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From portal hypertension to acute-on-chronic liver failure: New insights to pathophysiology and management of liver cirrhosis

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From portal hypertension to acute-on-chronic liver failure: New insights to pathophysiology and management of liver cirrhosis

Topic editors

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Plasma Cyclic Guanosine Monophosphate Is a Promising Biomarker of Clinically Significant Portal Hypertension in Patients With Liver Cirrhosis

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Introduction: Despite intensive research, reliable blood-derived parameters to detect clinically significant portal hypertension (CSPH) in patients with cirrhosis are lacking. As altered homeostasis of cyclic guanosine monophosphate (cGMP), the central mediator of vasodilatation, is an essential factor in the pathogenesis of portal hypertension, the aim of our study was to evaluate plasma cGMP as potential biomarker of cirrhotic portal hypertension.

Methods: Plasma cGMP was analyzed in cirrhotic patients with CSPH (ascites, $n = 39$; esophageal varices, $n = 31$), cirrhotic patients without CSPH ($n = 21$), patients with chronic liver disease without cirrhosis ($n = 11$) and healthy controls ($n = 8$). cGMP was evaluated as predictor of CSPH using logistic regression models. Further, the effect of transjugular intrahepatic portosystemic shunt (TIPS) placement on plasma cGMP was investigated in a subgroup of cirrhotic patients ($n = 13$).

Results: Plasma cGMP was significantly elevated in cirrhotic patients with CSPH compared to cirrhotic patients without CSPH [78.1 (67.6–89.2) pmol/ml vs. 39.1 (35.0–45.3) pmol/l, $p < 0.001$]. Of note, this effect was consistent in the subgroup of patients with esophageal varices detected at screening endoscopy who had no prior manifestations of portal hypertension ($p < 0.001$). Cirrhotic patients without CSPH displayed no significant elevation of plasma cGMP compared to patients without cirrhosis ($p = 0.347$) and healthy controls ($p = 0.200$). Regression analyses confirmed that cGMP was an independent predictor of CSPH (OR 1.042, 95% CI 1.008–1.078, $p = 0.016$). Interestingly, portal decompression by TIPS implantation did not lead to normalization of plasma cGMP levels ($p = 0.101$).

Conclusions: Plasma cGMP is a promising biomarker of CSPH in patients with cirrhosis, especially with respect to screening for esophageal varices. The lacking

normalization of plasma cGMP after portal decompression suggests that elevated plasma cGMP in cirrhotic portal hypertension is mainly a correlate of systemic and splanchnic vasodilatation, as these alterations have been shown to persist after TIPS implantation.

Keywords: cyclic guanosine monophosphate, liver cirrhosis, portal hypertension, varices, transjugular intrahepatic portosystemic shunt (TIPS)

INTRODUCTION

The development of clinically significant portal hypertension (CSPH) is a milestone in the disease progression of liver cirrhosis as it underlies numerous complications such as variceal bleeding, ascites and hepatorenal syndrome and is associated with significantly reduced survival (1). Accordingly, diagnosis of CSPH is of great prognostic relevance. The gold standard for the diagnosis of portal hypertension is invasive hepatic venous pressure gradient (HVPG) measurement (2). Despite intensive research on alternative, non-invasive tools to detect CSPH, no reliable blood-derived parameters or scoring systems for this purpose could be implemented into clinical care so far (3). Several studies in the animal model have demonstrated that altered homeostasis of cyclic guanosine monophosphate (cGMP), the central mediator of vasodilatation, is a substantial pathomechanism of cirrhotic portal hypertension: While intrahepatic cGMP activity is decreased, cGMP activity is increased in extrahepatic blood vessels, contributing to the state of sinusoidal constriction and systemic and splanchnic vasodilatation pathognomonic for advanced liver cirrhosis (4–7). These data suggest that altered plasma cGMP levels could be an indicator of the presence of portal hypertension in patients with cirrhosis. Therefore, the aim of our study was to investigate alterations of plasma cGMP in different stages of chronic liver disease and to evaluate cGMP as a potential biomarker of CSPH.

MATERIALS AND METHODS

Patient Selection

In total, 110 participants were enrolled in the study. This included 70 cirrhotic patients with CSPH: 39 patients had ascites and 31 patients had esophageal varices. All varices patients were free of ascites at the time of study inclusion and in the past. Twelve of the 31 patients had a history of variceal bleeding, while in 19 patients varices were not previously known, but detected during screening endoscopy (with no bleeding at the time of diagnosis). Further, 21 cirrhotic patients without CSPH, 11 patients with chronic liver disease without liver fibrosis or cirrhosis and eight healthy controls were included. Patients were recruited during in- or out-patient treatment at the University Medical Center Freiburg, Germany, between 06/2017 and 12/2019.

