high risk for liver-related complications, especially those with an HVPG <10 mmHg (14). The predictive factors for cirrhotic complications have received much attention in NASH cirrhosis, and identifying independent predictors for portal hypertensive complications is important for patients with NASH cirrhosis (30). In a study of a large cohort with 475 patients with biopsy-proven NASH from the simtuzumab trials, higher HVPG, both at baseline levels and elevated levels over time, was associated with a high risk of cirrhosis-related clinical events (14). With every 1 mmHg increase in HVPG, the associated risk of decompensation events increased by 15%. We also noticed a difference in the risk estimates of decompensation between our study and a previous study. Perhaps because this study only involves patients with decompensated cirrhosis, the risk of variceal bleeding is likely to be exaggerated. Our findings on the prognostic value of HVPG for risk stratification in NASH cirrhosis patients are consistent with those of Sanyal et al. (14), which showed the high prognostic value of HVPG for predicting cirrhotic decompensations and survival.

Few studies have focused on evaluating the predictive value of HVPG for rebleeding risk in NASH cirrhosis patients with variceal bleeding. According to our results, the accuracy of HVPG for predicting variceal rebleeding in NASH cirrhosis is superior to that in HBV cirrhosis. Our observation verifies a significant correlation between increased HVPG and high rebleeding risk. High portal pressure and high variceal pressure are recognized causes of variceal bleeding; hence, HVPG is a well-known useful predictor for variceal bleeding in cirrhotic patients. HVPG ≥20 mmHg correlates with grades of varices and increased risk of continued and recurrent variceal bleeding, which has been shown in many experimental and clinical studies (10, 28, 31, 32). Our findings suggest that an HVPG ≥17 mmHg is a valuable predictor for evaluating the risk of complications in patients with NASH cirrhosis. Thus, early evaluation of HVPG provides an opportunity for early intervention in these at-risk patients.

The limitation of this study is the relatively small sample size. Another limitation is that the HBV group is not comparable to the NASH group with respect to baseline characteristics, which might be inadequate to accurately describe the predictive value of HVPG in NASH cirrhosis. NASH cirrhosis has been projected to exceed virus cirrhosis and become the leading cause of cirrhosis worldwide (33). The number of NASH patients with cirrhosis is still limited due to slow disease progression, which impedes the assessment of long-term survival. Considering that the average follow-up in this study was approximately 2 years, whether HVPG has a good prognostic value for variceal bleeding in NASH cirrhosis awaits further investigation. We fully anticipate that further studies will explore the predictive value of HVPG for other complications of advanced cirrhosis due to NASH, including liver failure, hepatic encephalopathy, hepatorenal syndrome and hepatopulmonary syndrome. The hemodynamic measurement of portal pressure is invasive and relatively expensive, which limits its large-scale application. In that instance, a new minimally invasive and cost-effective method is expected to be a replacement for HVPG, showing a favorable prognostic value for long-term outcomes in NASH patients.

In conclusion, this study showed that patients with NASH cirrhosis had lower HVPG valus and lower PPG values and similar prevalence of cirrhosis-related complications after acute variceal bleeding compared with HBV cirrhosis. NASH cirrhotic patients with low HVPG values may present with variceal bleeding. According to the predictive value for variceal rebleeding, HVPG is a feasible accurate and valuable means of risk assessment in NASH cirrhosis. The presence of high HVPG contributes to stratifying high-risk patients and leads us to a deeper understanding of the management of NASH patients. Considering the rising trend in the prevalence of NAFLD, regular clinical evaluation and monitoring of liver-related events are recommended in these patients with high HVPG. Accordingly, stratification based on HVPG level is a promising risk stratification among patients with cirrhosis, especially in NASH cirrhosis.

A preprint has previously been published (34).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YS and BN conceived and wrote the manuscript. YS, XW, and GX participated in data acquisition and statistical analysis. WS and BN revised and edited the manuscript and supervised the study. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Association of C-type lectin-like receptor 2 and galectin-1 with portal vein system thrombosis in HBV-related liver cirrhosis

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Background and aims: Hepatitis B virus (HBV) infection is the most common cause of liver cirrhosis. Portal venous system thrombosis (PVST) is a major complication of liver cirrhosis. Recently, it has been shown that C-type lectin-like receptor 2 (CLEC-2) and galectin-1 participate in the activation and aggregation of platelets, thereby promoting the development of thrombosis. This cross-sectional study aims to evaluate the association of serum CLEC-2 and galectin-1 levels with PVST in patients with HBV-related liver cirrhosis.

Methods: Overall, 65 patients with HBV-related liver cirrhosis were included, of whom 23 had PVST and 42 did not have. Serum CLEC-2 and galectin-1 levels were measured using enzyme-linked immunosorbent assay kits. PVST was assessed by contrast-enhanced computed tomography and/or magnetic resonance imaging scans. Subgroup analyses were conducted according to the degree and location of PVST.

Results: Patients with PVST had significantly higher serum CLEC-2 ($p = 0.006$) and galectin-1 ($p = 0.009$) levels than those without. Patients with partial/complete PVST or fibrotic cord ($p = 0.007$; $p = 0.002$), but not those with mural PVST ($p = 0.199$; $p = 0.797$), had significantly higher serum CLEC-2 and galectin-1 levels than those without PVST. Patients with superior mesenteric vein thrombosis had significantly higher serum CLEC-2 ($p = 0.013$) and galectin-1 ($p = 0.025$) levels than those without PVST. Patients with main portal vein thrombosis had higher serum CLEC-2 ($p = 0.020$) and galectin-1 ($p = 0.066$) levels than those without PVST, but the difference in serum galectin-1 level was not significant between them.

Conclusion: Serum CLEC-2 and galectin-1 levels may be associated with the presence of PVST in HBV-related cirrhotic patients, but this association should be dependent upon the degree of PVST.

KEYWORDS

portal venous system thrombosis, C-type lectin-like receptor 2, galectin-1, hepatitis B virus infection, liver cirrhosis

1. Introduction

Hepatitis B virus (HBV) infection is the most common etiology of liver cirrhosis (1). Portal venous system thrombosis (PVST) is one of major complications of liver cirrhosis (2). Its prevalence ranges from 1 to 26%, which increases with the severity of liver disease (3, 4). In addition, PVST can increase the risk of long-term death, portal hypertension-related bleeding, ascites, and acute kidney injury in cirrhotic patients (5). Therefore, it is necessary to explore the mechanism regarding the development of PVST in HBV-related liver cirrhosis.

C-type lectin-like receptor 2 (CLEC-2) is a type II transmembrane receptor composed of an extracellular ligand-binding C-type lectin-like domain, stalk region, single transmembrane helix, and short cytoplasmic tail (6). It is encoded by the gene CLEC1B located on 12p13.31 with a molecular weight of 32-40 kDa (7). It is highly expressed in megakaryocytes and platelets and slightly expressed in myeloid cell subsets (8, 9). It has been shown that CLEC-2 mediates the activation and aggregation of platelets (10). Moreover, deep venous thrombosis is significantly inhibited in CLEC-2-deficient mice, suggesting that CLEC-2, especially platelet CLEC-2, should be critical for the development of venous thrombosis (11).

Galectin-1 is the firstly discovered member of the galectin family and belongs to the prototype galectin containing a carbohydrate-binding domain, which can exist as monomers or dimers (12). It is a 14.5 kDa protein encoded by the gene LSGALS1 located on 22q12 (13). Due to the lack of signal peptides, galectin-1 is secreted through a non-classical secretion pathway that translocate directly across the plasma membrane, which performs important biological functions both inside and outside cells (14). Galectin-1 knockout mice have a significantly longer median tail bleeding time than wild-type mice (15). Furthermore, galectin-1-deficient platelets exhibit impairment in fibrinogen adhesion and clot retraction, suggesting that galectin-1 should contribute to hemostasis and involve in thrombosis (15).

Some animal studies have demonstrated a relationship of CLEC-2 and galectin-1 with venous thrombosis, but human studies are lacking (10, 15–17). On the other hand, their association with PVST has never been explored yet. For this reason, this cross-sectional study aimed to evaluate the relationship between CLEC-2/galectin-1 and PVST in patients with HBV-related liver cirrhosis, and further explore the impact of degree and location of PVST on their relationship.

2. Materials and methods

2.1. Study design

This study followed the Declaration of Helsinki and was approved by the Medical Ethical Committee of the General Hospital of Northern Theater Command (approval number: Y2023-008). All patients who were admitted to the Department of Gastroenterology of the General Hospital of Northern Theater Command between January 2020 and August 2022 were selected. Inclusion criteria were as follows: (1) patients were diagnosed with HBV-related liver cirrhosis; (2) patients underwent contrast-enhanced computed tomography (CT) and/or magnetic resonance imaging (MRI); and (3) patients had already agreed to donate their blood samples and their blood samples remained. Exclusion criteria were as follows: (1) patients with a diagnosis of malignancy or history of splenectomy, splenic artery embolization, transjugular intrahepatic portosystemic shunt, or liver transplantation, which are well-known local risk factors of PVST; (2) patients with other thrombotic diseases; (3) patients with coronary heart disease (18) or acute ischemic stroke (19), which may affect serum CLEC-2/galectin-1 level; (4) patients took anticoagulants and antiplatelet drugs within one month prior to their admissions; and (5) PVST could not be accurately determined. Repeated admissions were not excluded.

2.2. Diagnosis and definition

Diagnosis of liver cirrhosis was based on medical history, clinical manifestations, cirrhosis-related complications, laboratory tests, imaging, and histology. HBV-related liver cirrhosis would be defined, if cirrhotic patients were diagnosed with HBV infection or had positive hepatitis B surface antigens. Based on the contrast-enhanced CT/MRI imaging, PVST was defined as thrombosis within portal venous system vessels, including left portal vein, right portal vein, main portal vein (MPV), superior mesenteric vein (SMV), splenic vein (SV), or the confluence of SMV and SV (20). Based on the most severe thrombosis in any vessel of the portal venous system, the degree of thrombosis was divided into mural thrombosis (<50%), partial thrombosis (50–80%), complete thrombosis (>80%), and fibrotic cord (20).

2.3. Clinical data collection

Demographics, laboratory tests (i.e., white blood cell, platelet, hemoglobin, total bilirubin, aspartate aminotransferase, albumin, and international normalized ratio), and major complications of liver cirrhosis (i.e., acute gastrointestinal bleeding, ascites, and hepatic encephalopathy) at admission were collected. Child-Pugh score and model for end-stage liver disease (MELD) score at admission were calculated (21). Data accuracy was checked by two researchers (XX and JC).

2.4. Measurement of CLEC-2 and galectin-1

Blood samples used in the present study were obtained from the remaining samples of cirrhotic patients prospectively collected by our group. After fasting for 12 h, venous blood samples were collected by gel-procoagulant tubes from cirrhotic patients and centrifuged at 3000 rpm for 10 min at room temperature. The supernatant was carefully collected to obtain serum and stored at a −80°C refrigerator until further analyses. Serum CLEC-2 (MM-2468H1, Meimian, Shanghai, China) and galectin-1 (MM-51147H1, Meimian, Shanghai, China) levels were measured using enzyme-linked immunosorbent assay kits according to the manufacturer's instructions.

Abbreviations: HBV, hepatitis B virus; PVST, portal venous system thrombosis; CLEC-2, C-type lectin-like receptor 2; CT, computed tomography; MRI, magnetic resonance imaging; MPV, main portal vein; SMV, superior mesenteric vein; SV, splenic vein; MELD, model for end-stage liver disease; IQR, interquartile range.

2.5. Statistical analyses

Continuous variables were expressed as median (interquartile range, [IQR]) and mean ± standard deviation, and their differences between groups were compared with the Mann–Whitney U test (if non-normal distribution) or T-test (if normal distribution). Categorical variables were expressed as frequency (percentage), and their differences between groups were compared with the Chi-square test or Fisher's exact test. Subgroup analyses were conducted according to the degree and location of PVST. A two-tailed $p < 0.05$ was considered statistically significant. SPSS version 20.0 (IBM, Armonk, New York, United States) and GraphPad Prism version 8.0.1 (GraphPad software Inc., San Diego, California, USA) were used for all statistical analyses.

3. Results

3.1. Patients' characteristics

Overall, 65 patients were included (Figure 1). Their median age was 53.30 years (IQR: 46.33–61.54) and 57 (87.69%) patients were male. The median Child-Pugh score and MELD score were 6.00 (IQR: 5.25–8.00) and 10.43 (IQR: 9.07–13.32), respectively (Table 1). Twenty-three patients (35.38%) had PVST (Figure 2A). Among patients with PVST, seven (30.43%) had mural PVST and 16 (69.57%) had partial/complete PVST or fibrotic cord (Figure 2B). The most common location of PVST was MPV (17/23, 73.91%), followed by SMV (13/23, 56.52%) (Figure 2C).

3.2. Difference according to the presence of PVST

Child-Pugh score (6.96 ± 1.49 vs. 7.07 ± 2.17, $p = 0.612$) and MELD score (11.27 ± 2.36 vs. 11.37 ± 3.62, $p = 0.900$) were statistically similar between patients with and without PVST (Table 2). Patients with PVST had significantly higher serum CLEC-2 (1035.17 ± 630.93 pg/mL vs. 753.14 ± 507.06 pg/mL, $p = 0.006$; Figure 3A) and galectin-1 (104.42 ± 73.37 pg/mL vs. 61.95 ± 56.32 pg/mL, $p = 0.009$; Figure 4A) levels than those without.

3.3. Difference according to the degree of PVST

Child-Pugh score (6.71 ± 1.38 vs. 7.07 ± 2.17, $p = 0.940$) and MELD score (10.76 ± 3.24 vs. 11.37 ± 3.62, $p = 0.681$) were statistically similar between patients with mural PVST and those without PVST (Table 3). Serum CLEC-2 (1147.65 ± 951.76 pg/mL vs. 753.14 ± 507.06 pg/mL, $p = 0.199$; Figure 3B) and galectin-1 (76.83 ± 87.03 pg/mL vs. 61.95 ± 56.32 pg/mL, $p = 0.797$; Figure 4B) levels were not significantly different between patients with mural PVST and those without PVST.

Child-Pugh score (7.06 ± 1.57 vs. 7.07 ± 2.17, $p = 0.490$) and MELD score (11.50 ± 1.94 vs. 11.37 ± 3.62, $p = 0.864$) were statistically similar between patients with partial/complete PVST or fibrotic cord and those without PVST (Table 3). Patients with partial/complete PVST or fibrotic cord had significantly higher serum CLEC-2 (985.96±461.53 pg/mL vs. 753.14±507.06 pg/mL, $p = 0.007$; Figure 3B) and galectin 1 116.49±65.98 pg/mL vs. 61.95±56.32 pg/mL, $p = 0.002$; Figure 4B) levels than those without PVST.

3.4. Difference according to the location of PVST

Child-Pugh score (6.88 ± 1.62 vs. 7.07 ± 2.17, $p = 0.834$) and MELD score (10.85 ± 2.37 vs. 11.37 ± 3.62, $p = 0.522$) were statistically similar between patients with MPV thrombosis and those without PVST (Table 4). Patients with MPV thrombosis had

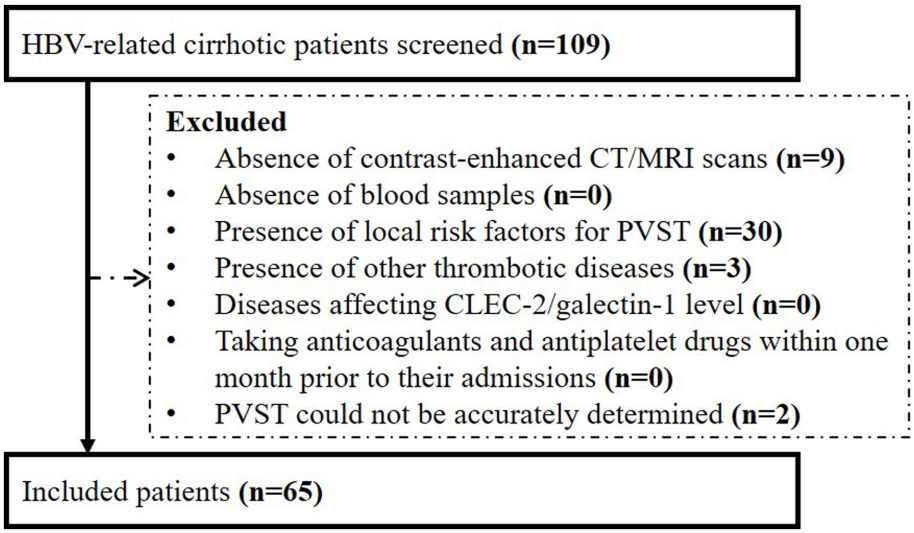

FIGURE 1
Flow chart of patients' selection. HBV, hepatitis B virus; CT, computed tomography; MRI, magnetic resonance imaging; PVST, portal venous system thrombosis; CLEC-2, C-type lectin-like receptor 2.

TABLE 1 Patients' characteristics.

Variables	No. Pts.	Median (IOR), mean ± SD, or frequency (%)
Demographics		
Age (years)	65	53.30 (46.33–61.54) 54.19 ± 10.05
Male	65	57 (87.69%)
Complications of liver cirrhosis		
Acute gastrointestinal bleeding	65	8 (12.31%)
Ascites	65	36 (55.38%)
Hepatic encephalopathy	65	4 (6.15%)
Portal vein system thrombosis	65	23 (35.38%)
Laboratory tests		
White blood cell (10^9/L)	65	2.80 (2.00–4.20) 3.08 ± 1.44
Platelet (10^9/L)	65	66.00 (50.50–87.00) 69.91 ± 29.56
Hemoglobin (g/L)	65	111.00 (82.50–133.50) 108.89 ± 33.81
Total bilirubin (μmol/L)	65	18.20 (13.45–31.55) 25.91 ± 17.40
Aspartate aminotransferase (U/L)	65	27.30 (21.81–49.44) 63.88 ± 147.15
Albumin (g/L)	65	34.30 (29.55–38.55) 34.19 ± 6.06
International normalized ratio	64	1.32 (1.20–1.51) 1.36 ± 0.22
MELD score	64	10.43 (9.07–13.32) 11.33 ± 3.20
Child-Pugh score	64	6.00 (5.25–8.00) 7.03 ± 1.94
Child-Pugh class A/B/C	64	33 (51.56%)/21(32.81%)/10(15.63%)
CLEC-2 (pg/mL)	65	622.89 (520.41–970.73) 852.93 ± 565.70
Galectin-1 (pg/mL)	65	38.12 (26.40–131.82) 76.98 ± 65.58

No. Pts, number of patients; IQR, interquartile range; SD, standard deviation; MELD, model for end-stage liver disease; CLEC-2, C-type lectin-like receptor 2.

higher serum CLEC-2 (1012.55 ± 674.39 pg/mL vs. 753.14 ± 507.06 pg/mL, $p = 0.020$; Figure 3C) and galectin-1 (96.67 ± 79.43 pg/mL vs. 61.95 ± 56.32 pg/mL, $p = 0.066$; Figure 4C) levels than those without PVST. The difference in serum CLEC-2 level was statistically significant between them, but not serum galectin-1 level.

Child-Pugh score (6.38 ± 1.12 vs. 7.07 ± 2.17, $p = 0.708$) and MELD score (10.67 ± 2.25 vs. 11.37 ± 3.62, $p = 0.416$) were statistically similar between patients with SMV thrombosis and those without PVST (Table 4). Patients with SMV thrombosis had significantly higher serum CLEC-2 (1084.65 ± 558.48 pg/mL vs. 753.14 ± 507.06 pg/mL, $p = 0.013$; Figure 3C) and galectin-1 (98.64 ± 61.17 pg/mL vs. 61.95 ± 56.32 pg/mL, $p = 0.025$; Figure 4C) levels than those without PVST.

4. Discussion

Some major findings of this study were as follows: (1) serum CLEC-2/galectin-1 level was positively associated with the presence of PVST in HBV-related cirrhotic patients; (2) patients with partial/complete PVST or fibrotic cord, but not those with mural PVST, had significantly higher serum CLEC-2 and galectin-1 levels than those without PVST; (3) patients with MPV or SMV thrombosis had a significantly higher serum CLEC-2 level than those without PVST; and (4) patients with SMV thrombosis, but not those with MPV thrombosis, had a significantly higher serum galectin-1 level than those without PVST.

Platelets are denucleated blood cells from megakaryocytes in the bone marrow (22). Thrombocytopenia, a common complication of liver cirrhosis, is mainly due to decreased synthesis of thrombopoietin in the liver and increased breakdown of platelets in the spleen (23). However, the coagulation system can be often compensated in people with liver disease (24). A reduction in the number of platelets can induce an increase in von Willebrand factor and a decrease in plasma metalloproteinase ADAMTS13, compensatively promoting platelets function (24). On the other hand, platelets adhesion, dissemination, and aggregation are essential for venous thrombosis (25). They also express phosphatidylserine, which provides procoagulant surface for prothrombinase complexes to activate coagulation cascade, and exert pro-inflammatory effects by inducing neutrophil extracellular trap formation, enhancing leukocyte recruitment, and secreting granular contents to activate coagulation system, which further promote the development of venous thrombosis (26, 27). Taken together, it should be reasonable to speculate that the change of platelets function in cirrhotic patients contributes to the development of PVST.

CLEC-2 is an important platelet-activating receptor (9). Its ligand, podoplanin, expresses near the lumen side of endothelial cell in the vascular wall, and the expression of podoplanin is increased in the presence of inflammation and blood flow obstruction (11). Systemic and local inflammation caused by viral hepatitis and increased portal pressure can damage vascular endothelial cells, allowing circulating platelet CLEC-2 to interact with subendothelial podoplanin and possibly with some other unidentified ligands (11, 28, 29). CLEC-2 binds to its ligand to induce phosphorylation of tyrosine residues in a single YXXL motif in the CLEC-2 intracellular domain (30, 31). Splenic tyrosine kinase is activated by binding its tandem Src homologous 2 domain to two tyrosine-phosphorylated YXXL motifs, subsequently triggering downstream signaling pathways that ultimately promote platelets activation and aggregation (7, 17, 32).

Extracellular galectin-1 can induce platelets activation by binding to different surface receptors on platelets (15). As potent platelets agonist, it activates the "inside-out" signal transduction pathway to promote a conformational change in the integrin $\alpha_{IIb}\beta_3$ on the platelets surface, changing from a low-affinity/resting state to a high-affinity/active state, exposing the high-affinity binding sites of fibrinogen (15, 33–35). At the same time, galectin-1 can also directly bind to platelets surface integrin $\alpha_{IIb}\beta_3$ through α_{IIb} subunit,

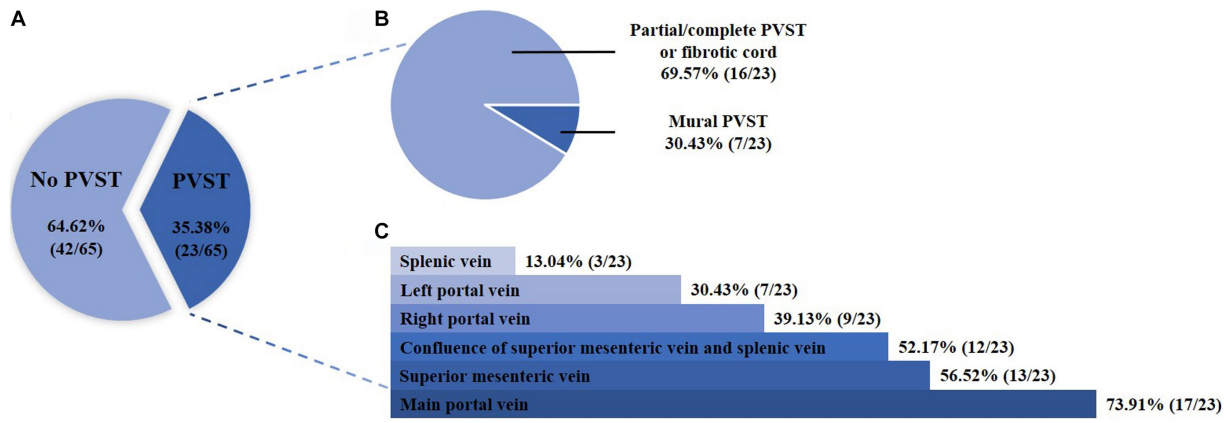

FIGURE 2
Proportion of patients according to the presence **(A)**, degree **(B)**, and location **(C)** of PVST. PVST, portal venous system thrombosis.

TABLE 2 Comparison between patients with versus without PVST.

Variables	No PVST		PVST		*p*-value
	No. Pts.	Median (IQR), mean ± SD, or frequency (%)	No. Pts.	Median (IQR), mean ± SD, or frequency (%)	
Demographics					
Age (years)	42	52.23 (43.41–61.47) 53.04 ± 10.35	23	55.16 (50.58–64.54) 56.27 ± 9.33	0.218
Male	42	37 (88.10%)	23	20 (86.96%)	1.000
Child-Pugh score	41	6.00 (5.00–9.00) 7.07 ± 2.17	23	7.00 (6.00–8.00) 6.96 ± 1.49	0.612
MELD score	41	10.03 (8.89–14.66) 11.37 ± 3.62	23	10.88 (9.81–13.22) 11.27 ± 2.36	0.900

No. Pts, number of patients; IQR, interquartile range; SD, standard deviation; PVST, portal vein system thrombosis; MELD, model for end-stage liver disease.

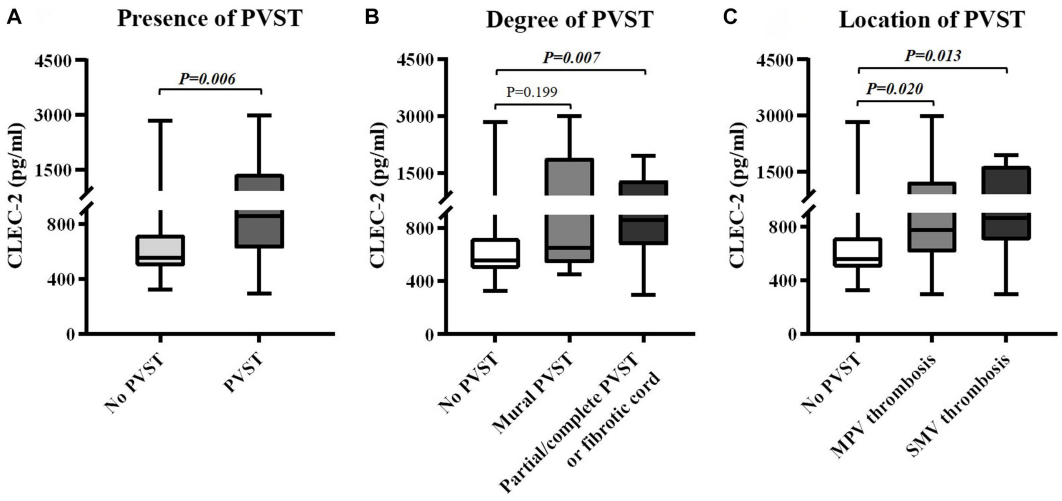

FIGURE 3
Differences in serum CLEC-2 levels between groups. **(A)** Differences in serum CLEC-2 levels between patients with versus without PVST. **(B)** Differences in serum CLEC-2 levels between patients with mural PVST and patients with partial/complete PVST or fibrotic cord versus those without PVST. **(C)** Differences in serum CLEC-2 levels between patients with MPV thrombosis and patients with SMV thrombosis versus those without PVST. CLEC-2, C-type lectin-like receptor 2; PVST, portal venous system thrombosis; MPV, main portal vein; SMV, superior mesenteric vein.

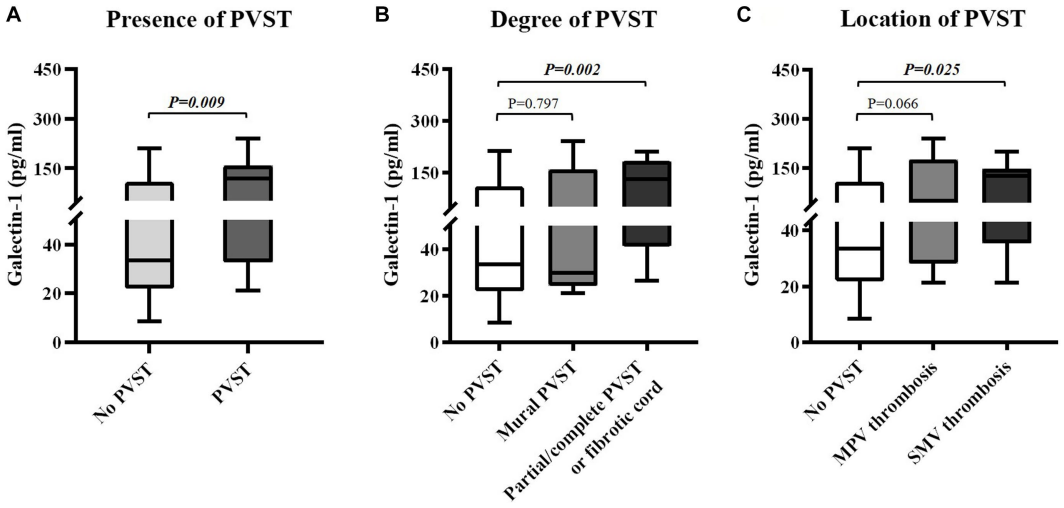

FIGURE 4
Differences in serum galectin-1 levels between groups. **(A)** Differences in serum galectin-1 levels between patients with versus without PVST. **(B)** Differences in serum galectin-1 levels between patients with mural PVST and patients with partial/complete PVST or fibrotic cord versus those without PVST. **(C)** Differences in serum galectin-1 levels between patients with MPV thrombosis and patients with SMV thrombosis versus those without PVST. PVST, portal venous system thrombosis; MPV, main portal vein; SMV, superior mesenteric vein.

TABLE 3 Comparison between patients with mural PVST and those with partial/complete PVST or fibrotic cord versus those without PVST.

Variables	No PVST		Mural PVST		*p*-value	Partial/complete PVST or fibrotic cord		*p*-value
	No. Pts.	Median (IQR), mean ± SD, or frequency (%)	No. Pts.	Median (IQR), mean ± SD, or frequency (%)		No. Pts.	Median (IQR), mean ± SD, or frequency (%)	
Demographics								
Age (years)	42	52.23 (43.41–61.47) 53.04 ± 10.35	7	58.95 (54.57–60.99) 59.29 ± 5.30	***0.027***	16	52.97 (46.33–66.19) 54.95 ± 10.51	0.534
Male	42	37 (88.10%)	7	5 (71.43%)	0.258	16	15 (93.75%)	1.000
Child-Pugh score	41	6.00 (5.00–9.00) 7.07 ± 2.17	7	7.00 (5.00–8.00) 6.71 ± 1.38	0.940	16	7.00 (6.00–7.75) 7.06 ± 1.57	0.490
MELD score	41	10.03 (8.89–14.66) 11.37 ± 3.62	7	10.34 (8.34–10.88) 10.76 ± 3.24	0.681	16	11.09 (9.99–13.31) 11.50 ± 1.94	0.864

No. Pts, number of patients; IQR, interquartile range; SD, standard deviation; PVST, portal vein system thrombosis; MELD, model for end-stage liver disease. Bold italic value indicates results are statistically significant.

trigger the "outside-in" signal transduction pathway, and then promote platelets activation and aggregation (15, 34). Moreover, platelets can also express galectin-1, and galectin-1 contained in platelets may play a role in platelets activation, which requires further studies (15, 35).

Our study has for the first time demonstrated a relationship between CLEC-2/galectin-1 level and PVST in cirrhotic patients, which may be dependent upon the degree of PVST. Similarly, an animal study also showed that the progression to stable large thrombosis in CLEC-2-deficient mice was almost completely eliminated compared to the control group, suggesting the critical role of CLEC-2 in pathologically occlusive thrombosis (10). At present, both the Chinese consensus (5) and Baveno VII consensus (36) indicate that the timing of anticoagulation should be related to the degree of PVST. Therefore, both CLEC-2 and galectin-1 levels may be valuable for deciding the initiation of anticoagulant therapy.

Our study also demonstrated that serum galectin-1 level was higher in patients with MPV thrombosis than those without PVST, but this difference was not statistically significant. There were two possible explanations for this unexpected phenomenon. First, Xu et al. (37) showed that galectin-1 level in the peripheral blood of mice decreased with its age. Indeed, the mean age was significantly higher in our patients with MPV thrombosis than those without PVST, suggesting that age might weaken the difference in serum galectin-1 level between the two groups. Second, the sample size of this study was relatively insufficient, thereby compromising the statistical power.

There were some limitations in our study. First, the number of patients included was limited, thus large-scale studies are needed to further verify our results. Second, only patients with HBV-related liver cirrhosis were selected, thus further validation is needed in liver cirrhosis secondary to other etiology.

TABLE 4 Comparison between patients with MPV thrombosis and those with SMV thrombosis versus those without PVST.

Variables	No PVST		MPV thrombosis		*p*-value	SMV thrombosis		*p*-value
	No. Pts.	Median (IQR), mean ± SD, or frequency (%)	No. Pts.	Median (IQR), mean ± SD, or frequency (%)		No. Pts.	Median (IQR), mean ± SD, or frequency (%)	
Demographics								
Age (years)	42	52.23 (43.41–61.47) 53.04±10.35	17	58.95 (52.97–67.41) 59.17±8.82	***0.036***	13	57.08 (45.57-62.77) 54.66±10.75	0.627
Male	42	37 (88.10%)	17	15 (88.24%)	1.000	13	12 (92.31%)	1.000
Child-Pugh score	41	6.00 (5.00–9.00) 7.07±2.17	17	7.00 (5.50–8.00) 6.88±1.62	0.834	13	6.00 (5.50-7.00) 6.38±1.12	0.708
MELD score	41	10.03 (8.89–14.66) 11.37±3.62	17	10.38 (9.25–11.34) 10.85±2.37	0.522	13	10.35 (8.76-12.11) 10.67±2.25	0.416

No. Pts, number of patients; IQR, interquartile range; SD, standard deviation; PVST, portal vein system thrombosis; MPV, main portal vein; SMV, superior mesenteric vein; MELD, model for end-stage liver disease. Bold italic value indicates results are statistically significant.