Diagnosis of liver cirrhosis was based on distinct sonographic, clinical and laboratory findings. Liver function was assessed using the Child-Pugh score and the Model of End Stage Liver Disease (MELD). In patients with CSPH, the presence of ascites was confirmed by sonography and the presence of clinically relevant varices according to the Baveno VI consensus definition (medium or large varices requiring treatment by non-selective betablockers or endoscopic band ligation) was assessed by endoscopy (8). In cirrhotic patients without CSPH, the absence of varices and ascites was verified by means of endoscopy and sonography and the medical records were reviewed to exclude a history of varices or ascites. In patients with chronic liver disease without liver fibrosis this was confirmed by sonography and transient elastography (liver stiffness < 6.5 kPa). Apart from chronic liver disease, patients had no other severe cardiovascular, respiratory, renal or metabolic conditions.

Assessment of CGMP Levels

Venous blood samples were obtained from all participants at the time of study inclusion. Blood samples were centrifuged immediately and plasma was stored at $-80°C$ until cGMP measurement. In 13 patients with cirrhosis, additional blood samples were obtained between one and 12 months after implantation of a transjugular intrahepatic portosystemic shunt (TIPS) to study the effects of portal decompression.

CGMP levels were determined in plasma using an enzyme-linked immunosorbent assay (ELISA) by Research & Diagnostic Systems Inc., MN, US (KGE003). Sample preparation and conduction of the assay were performed according to the manufacturer's specifications.

Patients' Consent and Ethics Approval

All patients gave written informed consent to their participation. The study was approved by the local ethics committee of the University of Freiburg, Germany, (no. EK 85/19) and is in accordance with the Declaration of Helsinki.

Statistical Analyses

The study was a comprehensive analysis of plasma cGMP levels of patients in different stages of chronic liver disease and portal hypertension. Categorical variables are expressed as absolute and relative frequencies, continuous variables as median with interquartile range. In the absence of a Gaussian distribution of the data, differences between patient groups were assessed by

Abbreviations: APRI, aspartate-aminotransferase to platelet ratio index; cGMP, cyclic guanosine monophosphate; CSPH, clinically significant portal hypertension; HVPG, hepatic venous pressure gradient; MELD, Model of End Stage Liver Disease; PC/SD, platelet count/spleen diameter; PSG, portosystemic pressure gradient; TIPS, transjugular intrahepatic portosystemic shunt.

TABLE 1 | Patient characteristics.

	Cirrhosis with CSPH (n = 70)		Cirrhosis no CSPH (*n* = 21)	No cirrhosis (*n* = 11)	Controls (*n* = 8)
	Ascites (*n* = 39)	Varices (*n* = 31)			
Age [years]	60 (56–72)	60 (55–66)	61 (54–67)	47 (41–63)	48 (42–58)
Sex					
Male	28 (71.8)	21 (67.7)	15 (71.4)	7 (63.6)	4 (50.0)
Female	11 (28.2)	10 (32.3)	6 (28.6)	4 (36.4)	4 (50.0)
Etiology					
Viral	6 (15.4)	6 (19.4)	15 (71.4)	8 (72.7)	
Alcoholic	29 (74.4)	16 (51.6)	1 (4.8)		
Other	4 (10.2)	9 (29.0)	5 (23.8)	3 (27.3)	
Child-Pugh					
A	4 (10.3)	20 (64.5)	18 (85.7)		
B	26 (66.7)	9 (29.0)	3 (14.3)		
C	9 (23.1)	2 (6.5)	0		
MELD	11 (8–13)	10 (8–14)	7 (7–8)		
Platelets [10^3/μl]	118 (83–161)	84 (54–119)	124 (98–190)	238 (221–270)	
INR	1.2 (1.1–1.2)	1.2 (1.1–1.3)	1.0 (1.0–1.1)	1.0 (0.9–1.0)	
Bilirubin [mg/dl]	1.0 (0.7–2.2)	1.3 (0.9–2.3)	0.6 (0.4–1.0)	0.5 (0.4–0.7)	
Albumin [g/dl]	3.1 (2.8–3.3)	4.2 (3.6–4.3)	4.6 (4.2–4.8)	4.6 (4.4–4.7)	
Creatinine [mg/dl]	1.2 (0.9–1.7)	0.8 (0.7–1.1)	0.9 (0.8–1.1)	1.0 (0.8–1.1)	
AST (U/l)	47 (36–72)	46 (34–63)	46 (30–58)	27 (22–39)	
ALT (U/l)	24 (20–35)	35 (28–42)	43 (28–63)	41 (25–85)	
Spleen diameter [mm]	130 (120–160)	150 (130–180)	125 (110–145)	110 (90–120)	
Lok index	1.51 (0.98–2.51)	2.11 (1.31–2.59)	0.63 (−0.42–1.07)	−1.63 (−2.21–1.20)	
APRI	0.71 (0.46–1.13)	1.26 (0.58–2.15)	0.53 (0.42–1.08)	0.23 (0.16–0.33)	
PC/SD ratio	804 (626–1.309)	590 (376–1.032)	946 (718–1.736)	2,164 (2046–2.700)	