5. Conclusion

Our current findings support a relationship between CLEC-2/galectin-1 and PVST in patients with HBV-related liver cirrhosis. Thus, CLEC-2/galectin-1 may predict the risk of more severe PVST, and they should be considered as potential targets for the prevention and treatment of PVST. In future, the mechanism regarding how CLEC-2/galectin-1 influences the development of PVST needs to be explored.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study followed the Declaration of Helsinki and was approved by the Medical Ethical Committee of the General Hospital of Northern Theater Command (approval number: Y2023-008).

Author contributions

XQ: conception and design, administrative support. YZ, XX, SX, JC, and XQ: provision of study materials or patients. YZ, XX, SX, and JC: collection and assembly of data. YZ and XQ: data analysis and interpretation. YZ, XZ, XX, XG, SX, SM, JC, and XQ: manuscript writing. YZ, XZ, XX, XG, SX, SM, JC, and XQ: final approval of manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Prognostic assessment of liver cirrhosis and its complications: current concepts and future perspectives

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Liver cirrhosis is an irreversible stage of chronic liver disease with varying clinical course. Acute decompensation of liver cirrhosis represents a watershed in prognosis and is characterized by the occurrence of clinical complications such as ascites, jaundice, hepatic encephalopathy, infections, or portal-hypertensive hemorrhages. Emergent data indicate that an acute decompensation can be subdivided into stable decompensated cirrhosis (SDC), unstable decompensated cirrhosis (UDC), pre-acute-on chronic liver failure (pre-ACLF) and acute-on chronic liver failure (ACLF), while the mortality risk varies greatly between the respective subgroups. ACLF is the most severe form of acutely decompensated cirrhosis and characterized by the development of organ failure(s) and a high short-term mortality. Due to the dynamic disease course of acute decompensation, it is paramount to detect patients at particular risk for severe complications those at high risk for developing ACLF as early as possible in order to initiate optimal management. This review describes new concepts and perspectives in the definition and classification of decompensated cirrhosis and provides on overview on emerging predictive scoring systems, non-invasive measurement methods and new biomarkers, which allow an early identification of patients with acute decompensation at risk.

KEYWORDS

cirrhosis, prognosis, scoring systems, ACLF, AD, portal hypertension, LSM, SSM

Introduction

Liver cirrhosis is the final stage of chronic liver disease and characterized by an irreversible replacement of liver parenchyma with fibrotic tissue and regenerative nodules (1). The major causes of cirrhosis include hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, alcohol-associated liver disease (ALD), and non-alcoholic fatty liver disease (NAFLD). Though viral hepatitis remains globally the leading cause of cirrhosis, the prevalence and incidence of alcoholic cirrhosis and NAFLD-related cirrhosis are rising in several regions of the world during the past decades due to an increased alcohol consumption and the ongoing epidemic of obesity (2, 3).

Clinical course of cirrhosis: from compensated cirrhosis to acute-on-chronic liver failure

The course of liver cirrhosis can be divided into a compensated and a decompensated stage (4). Compensated cirrhosis (CC) is considered as a stable state of disease where clinical symptoms are often absent or minor. The 1-year mortality rate of CC is estimated to be <5% (5).

The manifestation of clinical complications such as ascites, jaundice, hepatic encephalopathy, portal hypertensive bleeding (e.g., variceal hemorrhage), infections or any combination of these disorders define an acute decompensation (AD) (1, 6). An acute decompensating event represents a watershed in prognosis. Ascites occurs as the most common first decompensating event, with a drastic increase in 1-year mortality rising up to 20%, while 1-year mortality of acute decompensation due to infections even rises to over 50% (4, 7–9). Precipitating events for decompensation include bacterial or viral infections, aggravations of underlying liver disease (e.g., hepatitis B flare), alcoholic hepatitis, and drug-induced liver injury (4, 6, 9).

While the classification into a compensated and decompensated form has been established in everyday clinical practice for decades, recent data have shown that this dichotomous type of classification is an oversimplification (10). Since decompensated cirrhosis encompasses several different prognostic subgroups of patients with varying risk profiles, a more specific classification is needed.

Two landmark studies that have significantly improved the understanding of acute decompensation in recent years, have been the Consortium on Acute-on-Chronic Liver Failure in Cirrhosis (CANONIC) and the Predicting-of-Acute-on-Chronic Liver Failure in Cirrhosis (PREDICT) studies.

The results of the CANONIC and the PREDICT studies provided detailed insights on disease progression and its course and allow us today to classify different stages of decompensated liver cirrhosis more precisely.

The aim of the CANONIC study was to identify a definition and diagnostic criteria for the most severe form of acute decompensation, termed acute-on-chronic liver failure (ACLF). ACLF represents a distinct form of acutely decompensated liver cirrhosis, which is characterized by the onset of (extra-hepatic) organ failure(s) and a high short-term mortality (11–13). The CANONIC study found that ACLF is a dynamic syndrome, which can improve or conversely worsen and identified systemic inflammation as a major driver of ACLF.

The PREDICT study added further information on the course of AD and provides the basis for establishing a new terminology by uncovering three different clinical courses of acutely decompensated cirrhosis in patients without ACLF. According to the PREDICT study, AD can be categorized into stable decompensated cirrhosis (SDC), unstable decompensated cirrhosis (UDC), and a pre-ACLF stage.

Patients with SDC are characterized by the occurrence of typical complications such as ascites, low systemic inflammation, and facility of rapid recompensation. Patients with SDC typically do not require re-hospitalization due to further decompensation events, and serious organ failure is rarely observed in SDC. The 3-month mortality of SDC is about 0% and the 1-year mortality rate in SDC about 10% (9, 14).

UDC is associated with significant portal hypertension, gastrointestinal bleeding episodes and increased incidence of bacterial infections, resulting in further decompensation events. After the initial decompensation event, UDC is defined by the need for at least one further hospital readmission. Although organ dysfunction occurs more frequently in UDC than in SDC (29% brain dysfunction, 19% circulatory failure, and 16% liver dysfunction), patients with UDC do not usually develop ACLF. The 90-day mortality of UDC is about 21% and the 1-year mortality in UDC is about 36% (9, 14).

Patients in the pre-ACLF stage typically develop ACLF during follow-up and have a 3-month mortality of about 53% and a significantly higher 1-year mortality of more than 65%. While the UDC stage is characterized by increased portal hypertension and frequent occurrence of gastrointestinal bleeding, the pre-ACLF stage is characterized by significantly higher systemic inflammation and can thereby distinguished from SDC and UDC. Inflammatory biomarkers include leukocyte count, C-reactive protein (CRP), and interleukin-6 (IL-6), all of which showing a successive increase with progression of decompensation severity (12, 15).

Thus, a modern classification of cirrhosis should include the following disease stages: CC, SDC, UDC, Pre-ACLF, and ACLF (Figure 1). However, it must be kept in mind that these stages do not have to follow each other in an obligatory sequential manner, since the disease course of cirrhosis can vary tremendously.

Recently, the European Association for the Study of Liver Disease (EASL) published their updated ACLF clinical practice guidelines, whose recommendations for the classification of AD were largely based on the findings from the CANONIC and PREDICT studies (16). According to the recent EASL-guidelines, ACLF is characterized by functional organ failure of the six major organ systems (liver, kidney, brain, coagulation, circulation, and respiration) and systemic inflammation, induced by acute hepatic or extrahepatic precipitating factors, or both. Within the updated guidelines, AD is also divided into the subtypes, as described above (16).

Prognostic assessment of decompensated liver cirrhosis

In addition to establish a diagnosis, prognostic evaluation is an essential part of any disease. Besides its role in informing patients, prognostic evaluation also forms the basis for any decision-making process by clinicians.

In the field of liver cirrhosis, several different classification models exist. In clinical practice, the Child-Turcotte-Pugh (CTP) classification has become widely accepted for risk assessment in cirrhotic patients over the past decades (8, 15). Even the original purpose of this scoring system was the risk assessment in patients undergoing surgical porto-systemic shunt operation, it was later also used for stratifying patients for liver transplantation (17, 18). However, due to several limitations like the subjective assessment of different score components (e.g., ascites) the CTP score was subsequently superseded by the more reproducible Model for End-Stage Liver Disease (MELD) score (19).

The MELD score, which was initially developed for short-time mortality analysis in patients undergoing transjugular-intrahepatic-portosystemic-shunt (TIPS) implantation, is based on laboratory parameters (serum creatinine, bilirubin, INR) and ranges from 6 to 40. The MELD score can be used to predict 3-month mortality in

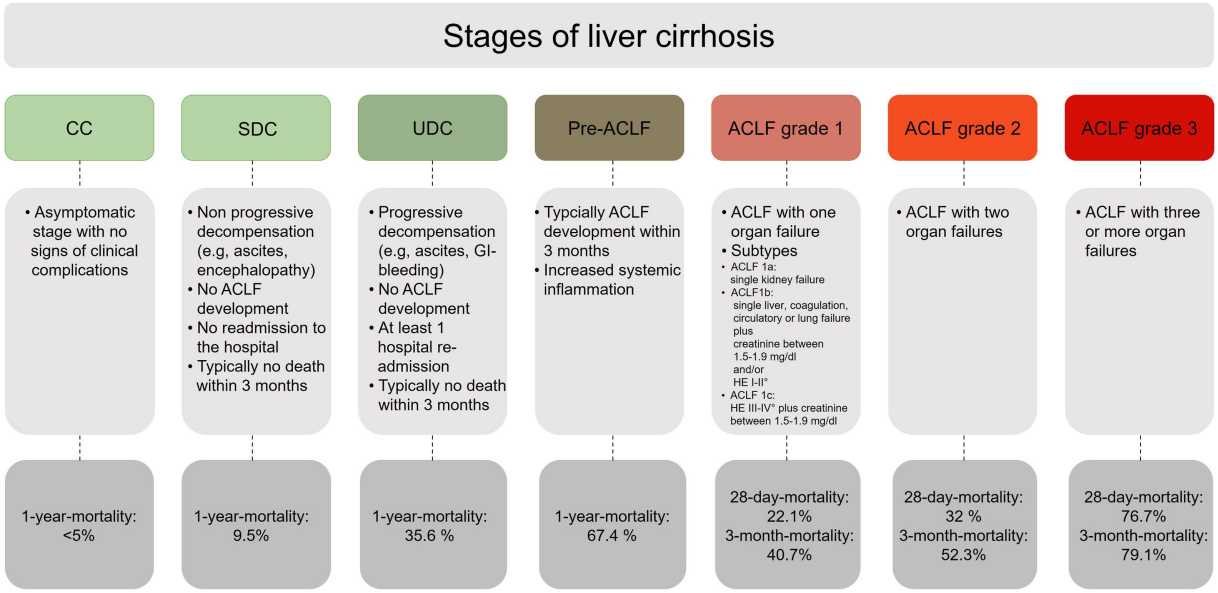


FIGURE 1
Overview of the different stages of liver cirrhosis. CC, compensated cirrhosis; SDC, stable decompensated cirrhosis; UDC, unstable decompensated cirrhosis; pre-ACLF, pre-acute-on-chronic liver failure; ACLF, acute-on-chronic liver failure.

patients with end-stage liver disease, even more accurately than the CTP score (8, 19, 20). Since sodium (Na) levels represent another independent predictor of mortality in cirrhosis, this marker can be incorporated into the ordinary MELD score and the MELD-Na score can be calculated. The MELD-Na score is currently used to determine organ allocation priorities for liver transplantation in the United States, whereas the ordinary MELD score is used in the European Eurotransplant (ET) region to allocate liver grafts (19).

However, the MELD score and MELD-Na score have some limitations that are currently the subject of debate. Studies have shown that renal function is not adequately reflected by serum creatinine and that individuals with lower muscle mass (e.g., cirrhotic patients with sarcopenia) as well as women who have less muscle mass compared with male counterparts are disadvantaged by using the MELD score for transplant allocation and prioritization. Furthermore, the MELD-Na score does not accurately predict risk in patients with ACLF (19).

Due to existing limitations new models of the MELD score, including but not limited to MELD-lactate, MELD-Na with transient elastography (TE), and the MELD 3.0 score including female sex and serum albumin as additional variables, has been developed in recent years to improve mortality prediction and allocation prioritization in liver transplantation (21).

Prognostic scoring systems in AD and ACLF

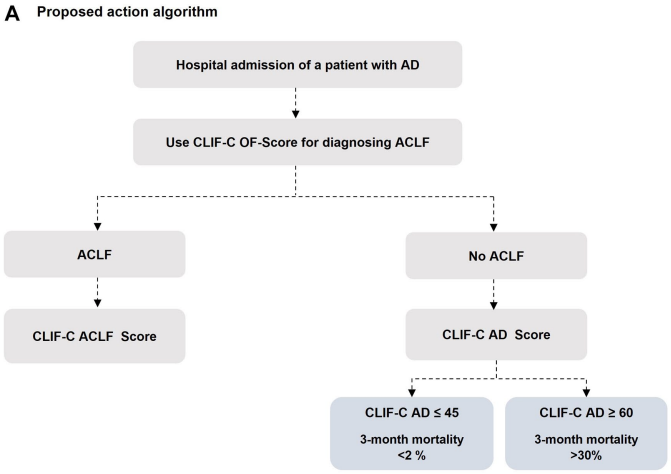
In the context of a dynamic acute decompensation and ACLF, new scoring systems have also been developed in recent years to identify patients at particular risk as early as possible. This scoring systems include the CLIF-C AD and CLIF-ACLF score.

Every patient who is admitted to hospital with acutely decompensated liver cirrhosis should evaluated immediately for the presence of (un-)complicated acute decompensation or ACLF (Figure 2A). The diagnosis of ACLF can be established if hepatic or extrahepatic organ failure is present, which should be defined according to the CLIF-C Organ Failure (CLIF-C-OF) score (24). The CLIF-C OF scoring system uses different clinical and biochemical criteria to define failure of the liver, kidneys, brain, coagulation, circulation, and/or respiration (Figure 2B).

If the diagnosis ACLF is established, the grade of an ACLF depends on the number of underlying organ failures. ACLF grade 1a is present in isolated renal failure with a serum creatinine level ≥ 2 mg/dL, while ACLF grade 1b is present in case of isolated liver, coagulation, circulatory, or respiratory failure with a serum creatinine level of 1.5–1.9 mg/dL and/or grade I-II encephalopathy. ACLF grade 1c, in turn, is present in isolated cerebral failure with hepatic encephalopathy grade III-IV and a serum creatinine level of 1.5–1.9 mg/dL. If there are two or three organ failures, a grade 2 or grade 3 ACLF can be diagnosed depending on the number of concomitant organ failures (13, 16).

To predict the mortality risk in patients with ACLF, it is recommended to apply the CLIF-C ACLF score, as this specific score reached a significantly higher predictive accuracy for mortality than the previously applied standard scoring systems, such as MELD, MELD-Na and the CTP score. After specific treatment measurements and multiorgan support have been initiated in patients with ACLF, a sequential use of the CLIF-C ACLF score during the hospital stay is recommended to evaluate treatment response, but also to identify patients in whom intensive care is likely to be futile despite maximal treatment efforts (25). Patients with a CLIF-C ACLF score ≥ 70 had a 100% mortality within 28-days after hospitalization (26), so that in particulary these cases, interdisciplinary evaluation is required to determine the extent to which intensive medical care should be continued or palliative regimens should be initiated (26).

If the diagnostic criteria of an ACLF are not met, an AD can be diagnosed, and the CLF-C AD score should be calculated for severity assessment.