APRI, aspartate-aminotransferase to platelet ratio index; ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; CSPH, clinically significant portal hypertension; INR, international normalized ratio; MELD, Model of End Stage Liver Disease; PC/SD ratio, platelet count/spleen diameter ratio.

Chi square tests for categorical variables and by Mann Whitney U, Wilcoxon rank sum or Kruskal Wallis tests, as applicable, for continuous variables. Predictors of CSPH were evaluated by fitting uni- and multivariable logistic regression models. Demographic data, etiology of liver disease and the MELD score as measure of liver function were included in the models. Further, the Lok index and the aspartate-aminotransferase to platelet ratio index (APRI) as fibrosis scores were included, as they showed good performance in the detection of CSPH previously (9). Due to the limited number of patients, the scores were entered into multivariable regression separately in order minimize bias by interactions. Further, the platelet count/spleen diameter (PC/SD) ratio was included (10). A p value of < 0.05 was considered significant.

RESULTS

Patient Characteristics

Table 1 summarizes patient characteristics. Cirrhotic patients with and without CSPH were of comparable age and gender distribution with a median age of 60 (55–67) and 61 (54–67) years ($p = 0.925$) and a majority of 49 (70.0%) and 15 (71.4%) males, respectively ($p = 0.900$). As to be expected, patients with CSPH had more advanced liver disease, highlighted by a MELD score of 11 (8–14) in comparison to patients without CSPH with a MELD score of 7 (7–8; $p = 0.007$). Alcoholic liver disease was the leading etiology in patients with CSPH ($n = 45$, 64.3 %), while cirrhotic patients without CSPH mostly had viral liver disease ($n = 15$, 71.4 %).

Elevated Plasma cGMP Levels in Patients With Clinically Significant Portal Hypertension

Plasma cGMP was significantly elevated in cirrhotic patients with CSPH in comparison to cirrhotic patients without CSPH [78.1 (67.6–89.2) pmol/ml vs. 39.1 (35.0–45.3) pmol/l, $p < 0.001$]; **Figure 1**. There was no significant difference in cGMP levels between cirrhotic patients without CSPH compared to patients with chronic liver disease without liver cirrhosis [40.3 (39.7–46.3) pmol/l, $p = 0.347$] or healthy controls [35.0 (32.9–39.1) pmol/l, $p = 0.200$]. The elevation in plasma cGMP was independent of the manifestation of portal hypertension,