B CLIF-C OF-Score

Organsystem	Subscore = 1	Subscore = 2	Subscore = 3
Liver	Bilirubin < 6 mg/dl	Bilirubin ≥ 6 mg/dl and < 12 mg/dl	Bilirubin ≥ 12 mg/dl
Kidney	Creatinine <2 mg/dl	Creatinine ≥2 mg/dl and <3,5 mg/dl	Creatinine ≥3,5 mg/dl or renal replacement therapy
Brain (West-Haven grade for HE)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	INR ≥ 2.0 and <2.5	INR ≥2.5
Circulatory	MAP ≥ 70 mmHg	MAP < 70mmHg	Use of vasopressors
Respiratory PaO_2/FiO_2 or SpO_2/FiO_2	>300 or >357	≤300 and >200 or >214 and ≤357	≤200 or ≤214

FIGURE 2
(A) A proposed algorithm for using the EASL CLIF-Consortium predictive score scoring systems for identifying cirrhotic patients with AD and ACLF [modified from Jalan et al. (22)]. The following formulas are used for calculation: CLIF-C Acute-on-chronic liver failure (CLIF-C ACLF) formula: CLIF ACLF score = 10 × (0.33 × CLIF-OF score + 0.44 × Age [years] + 0.63 × ln(WBC [109/L] − 2) (23); CLIF-C Acute decompensation (CLIF-C AD) formula: CLIF-C AD score = (10 × 0.03 × Age [years]) + (0.66 × ln(SCr [mg/dL]) + 1.71 × ln(INR) + 0.88 × ln(WBC [109/L]) − 0.05 × Na [mmol/L] + 8 (22).
(B) Overview of the Chronic Liver Failure-Consortium Organ Failure scale ()-score with definition criteria for organ failure. Shadowed areas define criteria for the diagnosis of organ failure. HE, Hepatic encephalopathy; FiO2, fraction of inspired oxygen; PaO2, partial pressure of arterial oxygen; SpO2, pulse oximetric saturation.

The CLIF-C-AD score was developed to predict the 3-month mortality in patients with acute decompensated liver cirrhosis (23) and is recommended to identify vulnerable patients at high mortality risk. With a potential scoring range of 0–100, patients with a CLIF-C AD ≤45 had a 3-month mortality less than 2%, whereas a score value ≥60 indicated a higher 3-month mortality of 30%. Since the CLIF-C-AD show superiority in predicting mortality to previously mentioned scores (MELD, CTP) it should be routinely calculated in all patients with AD (23).

In contrast to the previously available scores, the CLIF-C AD and CLIF-C ACLF scores also include age and leukocyte count, so that for the first-time systemic inflammation, which represents an important driver of disease progression in decompensation, is also considered as a prognostic parameter.

Emerging scoring systems and biomarkers

In addition to define and classifying the severity of AD and ACLF, scoring systems and biomarkers that predict the (future) risk of AD are also of great interest in everyday clinical practice, since early detection of transition to a decompensated stage of cirrhosis would enable timely targeted therapeutic interventions.

Recently Schneider and colleagues published the Early-Prediction-of-Decompensation (EPOD) score to predict the probability of decompensation within the next 3-years. In a large, multi-center cohort of 6,049 patients with compensated cirrhosis, the authors demonstrated that the EPOD score, which incorporates albumin level, platelet count, and bilirubin concentration, provides a good prediction tool for the risk of decompensation, and outperforms the established MELD and Child-Pugh score in predicting the risk of decompensation (27).

Other scoring systems to identify patients at risk for decompensation include the Albumin-Bilirubin- (ALBI) and the Firbosis-4 (FIB)-4 score. The ALBI score was developed to assess liver function in patients with hepatocellular carcinoma (HCC) undergoing liver surgery and has also been successfully applied to the prediction of survival and hepatic decompensation in patients with non-malignant liver diseases (22). Recent data has shown that the ALBI score can detect even slight deteriorations in liver function when compared to the Child-Pugh or MELD scores, making it a promising tool for early detection of AD.

Since the extent of fibrosis is closely associated with the risk of liver-related complications, non-invasive tests for fibrosis measurement such as the FIB-4 index, are also suitable for risk assessment of hepatic decompensation. The FIB-4 index is a reliable and useful predictor of the degree of liver fibrosis in patient with NAFLD and can also be used to predict the risk of liver-related complications and adverse outcomes (28–30). The combination of both scoring systems (ALBI-FIB-4 score) also shows a high predictive power for decompensation and represents a promising tool (31).

Nonetheless, the transferability or applicability of existing prognostic scores is not always satisfactory, as individual factors remain unconsidered. Therefore, it is essential to assess the overall situation of each patient individually and to use these scores as complementary tools for predicting mortality.

Besides scoring systems, there are also serum biomarker, which can be used for predicting prognosis and hepatic decompensation. Recently, Gurbuz et al. analyzed predictive biomarkers of decompensated cirrhosis by using untargeted serum proteomics and identified significantly lower serum concentrations of albumin, transferrin, pseudocholinesterase, transthyretin, and apolipoprotein AI in patients with cirrhosis compared with healthy individuals. Here, transferrin, pseudocholinesterase, and apolipoprotein AI show a stage-dependent decrease in serum concentration. In addition, the

authors demonstrated that patients with ACLF and higher serum levels of transthyretin have a better prognosis (32).

In a recent study including 444 hospitalized patients with decompensated cirrhosis, Juanola and colleagues demonstrated, that urinary fatty-acid-binding-protein (L-FABP) levels, as an indirect marker of hepatic inflammation, were independently associated with the 3-month clinical course in patients with decompensated cirrhosis, in terms of mortality and ACLF development. Therefore, urinary L-FABP seems to be another prognostic biomarker (33).

The role of serum bile acids as marker for AD and ACLF in patients with non-cholestatic cirrhosis, is also currently being investigated, since retention of bile acids and disrupted bile acid homeostasis plays a central role in hepatic damage. In a recent prospective cohort study including 143 patients with cirrhosis Horvatits et al. demonstrated, that serum bile acids were significantly associated with AD and ACLF and represents additional marker for risk stratification regarding new onset of AD and ACLF in cirrhotic patients (34).

However, many of these promising biomarkers are expensive and not available in clinical laboratory routine, and further prospective studies in Iarger cohorts are needed.

Non-invasive assessment of portal hypertension

Since, portal hypertension is the key driver and a proven predictor of hepatic decompensation in patients with advanced chronic liver disease, its invasive and non-invasive measurement is another important method for risk stratification.

Hepatic venous pressure gradient (HVPG) measurement is the current gold-standard procedure to determine the presence of clinically significant portal hypertension (CSPH), which is defined as an HVPG ≥10 mmHg. While a HVPG ≥10 mmHg is associated with an increased risk for the development of gastro-osephageal varices, a score of >12 mmHg with an increased risk of variceal bleeding (35–37). Though the concept of CSPH is HVPG-based, non-invasive testing methods, such as the measurements of liver and spleen stiffness, are also eligible to identify CSPH and of growing interest in clinical practice.

Liver stiffness measurement

In patients with virus- and/or alcohol-related CC and non-obese (BMI <30 kg/m^2) NASH-related advanced chronic liver disease, a LSM value by transient elastography of ≥25 kPa is sufficient to rule in CSPH, defining the group of patients at risk for endoscopic signs of portal hypertension (e.g., gastro-esophageal varices) and at higher risk of decompensation (35, 37). While a single baseline measurement is sufficient to identify patients at risk, repetitive measurements of LSM should be performed to predict the risk of future hepatic decompensation more precisely.

Recently, Semmler et al. demonstrated, that longitudinal dynamic changes in repeated LSM enables a more accurate risk prediction for decompensation and liver-related death in a retrospective cohort study including 2,508 patients than a single measurement (38). Specifically, a 20% increase/decrease of LSM in patients with advances liver disease indicates a ~ 50% increased/decreased risk of hepatic decompensation and liver-related death (38).

Spleen stiffness measurement

In addition to LSM, measurement of spleen stiffness measurement (SSM) may also be applied for risk prediction of AD, as splenomegaly results from passive congestion due to portal hypertension and recent data have shown a positive correlation between HVPG and spleen stiffness (37, 39). SSM seems to be an elegant and non-invasive method for detecting CSPH and objectifying short-time dynamic changes in portal circulation, i.e., after TIPS implantation (37, 40–42). According to the Baveno VII consensus guidelines, SSM can be also used in to rule-out and rule-in CSPH (SSM <21 kPa and SSM >50 kPa, respectively). Recently, Yu et al. developed an artificial intelligence-driven spleen-based model to identifying patients with compensated cirrhosis, which are at higher risk of decompensation using a quantitative 3-dimensional (3D) volumetric analysis of the spleen. In their study, a spleen volume > 364 cm^2 has been associated as a predictor for decompensation (43).

Unfortunately, the regional availability of TE to perform LSM and SSM is limited so far. However, if available, SSM should be performed in all cirrhotic patients in addition to LSM, and further investigation should be conducted to determine whether there are specific cut-offs that can optimally predict the risk of decompensation as well as success of re-compensation by specific treatment measurements lowering portal hypertension.

Conclusion

The early identification of the patients with acutely decompensated cirrhosis who are at high risk of mortality and ACLF development remains an unmet clinical need. Modern scoring systems such as the CLIF-C AD and ACLF scores are valuable tools in risk assessment and should be determined as standard in all cirrhotic patients after hospitalization. Future studies are needed to investigate the extent of sequential and non-invasive measurement to predict decompensation and whether a combination of a biomarker and non-invasive measurement method is an approach to optimize risk prediction in cirrhosis.

Author contributions

DG: Visualization, Writing – original draft. TG: Writing – review & editing, Supervision, Resources. PK: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Prevalence of chronic venous insufficiency and deep vein thrombosis in cirrhotic patients

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Summary: People with cirrhosis of the liver are at risk for complications that can worsen their quality of life and increase morbidity and mortality. Contrary to previous beliefs, cirrhosis does not protect against the development of thromboembolic events, and cirrhotic patients may have higher rates of deep vein thrombosis (DVT).

Background and aims: The study of chronic venous disease and its impact on patients with cirrhosis is unknown in the literature and may be an important fact since this condition also had impact on quality of life and morbidity. The aim of this study is to evaluate the prevalence of DVT (Deep Venous thrombosis) in outpatients with cirrhosis and the degree of chronic venous insufficiency, evaluating possible correlations between clinical and laboratory aspects of cirrhotic patients with these pathologies.

Methods: Patients with cirrhosis were evaluated in the outpatient clinic of the Liver Transplantation and Hepatology Service of HC-FMUSP from November 2018 to November 2022, with clinical evaluation, venous disease questionnaires, data collection of imaging and laboratory tests, and venous color Doppler ultrasound. The information was analyzed by the University of São Paulo (USP) Statistics Department.

Results: There was a prevalence of 7.6% of DVT in studied patients, VCSS score 6.73 and severe CEAP classification (C4-6) 32.1%. There was no association of DVT with qualitative variables by the Fisher test such as Child Turcotte Pugh Scale (CTP) (p = 0.890), dichotomized INR values (p = 0.804), etiology of cirrhosis (p = 0.650) and chronic kidney disease ($p > 0.999$), nor with quantitative variables by t-student's such as age (p = 0.974), Body Mass Index (BMI) (p = 0.997), MELD score (p = 0.555), Albumin (p = 0.150) and Platelets (p = 0.403). We found that as the severity of ascites increases, there is an increase in the proportion of patients classified in the category indicating more severe clinical manifestations of chronic venous disease (C4 to C6). The mean age (54 years) was higher in patients with DVT than in those without. The mean BMI of patients without DVT (25.7 kg/m^2) is lower than that of patients with DVT (27.0 kg/m^2). The prevalence of DVT is higher in patients with thrombophilia (20.0%) than in those without (7.0%). This suggests an association between the two variables. The descriptive measures of the MELD

score, the cirrhosis scale used for liver transplant waiting lists, did not indicate an association of this scale with the occurrence of DVT.

Conclusion: The incidence of VTE (Venous Thromboembolic Events) and CVD (Chronic Venous Disease) within the sample surpassed that of the general population; nevertheless, more studies are required to validate these results. Concerning venous thromboembolism, no correlation was observed between the variables within the sample and the augmented risk of VTE. Regarding chronic venous disease, studies have shown that edema and orthostatism are correlated with increased severity of CVD on the VCSS scales. Statistical dispersion methods suggest that patients with higher BMI and more severe liver disease (according to the Child-Pugh score) are more likely to experience worsening of CVD. About chronic venous disease, studies have shown that edema and orthostatism are correlated with increased severity of CVD on the VCSS scales.

KEYWORDS

venous incompetence, liver disease, deep vein thromboembolism, cirrhosis, venous disease

Highlights

- Prevalence of 7.6% of DVT.
- Venous Clinical Severity Score (VCSS) score 6.73.
- C4-6–Clinical signs, Etiology, Anatomic distribution, Pathophysiologic condition (CEAP) classification 32.1%.

Introduction

The liver plays a key role in coagulation, and liver disease, by causing profound changes in the synthesis of coagulation factors, altering the hemostatic balance with a reduction in both pro- and anticoagulant factors. This imbalance can lead to potentially fatal clinical changes due to hemorrhagic or thromboembolic states (1, 2). For decades, it was believed that the increase in International Normalized Ratio (INR) associated with thrombocytopenia in patients with advanced liver disease was a form of self-anticoagulation that protected these patients from thromboembolic episodes, but in recent years there have been changes in the understanding of coagulation mechanisms. The importance of the endothelium, platelets, and the reticuloendothelial system as mediators in the phases of thrombus formation, thrombus limitation and lysis (cell theory) has been observed and, on the contrary, studies show that there may be an increase in cases of thromboembolism in these patients (3). The incidence of venous thromboembolism in patients with cirrhosis ranged from 0.5 to 8.1% in different studies, according to a systematic review by Aggarwal et al., (1), 3.7% according systematic review of Ambrosino et al. (2) highlighting the importance of clinical suspicion in these patients and evaluating the benefits of prophylactic regimens. Qi et al. (4) performed a systematic review of 20 studies when they evaluated the incidence and prevalence of VTE using random effect models and analyzed subgroups by type of VTE [deep vein thrombosis (DVT), pulmonary embolism (PE)], type of liver disease (cirrhosis alone/unclassified liver disease or non-cirrhotic), region in which the study was conducted (USA/Europe/Asia), total number of patients observed with liver disease (greater or less than 1000), study quality (high/low), and method of case identification (ICD codes/clinical charts). The incidence of VTE ranged from 0.33 to 6.32% in 14 studies with a pooled value of 1% [95% confidence interval (CI) 0.7–1.3%]. The pooled incidence of DVT and PE was 0.6% (95% CI 0.4–0.8%) and 0.28% (95% CI 0.13–0.49%), respectively. The prevalence of VTE ranged from 0.6 to 4.69% in six studies, with a pooled prevalence of 1.0% (95% CI 0.7–1.2%). The pooled prevalence of DVT and PE was 0.7% (95% CI 0.6–0.9%) and 0.36% (95% CI 0.13–0.7%), respectively. Venous thromboembolism, with its potentially fatal consequences, is a major health problem worldwide, with at least 200,000 new cases per year in the United States and an incidence of 74.5 per 100,000 people per year in the United Kingdom. The global incidence of deep vein thrombosis is 5 cases per 100,000 people per year, and the risk of developing the disease varies with age, affecting approximately 2 to 3 cases per 100,000 people between the ages of 30 and 49 and approximately 20 cases per 100,000 people between the ages of 70 and 79 (5, 6).

The Virchow's triad implicates three main factors thought to contribute to VTE: hypercoagulability, endothelial damage, and venous stasis. Liver diseases potentially thrombogenic due to venous stasis caused by ascites, sarcopenia with immobility and high intrabdominal pressure resulting in venous stasis, as well as invasive procedures leading to inflammation and infectious conditions, which are other factors of Virchow's triad (7). Chronic venous insufficiency is defined as a dysfunction of the venous system caused by valvular incompetence associated or not with obstructed venous flow. It may affect the superficial venous system, the deep venous system, or both. In addition, venous dysfunction may be congenital or acquired (8).

Approximately 7 million people in the United States have chronic venous insufficiency. It is the cause of 70 to 90 percent of all leg ulcers (9).

Coagulation mechanism in liver disease

In patients with cirrhosis, because there are changes in coagulation proteins, with an increase in Prothrombin Activity Time and Activated Partial Thromboplastin Time, it was believed that there was protection against DVT and Pulmonary Thromboembolism (PE), but studies show that these data are not real, possibly because other procoagulant proteins may be at normal levels or increased, factors that inhibit thrombus formation may be reduced (protein C, protein S), in addition to other prothrombotic factors present in these patients (10).

Apart from Von Willebrand factor, Tissue Plasminogen Activator (TPA), Plasminogen Activator Inhibitor-1 (PAI-1), Thrombomodulin (TM), and Plasminogen Activated Urokinase (Upa), the liver synthesizes most coagulation factors (11).

Factor VIII is typically elevated in patients with cirrhosis, somewhat offsetting the decrease in other coagulation factors. At the same time, the procoagulants PtnC, PtnS, and antithrombin are decreased. However, normal thrombin generation activity has been demonstrated, as well as increased levels of Von Willebrand factor with greater platelet activation, although these are often reduced (12). The preservation of thrombin generation in cirrhosis is probably due to a rebalancing of coagulation mechanisms caused by the reduction of procoagulants (except factor VIII and Von Willebrand factor) and naturally occurring anticoagulants. The increase in Von Willebrand factor may also contribute to the increase in factor VIII by binding to it and preventing its cleavage and elimination (13). This results in acquired resistance to the anticoagulant action of thrombomodulin due to increased levels of factor VIII and decreased levels of its main physiological inhibitor, protein C (11, 12) **Figure 1**.

Venous insufficiency

Venous insufficiency usually results from venous hypertension secondary to valvular reflux and/or venous obstruction. Despite the wide variety of signs or symptoms associated with chronic venous insufficiency, the presence of venous hypertension appears to be very common. Venous hypertension can be caused by primary valve reflux or secondary to previous cases of DVT, and other causes of venous hypertension obstruction occur in obesity due to high abdominal pressure or failure of the calf muscle pump as occurs after ankle trauma, fibrosis secondary to chronic local inflammation, and severe loss of muscle mass. Reflux may occur in the superficial, deep, or both venous systems (15). Endothelial inflammation is known to be a major contributor to the development of CVD, which is perpetuated by venous hypertension and valvular insufficiency. Changes in shear stress affect endothelial cells, leading to their activation and recruitment of leukocytes with the release of pro-inflammatory agents.

The damaged endothelium remains a trigger for the ongoing inflammatory cascade leading to venous and valve wall damage and worsening CVD. Endothelial dysfunction is a central aspect linking chronic venous disease and DVT, for which endothelial dysfunction is also a risk factor (16).

Venous disease classifications

CEAP classification

The CEAP classification today is universally adopted to provide an orderly framework on venous pathology for communication between colleagues and decision-making. The use of this classification allows an organized categorization of the key elements of venous disease in each case and the interrelationships between clinical manifestations, causes and anatomical distribution. It describes static components that do not change with the response to treatment and are not used with the severity score of chronic venous disease, for this the American Venous Forum recommends the use of other scales and today the Villalta and the VCSS are the most used (17–19).

In the CEAP classification, clinical signs are divided into 7 classes ranging from C0 to C6, with C0 being total absence of signs related to venous disease and C6 being active venous ulcer (**Supplementary material**).

VCSS–Venous clinical severity score

The system of increasing classification of severity, proposed by *the American Venous Forum (Venous Clinical Severity Scale—VCSS),* consists of ten clinical descriptors: pain, presence of varicose veins, venous edema, skin pigmentation, induration (fibrosis, hypodermitis, white atrophy, lipodermatosclerosis), inflammation (erythema, eczema dermatitis), presence, diameter and duration of active ulcer and use of compressive therapy (20) (**Supplementary material**).

Primary endpoint

Measurement of the prevalence of deep vein thrombosis in patients with cirrhosis at the HCFMUSP gastroenterology outpatient clinic.

Secondary objectives

Evaluation of the degree and presence of chronic venous disease in patients with cirrhosis from the outpatient gastroenterology service of the HC-FMUSP.

Patients and methods

Ethical aspects

After receiving approval from the CEP of HC-FMUSP, a cross-sectional study aimed to assess the prevalence of venous disease in

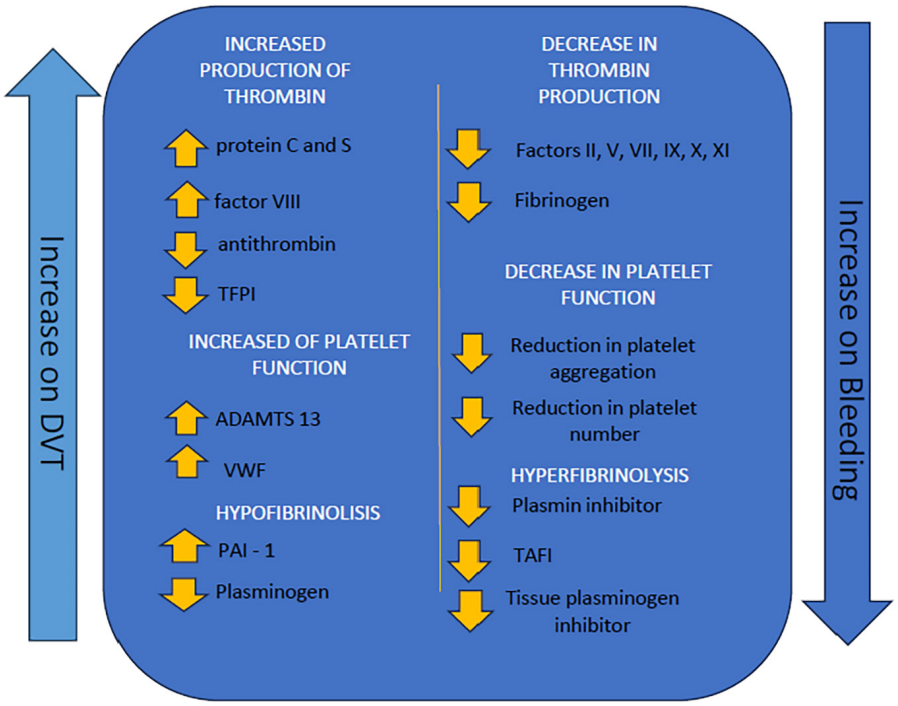

FIGURE 1
Illustrative scheme demonstrating factors that lead to greater coagulability or greater hemorrhagic tendency in patients with cirrhosis (14). TFPI, Tecidual factor pathway inhibitor; ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type 1 repeats 13; VWF, Von Willembrand factor; PAI-1, plasminogen inhibitor 1; TAFI, Thrombin activatable fibrinolysis inhibitor.

patients with cirrhosis. All patients provided their signature on the informed consent form. The HC-FMUSP CEP adopted this model to fulfill the requirements set forth by Resolution 196/96 of the National Health Council (CNS).

Population studied

Each patient was evaluated only once to verify the presence of venous disease. A total of 119 patients met all study inclusion criteria. We studied patients with cirrhosis diagnosed by the HC-FMUSP hepatology team using clinical, imaging, and laboratory criteria, all from the HC-FMUSP Liver Transplant, Ascites, and Hepatology Outpatient Clinic of the Gastrointestinal Surgery Service. Data collection started in November 2018 and ended in November 2022. In 2018, 03 patients were evaluated, in 2019, 40 patients, in 2020, 35 patients, in 2021, 27 patients, and in 2022, 27 patients were evaluated for a total of 119 patients. None of these patients developed DVT after evaluation. All patients with a history of DVT when already with clinically significant liver disease were DVT.

Inclusion criteria

Outpatients in the Liver Transplantation Outpatient Clinic and in the Ascites and Hepatology Clinics of the HCFMUSP (Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo–Brazil) with a confirmed diagnosis of cirrhosis.

Exclusion criteria

Patients who did not agree to sign the Free and Informed Consent Form were excluded.

Development of the study

This is a single-center observational study in which data collection, clinical examination, and venous Doppler ultrasound were performed by the same investigator. The medical record was used to collect laboratory and imaging data. Clinical assessment and physical examination of the lower extremities were performed to verify the functional status of the venous circulation. Classification was performed according to internationally established criteria (21). The physical examination was first performed with the patient in the orthostatic position. It was designed to evaluate edema, the presence of lipodermatosclerosis or hyperpigmentation, varicose veins, and active or healed ulcers. Patients signed the free and informed consent form and assisted in completing the anthropometric, demographic, and clinical data evaluation form. The clinical evaluation was performed immediately before or after the physical examination and consisted of specific standardized questionnaires to assess the integrity of the lower extremity venous system, a hepatic insufficiency scale, activities of daily living scales, and questionnaires to assess pain, discomfort, and disability related to varicose veins (22).

Questionnaires included the Karnofsky Performance Scale; the Child-Turcotte-Pugh Scale (CTP); and the VCSS Scale.

Doppler ultrasound was initially performed in the dorsal decubitus position to evaluate the inferior vena cava and iliac veins, the common, superficial, deep, and popliteal femoral veins for patency, presence or absence of reflux, venous wall thickness, presence, or absence of intraluminal fibrosis or partially recanalized thrombosis, and the patient was then placed in the orthostatic position for distal compression maneuvers to evaluate venous

reflux and the superficial venous system. The internal and external saphenous veins were scanned transversely and longitudinally for diameter measurement, assessment of pathologic reflux, and signs of previous or recent phlebitis. The popliteal, muscular, posterior, anterior tibial, and fibular veins were also evaluated in the orthostatism. Laboratory data for the dates closest to the time of data collection were obtained from medical records. BMI was calculated according to World Health Organization (WHO) criteria (23).

Statistical analysis

We performed a descriptive analysis of the data to characterize the patient profiles and summarize the results of the study. Then, appropriate statistical models were considered to estimate the prevalence and identify the risk factors for deep vein thrombosis and chronic venous insufficiency. The mean, standard deviation, median, minimum, maximum, and first and third quartiles were calculated. Fisher's exact test was used to evaluate the association between DVT and the qualitative variables. The *p*-values indicate that there is no association between any of the risk factors and the occurrence of DVT.

The associations of DVT with the quantitative variables: age, BMI, Meld score, albumin, and platelets, were evaluated using the t-Student's test (24), and the results obtained indicate that there is no difference in the means of the variables in the two categories of DVT.

The Kruskal-Wallis test was used to evaluate the association between the VCSS and each of the qualitative variables (25). A non-parametric approach was used because the descriptive analysis of the variables showed that the distributions of the VCSS in the categories of categorical risk factors may not be normal, with the same variance in all categories.

A logistic regression model was adjusted (26) to study the association between the occurrence of DVT and the risk factors together. Automatic backward and forward variable selection methods were used to fit the model (27). However, in both backward and forward selection, no variable was selected that contributed significantly to the explanation of the occurrence of DVT. A LASSO model was also fitted (25), but no variable was selected.

The associations between the VCSS scale and the quantitative variables of age, BMI, and Meld Score were assessed by calculating Spearman's correlation coefficient (28). The coefficient values and corresponding *p*-values shown in **Table 10**. A total of 73 indicate that there is no correlation between the VCSS and the quantitative variables individually. The statistical software RStudio for Windows (version 2022.07.2 + 576) was used for analysis.

Multiple linear regression models were then adjusted (29), with VCSS as the response variable and risk factors as explanatory variables.

Results

Baseline characteristics are summarized in **Table 1**. The mean body mass index is 27 kg/m^2. The first quartile of the Karnofsky Performance is equal to 60. This means that less than 25.0% of the patients are unable to perform manual and/or domestic activities.

TABLE 1 Baseline characteristics.

	N	Mean ± Dp	Median (Min–Max)
Age	119	53.00 ± 13.23	57 (21–76)
BMI	115	27.00 ± 5.07	25.7 (19.5–40.4)
MELD	119	14.77 ± 4.5	15 (6–25)
Albumin	119	3.30 ± 0.79	3.4 (0.7–5)
Urea	118	46.0 ± 35.0	35 (10–232)
Platelets	118	99.5 ± 64.0	89 (10–299)
INR	119	1.41 ± 0.43	1.25 (0.9–3.6)

DVT was present in 9 (7.6%) patients. A total of 5 (4.2%) patients had thrombophilia. Child-Pugh score was classified as category B in most patients (58%). The median Model for End-Stage Liver Disease (MELD) score was 15.

TABLE 2 Results of fisher's test applied to evaluate the occurrence of association between DVT and risk factors (qualitative variables).

Variable	*p*-value
Thrombophilia	0.330
Child-Pugh	0.890
Etiology	0.650
CKD	>0.999
INR	0.804

TABLE 3 Results of student's *t*-test applied to evaluate association between DVT with risk factors in patients with cirrhosis (quantitative variables).

Variable	*p*-value
Age	0.974
BMI	0.997
MELD score	0.555
Albumin	0.105
Platelets	0.403

A total of 61.2% of patients did not consume alcohol. A total of 63% of the patients did not smoke and 58.0% had no previous abdominal or hernia surgery.

Deep vein thrombosis (DVT)

In this study, we found alcoholic cirrhosis in 23.5%, NASH in 22.7%, autoimmune hepatitis in 16.8%, and viral hepatitis in 12.6%, with DVT found in 7.1% of patients with alcoholic cirrhosis, 7.4% of patients with NASH, 16.8% of patients with autoimmune hepatitis, and 7.7% of patients with viral hepatitis. We evaluated the association between DVT and possible risk factors such as thrombophilia, Child-Pugh, MELD score, cirrhosis etiology, chronic kidney disease, and International Normalized Ratio (INR). Fisher's exact test was used to evaluate the association between DVT and the qualitative variables. The results obtained are presented in **Table 2**. The *p*-values indicate that there is no association between any of the risk factors and the occurrence of DVT.

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TABLE 4 Factors impacting VCSS score- descriptive measures.

Child-Pugh	*n*	Mean	Std derivation	Min	1° quartile	Median	3° quartile	Max
Venous clinical severity scale by child-Pugh–Liver cirrhosis severity scale								
A (5–6)	31	5.03	4.01	0.0	2.5	5.0	6.5	19
B (7–9)	69	7.28	4.54	0.0	4.0	6.0	10	20
C (10–15)	19	7.53	3.52	1.0	4.0	8.0	10	14
Ascites	***n***	**Mean**	**Std derivation**	**Min**	**1° quartile**	**Median**	**3° quartile**	**Max**
Venous clinical severity scale for ascites								
Absence	23	6.13	4.49	0.0	3.0	5.0	8.5	16
Low/mild	40	6.45	4.87	0.0	3.0	5.0	10	19
Bulky/refractary	56	7.18	3.90	1.0	4.0	6.5	10	20
Ortostatism	***n***	**Mean**	**Std derivation**	**Min**	**1° quartile**	**Median**	**3° quartile**	**Max**
Orthostatism venous clinical severity scale								
Less than 3 h	10	4.50	3.34	0.0	3.25	4.0	5.75	11
Between 3 and 6 h	35	5.77	3.72	0.0	3.50	5.0	8.00	14
More than 6 h	73	7.47	4.61	0.0	4.00	6.0	7.47	10

The relationships between DVT and the quantitative variables presented in Table 3, indicate that there is no difference in the means of the variables in the two categories of DVT and in the association with the risk facts in the population of patients with cirrhosis.

Chronic venous disease (CVD)

The descriptive measures of age and BMI do not suggest an association of these variables with CEAP. The median Child-Pugh for patients classified in classes C4 to C6 (9.00) is higher than the median for patients classified in classes C0 to C3 (7.00).

The descriptive measures of the Meld Score also do not suggest an association of this variable with CEAP, and we found that as the severity of ascites increases, there is an increase in the proportion of patients classified in the category indicating more severe clinical manifestations of chronic venous disease (C4 to C6). The percentage of patients classified in classes C4 to C6 in those without hernia is lower than in those with hernia, and the highest percentage of patients classified in classes C4 to C6 was observed in those who remained standing (orthostatism) for more than 6 h.

The joint behavior of the candidate variables for risk factors for chronic venous insufficiency and the VCSS–Venous Clinical Severity Scale is shown in Table 4. The Spearman correlation coefficient between VCSS and age is 0.123, indicating no association between the variables and the same for body mass index, with the value of Spearman's correlation coefficient of 0.075. It is observed that as the value of VCSS increases, Child-Pugh tends to increase.

The Spearman correlation coefficient between the Meld Score scale and VCSS was 0.078, indicating no association between the two scales.

TABLE 5 Descriptive measures for MELD Score and VCSS.

	N	Mean ± SD	Median (Q1–Q3)	Min–Max
MELD	119	14.77 ± 4.50	15.00 (11.00–18.00)	6.00–25.00
VCSS	119	6.73 ± 4.34	6.00 (4.00–10.00)	0.00–20.00

The median of the VCSS scale observed in patients with refractory ascites is higher than that observed in the other grades of ascites.

Descriptive measures of the VCSS scale observed in the groups with and without hernias do not suggest an association between these variables. The mean and median of the VCSS scale increase with increasing orthostatism, and descriptive measures of the presence of an extracardiac shunt on transthoracic echocardiography also do not suggest an association of this condition with VCSS. Table 5 shows the descriptive measures for the severity scales of liver disease, chronic venous disease VCSS. Remembering that this scale varies between 0 and 30, with increasing severity, at least 50.0% of the patients have a VCSS scale value below.

The mean age (54 years) was higher in patients with DVT than in those without. The mean BMI of patients without DVT (25.7 kg/m^2) is lower than that of patients with DVT (27.0 kg/m^2). The prevalence of DVT is higher in patients with thrombophilia (20.0%) than in those without (7.0%). This suggests an association between the two variables (Table 6). The descriptive measures of the MELD score, the cirrhosis scale used for liver transplant waiting lists, did not indicate an association of this scale with the occurrence of DVT.

TABLE 6 Descriptive measures for age, BMI and MELD for DVT.

		N	Mean ± SD	Median (Q1–Q3)	Min–Max
Age	No DVT	110	53.99 ± 13.48	57.00 (46.00–64.00)	21.00–76.00
	DVT	9	54.11 ± 10.20	55.00 (46.00–59.00)	38.00–70.00
BMI	No DVT	106	27.06 ± 5.07	25.71 (23.08–30.40)	19.50–40.39
	DVT	9	27.07 ± 4.07	27.03 (24.00–28.65)	21.22–35.15
MELD score	No DVT	110	14.85 ± 4.51	15.00 (11.25–18.00)	6.00–25.00
	DVT	9	13.89 ± 4.51	15.00 (9.00–18.00)	8.00–20.00

TABLE 7 Descriptive measures for age and CEAP, BMI for CEAP, and Child-Pugh for CEAP.