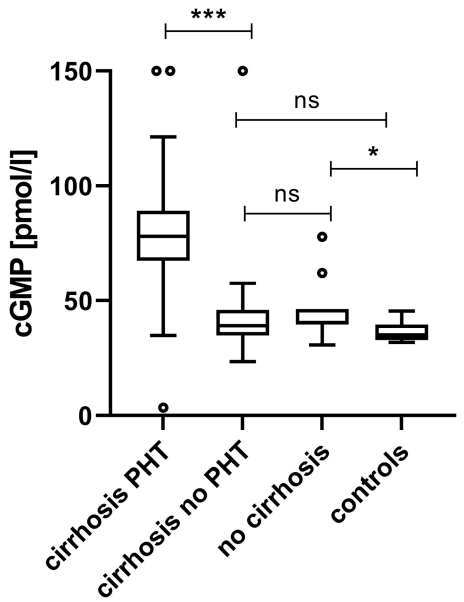

FIGURE 1 | Plasma cGMP in patients in different clinical states of chronic liver disease and clinically significant portal hypertension. Plasma cGMP was significantly elevated in cirrhotic patients with CSPH in comparison to cirrhotic patients without CSPH [78.1 (67.6–89.16) pmol/ml vs. 39.1 (35.0–45.3) pmol/l, $p < 0.001$]. There was no significant difference in cGMP levels between cirrhotic patients without CSPH compared to patients with chronic liver disease without liver cirrhosis [40.3 (39.7–46.3) pmol/l, $p = 0.347$] or healthy controls [35.0 (32.9–39.1) pmol/l, $p = 0.200$]. For better visualization cGMP measurements of two patients with CSPH (320.1 pmol/ml and 249.6 pmol/l) and one cirrhotic patient without CSPH (249.2 pmol/l) are plotted at 150 pmol/l. ***$p < 0.001$; *$p < 0.05$; ns, not significant.

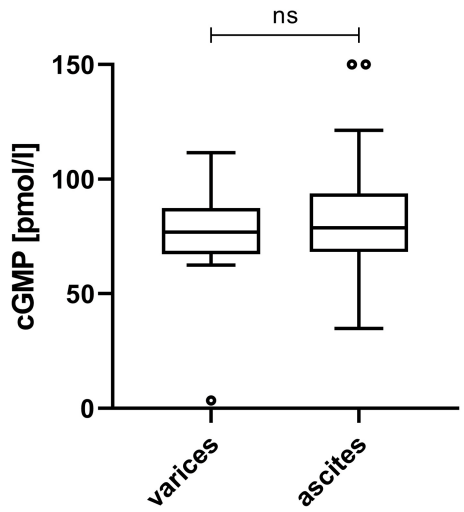

FIGURE 2 | Plasma cGMP in cirrhotic patients with varices and ascites. Plasma cGMP levels were independent of the manifestation of portal hypertension, as there was no significant difference between varices patients with varices and patients with ascites [76.9 (70.3–93.1) pmol/ml vs. 78.9 (69.1–93.0) pmol/l, $p = 0.537$]. For better visualization cGMP measurements of two ascites patients (320.1 pmol/ml and 249.6 pmol/l) are plotted at 150 pmol/l. ns, not significant.

as there was no significant difference between patients with varices and patients with ascites [76.9 (70.3–93.1) pmol/ml vs. 78.9 (69.1–93.0) pmol/l, p = 0.537]; **Figure 2**. Of note, the subgroup of patients with esophageal varices detected during screening endoscopy without other manifestations of portal hypertension also displayed significantly elevated plasma cGMP [74.3 (67.0–85.0) pmol/l] in comparison to cirrhotic patients without CSPH ($p < 0.001$); **Figure 3**. Comparison of etiologies of liver disease among patients with CSPH revealed no significant difference in plasma cGMP between patients with alcoholic liver disease [76.8 (69.1–87.7 pmol/l)], viral liver disease [75.5 (61.6–87.0 pmol/l)] and other etiologies [87.5 (71.8–97.5) pmol/l], $p = 0.246$.

Evaluation of Plasma cGMP as Predictor of Clinically Significant Portal Hypertension

To explore the predictive effect of plasma cGMP for the presence of CSPH and to adjust for differences between patient groups, potential predictors of portal hypertension were included in a regression model. Multivariable regression demonstrated that plasma cGMP indeed was an independent predictor of CSPH (OR 1.042, 95% CI 1.008–1.078, $p = 0.016$), besides viral liver disease (OR 0.032, 95% CI 0.003–0.415, $p = 0.008$) and the Lok index (OR 1.650, 95% CI 1.006–2.708, $p = 0.047$); **Table 2**.

Effects of Non-selective Beta Blocker Treatment and Transjugular Intrahepatic Portosystemic Shunt Placement on Plasma cGMP

Of the 12 included patients with a history of variceal bleeding, seven patients (58.3%) received treatment with non-selective beta blockers (NSBBs) for secondary prophylaxis of variceal hemorrhage. Comparison with the five patients (41.7%) without NSBB treatment showed no significant difference in plasma cGMP between the groups [84.7 (65.1–87.5) pmol/l vs. 85.1 (75.9–87.1), $p = 0.999$].