		N	Mean ± SD	Median (Q1–Q3)	Min–Max
Age	C0–C3	81	53.47 ± 13.78	57.00 (44.00–64.00)	21.00–75.00
	C4–C5	38	55.13 ± 12.06	56.00 (46.50–63.00)	28.00–76.00
BMI	C0–C3	78	27.11 ± 5.25	25.75 (22.89–30.85)	19.50–40.39
	C4–C5	37	26.96 ± 4.75	25.70 (23.24–29.28)	20.28–39.44
Child-Pugh	C0–C3	81	7.59 ± 1.63	7.00 (6.00–9.00)	5.00–11.00
	C4–C5	38	8.32 ± 1.61	9.00 (7.00–9.00)	5.00–11.00

The prevalence of DVT can be considered high in all etiologies of cirrhosis except those related to the hepatitis B virus.

Chronic venous disease risk factors identification

We evaluated possible associations of chronic venous insufficiency with the following variables that may be risk factors for the disease: age, BMl, Child-Pugh, MELD score, ascites, hernia, orthostatism. The joint behavior of the candidate variables for risk factors for chronic venous insufficiency and the categorized CEAP is illustrated in Table 7. The descriptive measures of age and BMI do not suggest an association of these variables with CEAP. Orthostatism was evaluated according to the patient's report related to his daily habits during life, especially at work, evaluating the time in which he was mainly standing or with the limbs hanging without walking. Ranged from <3 h, 3–6 h and >6 h.

The mean Child Pugh for patients classified in classes C4 to C6 (9.00) is higher than the mean for patients classified in classes C0 to C3 (7.00).

Descriptive measures of the MELD score also do not suggest an association of this variable with CEAP. As the severity of ascites increases, the proportion of patients classified as having more severe clinical manifestations of chronic venous disease (C4 to C6) increases.

The percentage of patients classified in categories C4 to C6 is lower in those without hernia than in those with hernia. The highest percentage of patients classified in category C4 to C6 was observed in those who remained standing (orthostatism) for more than 6 h. The joint behavior of the candidate variables for risk factors for chronic venous insufficiency VCSS—venous clinical severity scale and MELD is illustrated in Table 8.

Multiple linear regression models with VCSS as the response variable and risk factors as explanatory variables. In the adjustment

TABLE 8 Descriptive measures of MELD and VCSS.

	N	Mean ± SD	Median (Q1–Q3)	Min–Max
MELD	119	14.77 ± 4.50	15.00 (11.00–18.00)	6.00–25.00
VCSS	119	6.73 ± 4.34	6.00 (4.00–10.00)	0.00–20.00

of the model, the automatic procedures of selection of forward and backward variables were adopted. The variables Child-Pugh, Edema and Orthostatism were selected to compose the final model by both methods. The adjusted model is presented in Table 9.

Fixed edema and the degree of orthostatism for patients classified as Child-Pugh B, an increase of 3.037 on the VCSS is expected, on average, in relation to those classified as Child-Pugh A; for patients classified as Child-Pugh C, a mean increase of 2.743 on the VCSS scale is expected in relation to those with Child-Pugh A.

The associations of the VCSS with the quantitative variables, Age, BMI and Meld Score were evaluated by calculating the Spearman correlation coefficient. The values of the coefficients and corresponding *p*-values presented in Table 10 indicate that there is no correlation between Villalta and the quantitative variables individually.

Discussion

In this study, the collection of history, questionnaires, and physical examination were performed in the field, which represents an advantage in relation to other studies published on this topic, with a much larger number of patients, but using an administrative database, based on the International Classification of Diseases (ICD) (30). This represents an important bias since such databases

TABLE 9 Results obtained in the adjustment process of the linear regression model with VCSS response variable and the risk factors for CVI as explanatory.

	Estimates of coefficients	Standard error	*P*-value
Intercept	3.698	2.534	0.147
Child-Pugh B	3.037	1.549	0.052
Child-Pugh C	2.743	2.141	0.203
Edema	7.411	1.332	<0.001
Orthostatism 1	3.372	2.559	0.190
Orthostatism 2	4.573	2.398	0.059

Selected variables.

TABLE 10 Spearman correlation coefficient of the VCSS with age, BMI and MELD.

	Statistics	*p*-value
Age	253,682	0.296
BMI	228,462	0.294
MELD	266,024	0.569

do not have the primary objective of serving the research and with large heterogeneity in the population studied, making it difficult to perform meta-analyses. Qi et al. (4) in their systematic review showed that about 1% of patients with liver diseases develop or are diagnosed with VTE during their hospitalizations. However, the epidemiological data are very heterogeneous among studies. They demonstrated that the incidence of VTE appeared to be higher in studies including fewer than 1,000 patients than in those including more than 1,000 patients.

The cirrhotic patient is often malnourished, with great loss of muscle mass, decreased calf muscle pump, and decreased venous return. This may contribute to the development of venous disease in the lower limbs (31, 32). The analysis of the degree of chronic venous insufficiency associated with cirrhosis is a novelty based on hypotheses that can be partially validated by the data found. However, it's clear that studies with a larger number of patients, controlled and using methods such as plethysmography and accurate analyses of the degree of sarcopenia, would better confirm such hypotheses. Laboratory findings of albumin and platelets in cirrhotic cases do not show the same pattern of findings in the literature, but it should be remembered that patients in this study are outpatients, and these studies are mostly inpatient. The prevalence of detected DVT and degree of CVD are consistently higher than in the general population. In 119 patients evaluated in this study, the prevalence of previous VTE was 7.6%. Dabbagh et al. (33), during 7 years of evaluation, included 190 patients, and 12 had VTE, or 6.3%, in hospitalized patients. Barba et al. (34) found 2.7% VTE in 157,654 patients. They were all hospitalized.

Bogari et al. (35) reported 18 cases of VTE, or 11%, in a total of 163 patients with severe liver disease. However, this was a sample of critically ill patients admitted to the hospital. The prevalence of VTE was 7.6, 8.1, and 8.2% in patients without cirrhosis, compensated cirrhosis, and decompensated cirrhosis, respectively, in a study by Nguyen et al. (36). In the present study, 9.7% were found to be compensated (CTP A), and 12.5% were found to be decompensated (CTP B and C). Stine considered the presence of NASH *per se* as a hypercoagulable state due to the associated inflammatory state, and in his study, the most common etiologies of cirrhosis were NASH (38%), HCV (26%), and alcoholism (24%). Among the patients admitted with liver dysfunction, 7% had a VTE, but portal vein thrombosis was also included in this article (37). The worst degrees of cirrhosis would be associated with higher CVD and DVT rates. Prophylactic measures could be taken in patients on the transplant list once these poor prognostic factors are established. The literature lacks studies evaluating the degree of chronic venous insufficiency in cirrhotic patients.

Study limitations

The study is cross-sectional, and therefore it is not possible to evaluate the evolution of the chronic venous disease in the same patient together with the concomitant worsening of the cirrhosis. It is only possible to observe the patient at a given moment and thus to correlate the venous and hepatic picture. Chronicity, subjectivity, and periodicity characterize the complaints of chronic venous disease. The questionnaires used, such as the VCSS scale, may have measures that are confused with those of cirrhosis. For example, edema is common in cirrhosis and venous disease, paresthesia may occur due to alcoholic neuropathy in patients with cirrhosis, and pruritus may occur in advanced stages of CVD but also in liver disease with jaundice. Spasms are very common in cirrhotic patient with ascites using diuretics, and their occurrence in chronic venous disease is considered in the VCSS scale.

Patients with portal vein thrombosis often, but not always, use oral anticoagulants or subcutaneous enoxaparin, which may reduce DVT occurrence in this population.

Conclusion

The incidence of VTE and CVI within the sample surpassed that of the general population; nevertheless, more studies are required to validate these results.

Concerning venous thromboembolism, no correlation was observed between the variables within the sample and the augmented risk of VTE. Regarding chronic venous disease, studies have shown that edema and orthostatism are correlated with increased severity of CVD on the VCSS scales. Regarding chronic venous disease, studies have shown that edema and orthostatism are correlated with increased severity of CVD on the VCSS scales. Statistical dispersion methods suggest that patients with higher BMI and more severe liver disease (according to the Child-Pugh score) are more likely to experience worsening of CVD.

About chronic venous disease, studies have shown that edema and orthostatism are correlated with increased severity of CVD on the VCSS scales.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**. Further inquiries can be directed to the corresponding author.

Author contributions

LC, FT, and WA contributed to conception and design of the study. LC, DW, VS, and RP organized the database. LC, RM, and LN performed the statistical analysis. LC wrote the first draft of the manuscript. LC, LD, RD, AT, and LD'A wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1214517/full#supplementary-material

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Primary biliary cirrhosis and osteoporosis: a bidirectional two-sample Mendelian randomization study

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Background: Observational studies have identified a heightened risk of osteoporosis and fractures in patients with primary biliary cholangitis (PBC). However, conclusive evidence establishing a causal relationship between the two, and a clear mechanism explaining this association, remains elusive.

Methods: We conducted a bidirectional two-sample Mendelian randomization (MR) analysis to investigate the causal relationship between PBC and osteoporosis. This analysis utilized five MR methods: inverse-variance weighted (IVW), MR-Egger, weighted median, weighted mode, and simple mode. Sensitivity analyses were performed, employing various models and testing methods, to assess the impact of heterogeneity and pleiotropy on the results and to confirm their robustness.

Results: A causal relationship between PBC and osteoporosis risk was established through IVW analysis (OR: 1.049, 95%CI: 1.017–1.082, P=0.002). Three other MR analyses corroborated these findings. Conversely, osteoporosis was not found to causally affect PBC risk, as evidenced by IVW analysis (OR: 0.941, 95%CI: 0.783–1.129, P=0.511). Across all MR analyses, no heterogeneity or horizontal pleiotropy was detected among the instrumental variables (IVs). Furthermore, the leave-one-out analysis indicated that no single SNP disproportionately influenced the results, affirming the reliability of the bidirectional MR findings.

Conclusion: This study establishes a positive causal relationship between PBC and the risk of osteoporosis, while no definitive causal link was found from osteoporosis to PBC. These findings offer new insights and guidance for managing bone health in PBC patients.

KEYWORDS

primary biliary cirrhosis, osteoporosis, Mendelian randomization study, genome-wide association studies, causal relationship

1 Introduction

Primary biliary cholangitis (PBC), formerly known as primary biliary cirrhosis, is an autoimmune disease characterized by chronic inflammatory damage to the liver. The precise etiology and mechanisms underlying PBC are not yet fully understood. Its hallmark is chronic non-suppurative destructive cholangitis, primarily affecting the interlobular and septal bile ducts, resulting in periductal inflammation and necrosis (1). The incidence and prevalence of PBC show significant variation across different regions and over time, influenced by genetic, environmental, and socio-economic factors. According to recent data (2), the incidence of PBC increased until 2000, then stabilized in North America and Europe, but continued to grow in the Asia-Pacific region. The global prevalence of PBC is on the rise, with North America reporting the highest rate of 21.81 per 100,000 individuals, followed by Europe at 14.59 per 100,000, and the Asia-Pacific region recording the lowest at 9.82 per 100,000. White non-Hispanic individuals exhibit the highest incidence rate, although the incidence among Black and Asian populations is also considerable. Clinical manifestations of PBC, such as cholestatic pruritus, abdominal discomfort, and fatigue, significantly impair patients' quality of life (3).

Osteoporosis is a prevalent skeletal disorder marked by the deterioration of bone tissue microstructure and a decrease in bone mineral density (BMD) (4). This condition leads to reduced bone strength and increased fragility, consequently elevating the risk of fractures. Established risk factors for osteoporosis include aging, endocrine disorders, malnutrition, obesity, and the use of medications that impact bone metabolism. Osteoporosis-related fractures represent a substantial economic burden; for instance, annual expenditures amount to approximately $17.9 billion in the United States and £4 billion in the United Kingdom (5). Therefore, identifying the causes and risk factors for osteoporosis is crucial for early diagnosis and treatment, reducing fracture risk, and enhancing the quality of life.

A review study reported that the prevalence of osteoporosis in PBC ranged from 20% to 44%, with an increase in prevalence correlating to disease progression and a high incidence of fractures (10-20%) (6). A comprehensive analysis of 3,980 PBC patients revealed a significantly higher fracture risk and post-fracture mortality in these patients compared to matched controls from the general population (7). However, the relationship between PBC and osteoporosis is subject to debate. A retrospective study indicated that the susceptibility to osteoporosis in PBC patients was not higher than in the general population, with factors such as age and gender being more influential (8). Some researchers have also noted no significant difference in bone loss between PBC patients and healthy controls, suggesting that the risk of osteoporosis in PBC patients is more closely related to age and menopausal status than to the severity of liver disease (9).

The pathogenesis of osteoporosis in PBC is multifactorial, primarily involving reduced bone formation and increased bone resorption in the later stages of the disease. Studies suggest that elevated bile acids and bilirubin may contribute to osteoporosis by inducing apoptosis in osteoblasts and stimulating osteoclast activity (10). Osteoporosis can be considered an extrahepatic complication of PBC, with its etiology and pathogenesis still not fully understood. A study involving European and Chinese Han populations identified a genetic link between PBC and osteoporosis (11). The CLDN14 gene, encoding the tight junction protein claudin-14, plays a role in regulating epithelial cell tight junctions and bile secretion. A variant, rs170183, in CLDN14 has been linked to kidney stones and reduced BMD. Thus, the CLDN14 gene may serve as a potential molecular connection between PBC and osteoporosis, though its function and mechanism require further experimental validation and clarification. To date, no Mendelian randomization studies have explored the inherent causal relationship between these two conditions, leaving open the possibility of confounding or reverse causality. Further investigation into the causal relationships underlying these associations is warranted.

Traditional observational studies are often limited by confounding factors, reverse causality, and selection bias. Mendelian randomization (MR), utilizing genetic variants as instrumental variables (IVs) derived from genome-wide association studies (GWAS) data, offers a method to estimate the causal effect between exposure and outcome (12). Genetic variants, randomly assorted during meiosis and fixed at conception, act as long-term stable exposure factors unaffected by environmental, social, or other factors. This method thus overcomes the limitations inherent in conventional observational studies. In our study, we employed a bidirectional two-sample MR analysis to evaluate the causal relationship between PBC and osteoporosis.

2 Materials and methods

2.1 Data source

To investigate the causal relationship between PBC and osteoporosis, we selected single nucleotide polymorphisms (SNPs) as IVs from the GWAS database. This database is publicly accessible, eliminating the need for additional ethical approval. The PBC GWAS data were derived from a dataset published by Cordell et al. in the Journal of Hepatology (https://www.ebi.ac.uk/gwas/labs/publications/34033851) (13), representing one of the largest PBC GWAS datasets available. This study identified multiple genetic loci and genes associated with PBC. We included all five European cohorts, encompassing 8,021 PBC cases, 16,489 controls, and 5,186,747 SNPs. For PBC definition, these cohorts adhered to the criteria set by the European Association for the Study of the Liver (EASL) (3). The osteoporosis GWAS data were obtained from the FinnGen R9 database (https://www.finngen.fi/en), including 7,300 cases and 358,014 controls from the European population. The dataset employed ICD-10, ICD-9, and ICD-8 codes for osteoporosis diagnosis (14).

2.2 Genetic instrument selection

The selected genetic IVs had to fulfill three core assumptions of MR analysis to minimize result bias (15): 1. Relevance assumption: direct association with the exposure. 2. Independence assumption:

independence from confounders of the exposure-outcome association. 3. Exclusion restriction assumption: influence on the outcome solely through the exposure (Figure 1). To mitigate potential interference from linkage disequilibrium between SNPs and ensure accurate and reliable causal inference for PBC and osteoporosis, we implemented various restrictive measures for SNP selection. We chose SNPs that were genome-wide and significantly associated with the exposure ($P<5\times10^{-8}$) and excluded those exhibiting high linkage disequilibrium ($r^2<0.001$, kb<10,000) (16). To maintain the accuracy of the MR analysis, it was necessary to filter out palindromic SNPs, which are SNPs with effect alleles and other alleles as complements. Additionally, we evaluated the strength of the association between each IV and the exposure, excluding weak IVs. The F-statistic for each IV was calculated using the formula: $F=Beta^2/SE^2$ (17), where Beta represents the estimated effect of the allele on the exposure, and SE is the standard error. IVs with an F-statistic less than 10 were excluded due to potential genetic confounding or measurement error. Ultimately, we used these rigorously selected SNPs as the final IVs for subsequent Mendelian randomization analysis.

2.3 Statistical analysis

In our study, we employed five different methods for MR analysis to explore the causal relationship between PBC and osteoporosis: the inverse-variance weighted (IVW) method, MR-Egger, weighted median, weighted mode, and simple mode. The IVW method, which uses all valid SNPs, is advantageous for increasing statistical power. In the absence of heterogeneity and pleiotropy, IVW can provide the most accurate and efficient causal estimate, making it our primary method (18). The MR-Egger method introduces an intercept term to the IVW, enabling the detection and adjustment for horizontal pleiotropy. However, it may yield unstable or inaccurate estimates due to its susceptibility to outliers or unevenly distributed IVs (19). The weighted median offers better robustness and reduced finite-sample bias compared to the IVW but tends to have larger standard errors and lower efficiency (20). We also employed the simple mode and weighted mode methods to further validate the potential causality between exposure and outcome. A *P*-value threshold of <0.05 was applied in all MR analyses to ascertain the causal effects of exposure on the outcome.

2.4 Pleiotropy, heterogeneity, and sensitivity evaluation

Given that the IVW method cannot adequately address horizontal pleiotropy and confounding among SNPs, and may be influenced by outliers or influential SNPs, it was necessary to check for heterogeneity and pleiotropy before using IVW, and to exclude outliers or influential SNPs (18). We utilized Cochran's Q test to examine heterogeneity among estimates from different genetic variants. A *P*-value of less than 0.05 indicated significant heterogeneity (21). The MR-PRESSO and MR-Egger's intercept tests were used to provide valid MR estimates in the presence of horizontal pleiotropy. MR-PRESSO identifies genetic variants significantly impacting the causal estimate, eliminates the interference from outliers, and offers corrected results after their removal (22). MR-Egger's intercept test serves to detect and estimate horizontal pleiotropy, and also provides a sensitivity analysis for the robustness of MR results (19). A leave-one-out analysis was conducted to evaluate the influence or bias of each SNP on the pooled estimate. All MR analyses and tests were performed using the "TwoSampleMR" and "MRPRESSO" packages in R software (version 4.3.1).

3 Result

3.1 MR analysis

We identified 47 SNPs significantly associated with the risk of PBC. These SNPs exhibited no linkage disequilibrium ($r^2<0.001$) and were not considered weak instrumental variables, as their

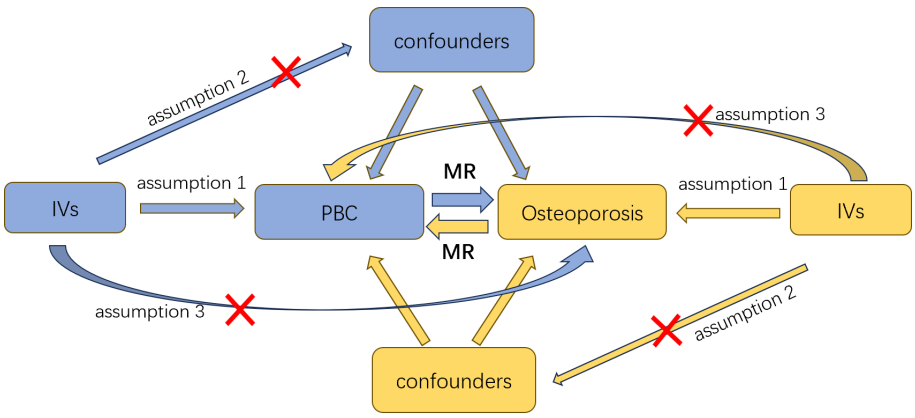

FIGURE 1
An overview of this Mendelian randomization (MR) study design; IVs, instrumental variables; MR, Mendelian randomization.

F-statistics all exceeded 10, fulfilling our previously established selection criteria. Nine of these SNPs did not have corresponding results in the osteoporosis-related GWAS database. Additionally, six palindromic SNPs were excluded. Consequently, we utilized the remaining 32 SNPs as IVs for MR analysis. To compute the proportion of phenotypic variance explained by the IVs, the following formula was used: $R^2 = [2 \times Beta^2 \times (1 - EAF) \times EAF]/[2 \times Beta^2 \times (1 - EAF) \times EAF + 2 \times SE^2 \times N \times (1 - EAF) \times EAF]$. Here, EAF is the effect allele frequency, N is the GWAS sample size of the exposure. The analysis revealed that these SNPs accounted for 14.55% of the PBC risk variance, indicating that the selected SNPs possessed strong predictive power and were capable of effectively minimizing confounding effects. Detailed information on these SNPs is presented in the appendix (Supplementary Table S1).

Based on the IVW method (OR: 1.049, 95%CI: 1.017–1.082, *P*=0.002), we found a statistically significant positive causal relationship between the risk of PBC and osteoporosis. The weighted median method also indicated a significant causal effect (OR: 1.045, 95%CI: 1.002–1.089, *P*=0.038). However, the MR-Egger method (OR: 1.071, 95%CI: 0.984–1.165, *P*=0.121) and the simple mode (OR: 1.080, 95%CI: 0.974–1.199, *P*=0.155) showed a directionally consistent but statistically non-significant positive causal relationship. The weighted mode (OR: 0.985, 95%CI: 0.872–1.114, *P*=0.815) yielded a non-significant effect estimate, diverging from the other methods' results (Table 1). The weighted mode and simple mode are two distinct causal effect estimation methods in MR analysis. The weighted mode assigns weights based on the variance of the genetic instruments, whereas the simple mode does not consider variance. Typically, the simple mode's accuracy is low, while the weighted mode can be inaccurate in small samples and is affected by the degree of association between genetic instruments and the exposure and outcome variables. In cases where the degree of association is inconsistent, the weighted mode may introduce bias, whereas the simple mode is more robust (23). In this study, the weighted mode's results were the only ones inconsistent with the other methods and lacked statistical significance, potentially due to the influence of certain genetic instruments. Our primary findings are derived from the IVW method, which utilizes all valid genetic instruments, thereby enhancing statistical power and offering the most accurate and efficient results in the absence of heterogeneity and horizontal pleiotropy. Consequently, the results from the weighted mode, which diverged from the findings of other methods and lacked statistical significance, are deemed unreliable and do not significantly impact the overall conclusion of the Mendelian randomization analysis. Instead, more weight is given to the IVW method, widely considered the most robust and reliable among the available approaches. We conducted heterogeneity tests for all IVs, using Q statistics to evaluate the differences among them. The Q statistic was calculated to be 41.27 (*P*=0.103), indicating no significant heterogeneity. Additionally, the MR-PRESSO global test did not detect any outlier SNPs or horizontal pleiotropy effects of PBC on osteoporosis (*P*=0.156), and MR-Egger's intercept test also found no evidence of horizontal pleiotropy (Egger intercept = -0.0057, *P*=0.610). These results suggest that the analysis is less likely to be influenced by potential confounding biases. A sensitivity analysis was conducted using the leave-one-out method, which involved sequentially removing SNPs, recalculating the causal effect with the remaining SNPs, and observing whether the results varied with each SNP removal. This analysis demonstrated stable results, further affirming the reliability of our findings (Table 2; Figures 2–5).

3.2 Further analyses

To delve deeper into the causal relationship between osteoporosis and PBC, we carried out a two-sample MR analysis with osteoporosis as the exposure and PBC as the outcome. We employed the same GWAS databases, IV selection methods, analysis techniques, and test procedures as previously described. When exposure-related SNPs were absent in the result dataset, we utilized alternative SNPs that demonstrated a high correlation with the relevant SNPs (r^2>0.8). We loosened the relevance assumption and initially identified 14 SNPs significantly associated with disease risk from GWAS ($P<5\times10^{-7}$, r^2<0.001). However, 8 of these SNPs were excluded because they lacked corresponding results in the PBC GWAS database, and no proxy SNPs were available. The F-statistics for the remaining SNPs were all above 10, indicating the absence of weak IV bias. Consequently, we used these 6 SNPs as IVs for our MR analysis (Supplementary Table S2).The results indicated no causal relationship between osteoporosis and the risk of PBC, as evidenced by the IVW analysis (OR: 0.941, 95%CI: 0.783–1.129, *P*=0.511), and similar conclusions were drawn from the other four methods (Table 3). Furthermore, we conducted heterogeneity tests on all IVs using the Q statistic to assess differences among them. The Q statistic was 1.434 (*P*=0.921), indicating no significant heterogeneity. MR-PRESSO global test did not detect any outlier

TABLE 1 Mendelian randomization estimates for PBC on osteoporosis.

Exposure	Outcome	No. of IVs	Methods	Beta	SE	OR (95%CI)	P
PBC	Osteoporosis	32	MR Egger	0.068	0.043	1.071 (0.984~1.165)	0.121
			Weighted median	0.044	0.021	1.045 (1.002~1.089)	0.038
			IVW	0.047	0.016	1.049 (1.017~1.082)	0.002
			Simple mode	0.077	0.053	1.080 (0.974~1.199)	0.155
			Weighted mode	-0.015	0.063	0.985 (0.872~1.114)	0.815

PBC, primary biliary cholangitis; IVs, instrumental variables; IVW, inverse variance weighting; SE, standard error; OR, odds ratio; CI, confidence interval.
P < 0.05 was considered statistically significant.

TABLE 2 Heterogeneity and horizontal pleiotropy for Mendelian randomization analysis.

Exposure	Outcome	Heterogeneity		Horizontal pleiotropy		
		Cochran's Q	P	Egger intercept	SE	P
PBC	Osteoporosis	41.27	0.103	-0.0057	0.0110	0.610
Osteoporosis	PBC	1.434	0.921	0.1067	0.0994	0.342

PBC, primary biliary cholangitis.
P < 0.05 was considered statistically significant.

SNPs or horizontal pleiotropic effects of PBC on osteoporosis (*P*=0.922), and MR-Egger's intercept test also found no evidence of horizontal pleiotropy (Egger intercept = 0.1067, *P*=0.342). These findings suggest that our analysis is credible (Table 2; Figures 6–9).

4 Discussion

Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease frequently associated with the development of osteoporosis. In our study, we employed bidirectional two-sample Mendelian randomization (MR) analysis to investigate the potential causal relationship between PBC and osteoporosis. This approach complements previous observational studies that have explored the linkage between these conditions. For instance, a study by Menon et al., published in the Journal of Hepatology in 2001, found a significant positive correlation between bone loss rate and bilirubin level in PBC patients. This finding suggests that the severity of liver disease is a crucial factor influencing the onset and progression of osteoporosis in these patients (24). Another study involving 176 PBC patients assessed the incidence, risk factors, and progression rate of osteoporosis, concluding that PBC is significantly associated with osteoporosis, with the severity of liver disease being an influential factor affecting the occurrence and progression of osteoporosis (25). These observational studies are consistent with the findings of our study.

Saeki et al. conducted research evaluating skeletal muscle disease in 117 PBC patients (26). Their findings revealed higher rates of osteoporosis, muscle atrophy, and vertebral fractures in this group, indicating a close interrelationship among these complications. Additionally, an article focusing on PBC and osteoporosis highlighted the strong connection between the two and analyzed various factors that may impact bone metabolism in PBC patients, such as vitamin deficiencies, IGF-1, BMP, bile acids, and bilirubin. This study concluded that individuals with PBC are more susceptible to osteoporosis and fractures than the general population, and the risk escalates as the disease progresses (6). In recent years, the exploration of the intestinal microbiota has gained

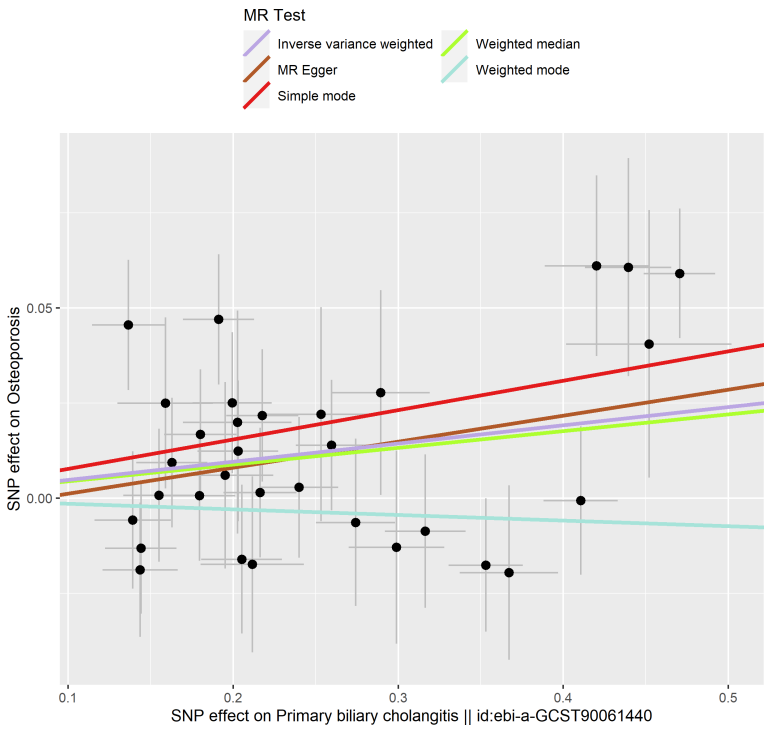

FIGURE 2
Scatter plot of the effect of primary biliary cholangitis on osteoporosis.

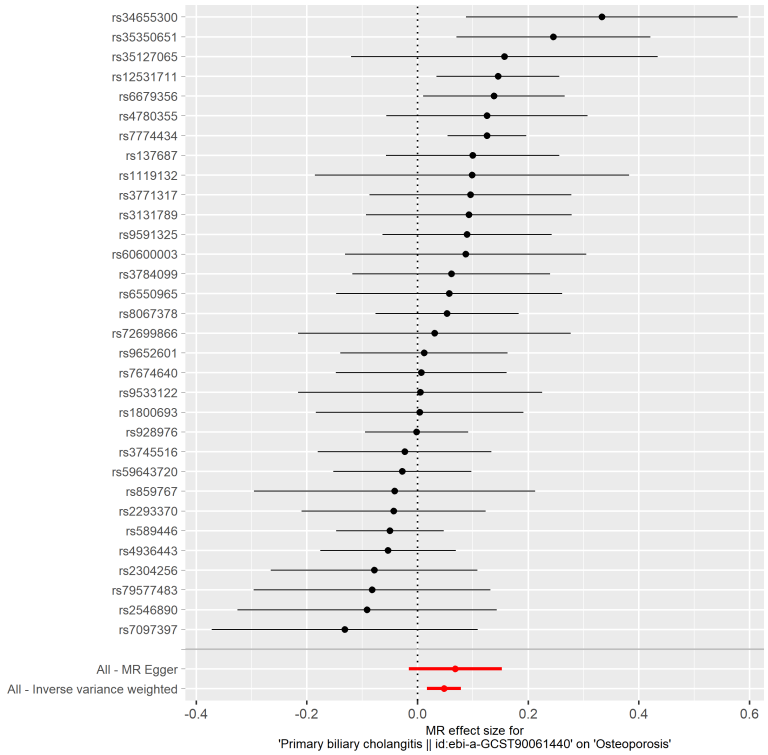

FIGURE 3
Forest plot of the effect of primary biliary cholangitis on osteoporosis.

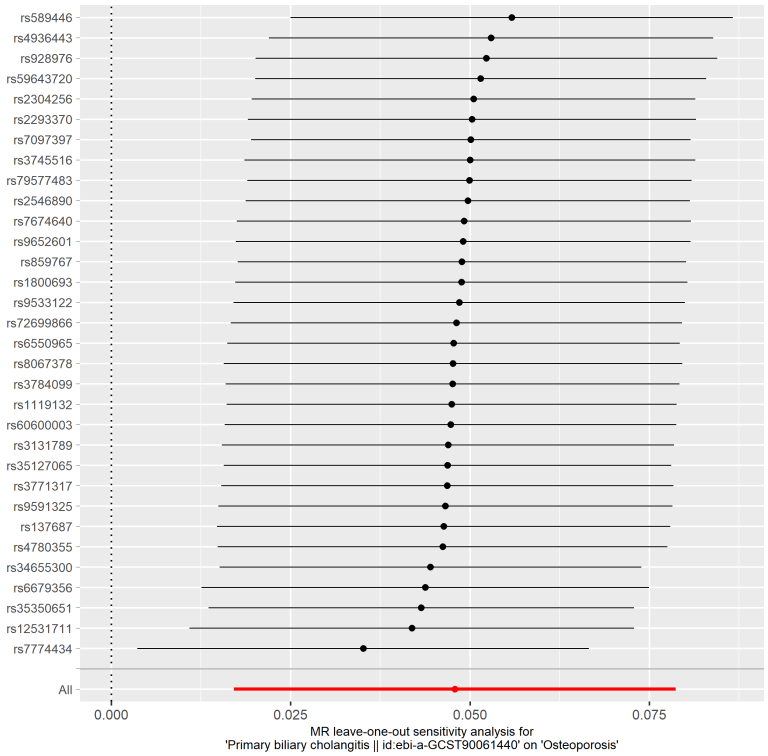

FIGURE 4
Leave-one-out plot of the effect of primary biliary cholangitis on osteoporosis.

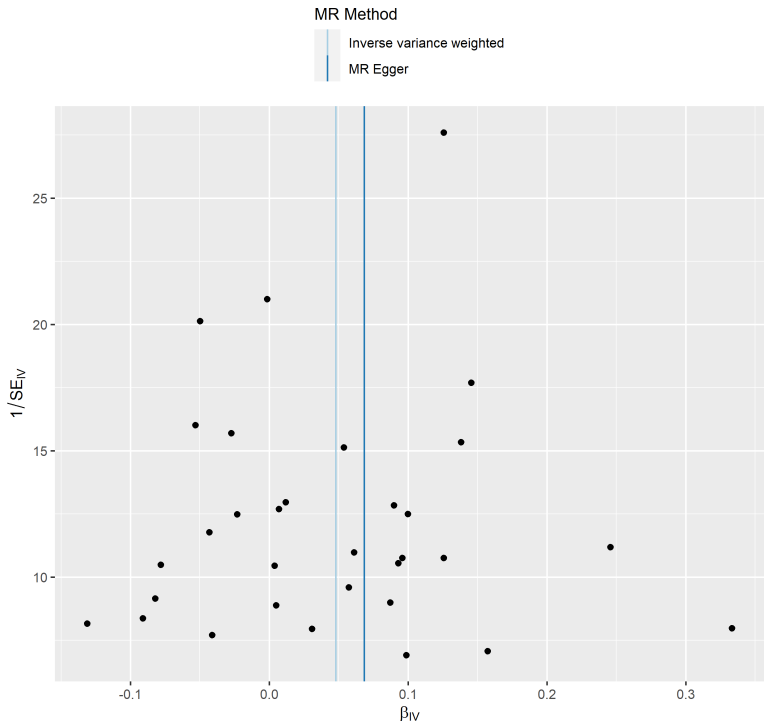

FIGURE 5
Funnel plot of the effect of primary biliary cholangitis on osteoporosis.

prominence in research. PBC and osteoporosis share common intestinal microbial groups, including Candidatus_Soleaferrea, Eubacterium_coprostanoligenes_group, Allisonella, and Peptococcus. These microbes are significantly associated with both PBC and osteoporosis and may influence liver function, calcium absorption, intestinal barrier integrity, immune response, and bone density through various metabolic products or mechanisms (27). These studies collectively indicate that PBC is a systemic metabolic disease affecting not only the liver but also bone health. This is in alignment with the findings of our MR analysis.

The causal relationship between PBC and osteoporosis is underpinned by several hypotheses, reflecting the complexity of this association. Initially, the issue of osteoporosis in PBC patients was acknowledged and attributed to hepatic osteodystrophy, but further exploration was limited by the technological and hardware constraints of the time (28). One key factor in normal bone metabolism involves the interaction of a three-molecule complex comprising osteoprotegerin (OPG), nuclear factor-kB receptor activator ligand (RANKL), and RANK. It has been observed in some studies that the progression of PBC can reduce OPG secretion by the liver, leading to uncontrolled osteoclast activity and increased bone resorption. This is considered one of the potential mechanisms. However, there is also evidence suggesting that PBC-related osteoporosis primarily results from impaired bone formation, though excessive bone resorption might also play a role (29). Thus, the precise mechanism of osteoporosis linked to PBC remains not fully understood and likely varies depending on the severity of liver disease. Patients with PBC exhibit elevated levels of inflammatory cytokines, including IL-1, IL-6, TNF-α, which can directly or indirectly promote osteoclast formation and activation via the RANKL-RANK signaling pathway, leading to increased bone resorption and osteoporosis (30). Additionally, cholestasis in

TABLE 3 Mendelian randomization estimates for osteoporosis on PBC.

Exposure	Outcome	No. of IVs	Methods	Beta	SE	OR (95%CI)	P
Osteoporosis	PBC	6	MR Egger	-1.168	1.035	0.311 (0.041~2.363)	0.322
			Weighted median	-0.070	0.112	0.932 (0.748~1.161)	0.530
			IVW	-0.061	0.093	0.941 (0.783~1.129)	0.511
			Simple mode	-0.065	0.162	0.937 (0.682~1.287)	0.703
			Weighted mode	-1.168	1.035	0.311 (0.041~2.363)	0.322

PBC, primary biliary cholangitis; IVs, instrumental variables; IVW, inverse variance weighting; SE, standard error; OR, odds ratio; CI, confidence interval.
P < 0.05 was considered statistically significant.

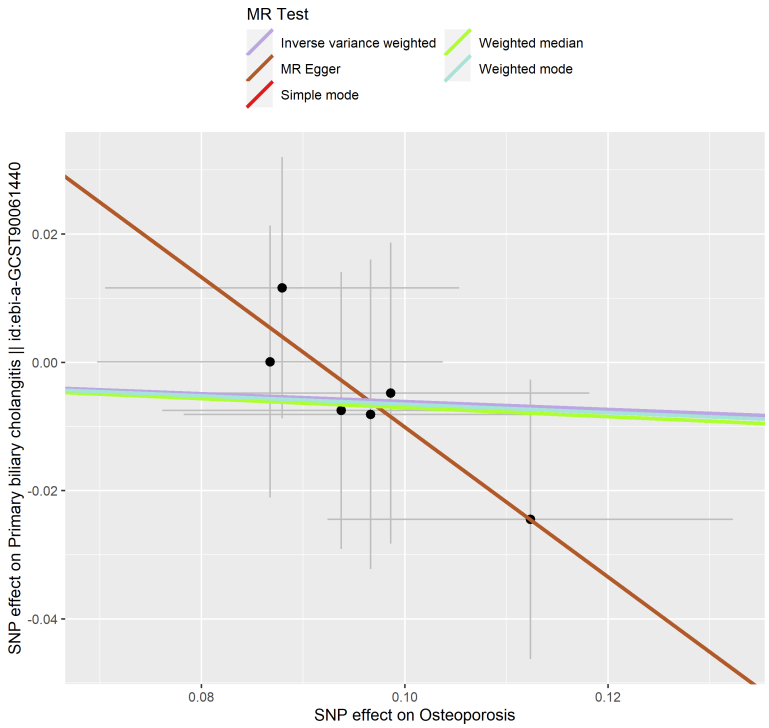

FIGURE 6
Scatter plot of the effect of osteoporosis on primary biliary cholangitis.

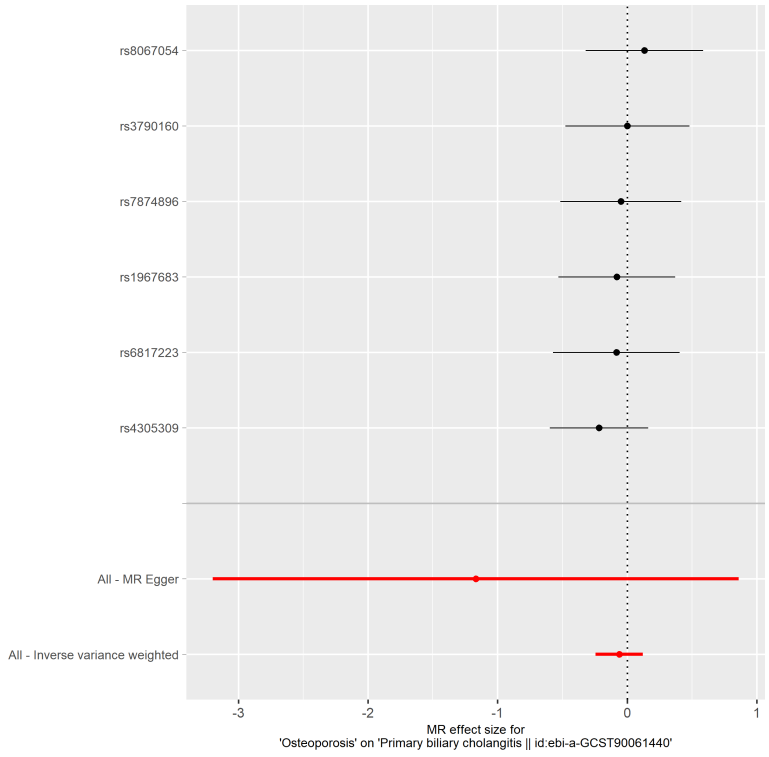

FIGURE 7
Forest plot of the effect of osteoporosis on primary biliary cholangitis.

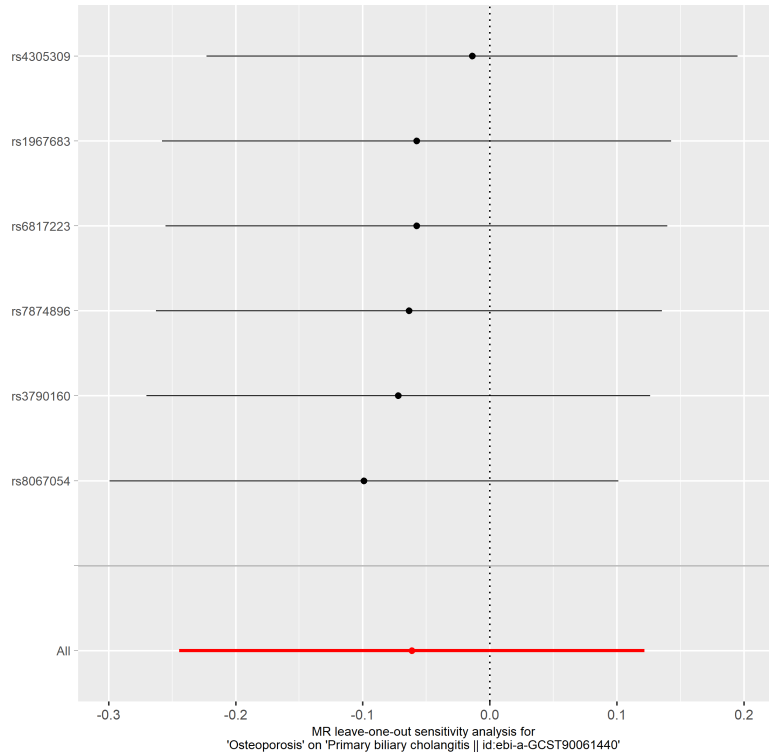

FIGURE 8
Leave-one-out plot of the effect of osteoporosis on primary biliary cholangitis.

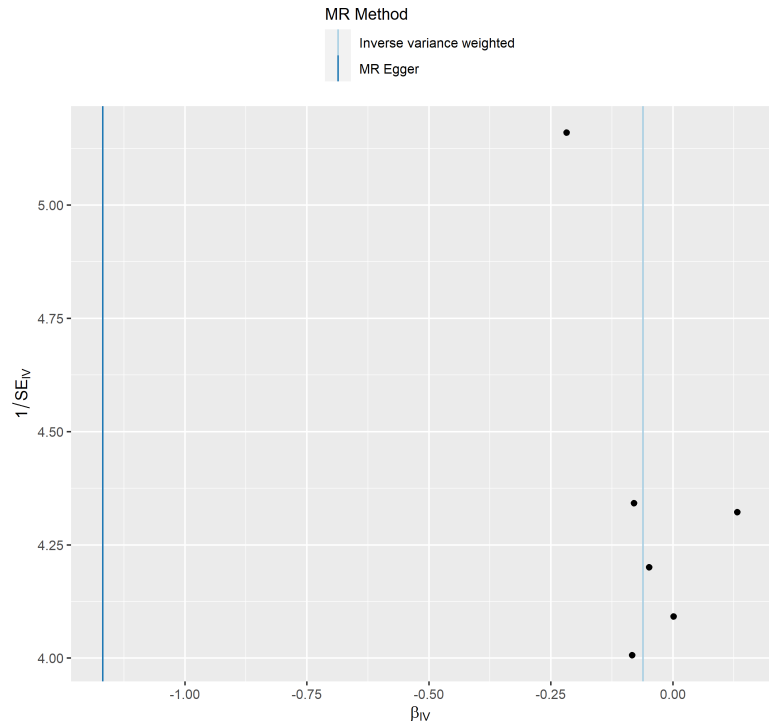

FIGURE 9
Funnel plot of the effect of osteoporosis on primary biliary cholangitis.

PBC patients raises levels of lithocholic acid and bilirubin, which can adversely affect osteoblasts and osteoblast-like cells, such as SAOS-2, through bile acid transport proteins like bile acid transport protein (BSEP) and multidrug resistance-associated protein (MRP). This impact results in reduced differentiation and mineralization, as well as increased apoptosis of these cells, contributing to osteoporosis (31). PBC also increases the risk of malabsorption and deficiencies of fat-soluble vitamins like A, D, E, and K, impacting bone metabolism and coagulation. Vitamin D is crucial for regulating bile acid metabolism and transport, inhibiting inflammation due to bile stasis, protecting bile duct cells, and suppressing liver fibrosis. Vitamin D deficiency, commonly observed in PBC patients, is associated with bone complications of the disease (32). Vitamin K acts as a coenzyme to activate bone formation proteins like osteocalcin (OC) and matrix Gla protein (MGP), maintaining bone stability and strength. A deficiency in Vitamin K can impair the function of OC and MGP, increasing the risk of osteoporosis and fractures (33). Moreover, estrogen, which inhibits osteoclasts, plays a role. PBC is more prevalent in middle-aged and older women, and post-menopausal decreases in estrogen levels can lead to excessive bone resorption and a heightened risk of osteoporosis (34).

Patients with PBC require comprehensive assessment and treatment strategies to improve their bone and muscle function and overall quality of life. The higher incidence and mortality of fractures observed in PBC patients underscore the need for early bone density testing to mitigate the risk of osteoporosis and fractures (10). Parés A, Guañabens N, et al. have also emphasized the importance of regular bone density monitoring and the supplementation of calcium and vitamin D in PBC patients (35). As PBC progresses, the associated bone loss intensifies, necessitating drug treatments that address both PBC and osteoporosis. Calcium and vitamin D supplements can be beneficial for bone health in PBC patients. However, the treatment of osteoporosis in this group is not well-explored, and the effectiveness of current medications remains uncertain. A systematic review and meta-analysis indicated that hormone replacement therapy and other drugs like calcitriol do not significantly reduce fracture risk or increase bone density in PBC patients (36), as the pathogenesis of PBC-related osteoporosis differs from that of postmenopausal osteoporosis, primarily stemming from reduced bone formation.

This study has limitations that should be addressed in future research. Firstly, it utilized GWAS data from European populations, limiting the generalizability of the results to other ethnicities or populations. Secondly, gender may significantly influence PBC and osteoporosis, as both conditions are more prevalent in middle-aged and elderly women. Due to the data sets' limited sample size, this study did not stratify by gender and age, potentially introducing confounders such as gender and age. Future studies could address these issues with larger, more diverse sample sizes and stratification by gender and age. Despite these limitations, our study has notable strengths. Firstly, it is the first to apply MR analysis to investigate the bidirectional causality between PBC and osteoporosis. Secondly, the MR method design allows us to overcome the interference of confounding factors on results and reverse causality, which are common issues in observational studies. Our sensitivity analysis further ensures the consistency and robustness of the causal estimates and results.

The future direction for treating osteoporosis induced by PBC remains an area of active exploration, highlighting the need for drugs that can both manage PBC and protect bone health. Denosumab, a medication that blocks the binding of RANKL to RANK and inhibits osteoclasts, thereby reducing bone resorption and increasing bone strength, shows potential (37). Intriguingly, some researchers have noted high expression of RANK in the bile duct cells of PBC patients, implicating the RANKL-RANK axis in the disease's pathogenesis. This suggests that Denosumab might not only improve bone health but also prevent the progression of PBC and protect liver function (38). Another promising drug is abaloparatide, a synthetic peptide analog of parathyroid hormone-related protein. It is used for postmenopausal osteoporosis and has demonstrated superior efficacy and safety compared to teriparatide (39). Denosumab and abaloparatide may therefore be effective options for treating osteoporosis associated with PBC, but their impacts in this specific context are yet to be fully assessed and require further clinical trials for validation. Additionally, the development of new bone-forming medications tailored to PBC-related osteoporosis should be a focus of future research.

5 Conclusion

Our study concludes that PBC is a positive causal factor for the risk of osteoporosis. This association may be attributed to chronic inflammation, bile acid metabolism disorders, and vitamin D deficiency commonly seen in PBC patients. However, we did not establish a clear causal link between osteoporosis and the risk of developing PBC, indicating that osteoporosis does not appear to be a risk factor or early marker for PBC. These findings offer new perspectives and guidelines for managing the bone health of patients with PBC. We recommend regular bone density testing, calcium and vitamin D supplementation, and appropriate physical activity for these patients.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

DZ: Data curation, Methodology, Software, Writing – original draft. GL: Validation, Writing – review & editing. WB:

Conceptualization, Data curation, Investigation, Methodology, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. JT: Conceptualization, Data curation, Investigation, Project administration, Supervision, Validation, Writing – review & editing. BY: Methodology, Software, Supervision, Writing – original draft, Writing – review & editing. CH: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1269069/full#supplementary-material

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