The effect of portal decompression on plasma cGMP was studied in 13 cirrhotic patients who underwent TIPS implantation. The patients' pre-TIPS portosystemic pressure gradient (PSG) was 20 (19–28) mmHg. Graphical exploration revealed no unequivocal link of plasma cGMP to pre-TIPS PSG measurements; **Supplementary Figure 1**. TIPS placement reduced the patients' PSG to 11 (10–12) mmHg. Following TIPS implantation, plasma cGMP showed a decrease in 10 out of 13 patients (76.9 %); **Figure 4**. However, the intra-individual changes in cGMP levels were not significant ($p = 0.101$).

DISCUSSION

Besides fibrotic re-modeling of the liver tissue, impaired vasotonus regulation is the most important factor in the

pathogenesis of portal hypertension in liver cirrhosis (11). Various studies in the animal model of portal hypertension have demonstrated decreased hepatic cGMP activity with reflectively increased splanchnic and systemic cGMP activity (4–7). These alterations are believed to be a major contributing factor to the state of profuse hepatic vascular resistance and hyperdynamic splanchnic and systemic circulation that characterizes cirrhotic portal hypertension (12, 13). This pathophysiological background suggests that cGMP could be a biomarker of portal hypertension. As the diagnosis of CSPH in patients with cirrhosis is of great prognostic relevance, means to detect and monitor CSPH foregoing the invasive gold standard of HVPG measurement are subject to intensive research. First, a variety of promising instrument-based parameters such as transient elastography or magnetic resonance imaging have been investigated in this context (14–16). Second, different blood-derived parameters and scoring systems have been evaluated (9, 17). In comparison to instrument-based methods, a broad availability and uncomplicated conduction may be considered potential benefits of blood-derived tests. However, no reliable blood-derived parameters could be incorporated into clinical routine so far (3). Against this background, we set out to investigate alterations of plasma cGMP in chronic liver disease with special focus on evaluating its potential as a biomarker of CSPH.

Indeed, we observed significantly increased plasma cGMP in patients with cirrhosis who had CSPH compared to cirrhotic

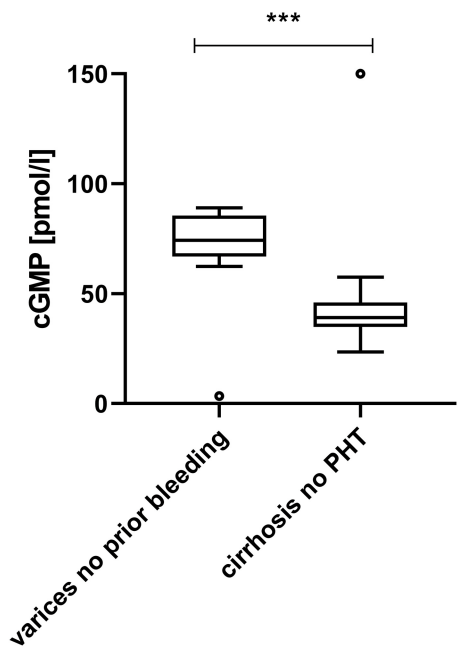

FIGURE 3 | Plasma cGMP in patients with varices diagnosed at screening endoscopy in comparison to cirrhotic patients without clinically significant portal hypertension. Patients with esophageal varices detected by screening endoscopy without other manifestations of portal hypertension displayed significantly elevated plasma cGMP in comparison to cirrhotic patients without CSPH [74.3 (67.0–85.0) pmol/l vs. 39.1 (35.0–45.3) pmol/l, $p < 0.001$]. For better visualization the cGMP measurement of one patient without CSPH (249.2 pmol/l) is plotted at 150 pmol/l. ***$p < 0.001$.

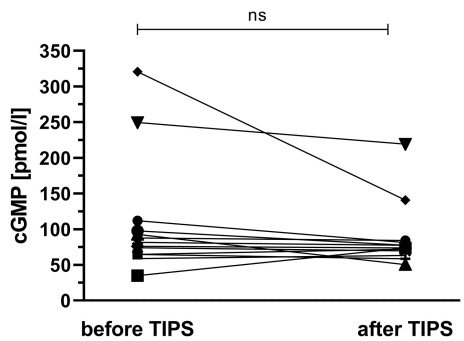

FIGURE 4 | Effect of transjugular intrahepatic portosystemic shunt placement on plasma cGMP. Following TIPS implantation, plasma cGMP showed a decrease in 10 out of 13 patients (76.9 %). However, the intra-individual changes in cGMP levels did not reach significance ($p = 0.101$). ns, not significant.

TABLE 2 | Logistic regression models of predictors of clinically significant portal hypertension.

	Univariable regression			Multivariable regression		
Parameters	**OR**	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**
Male gender	1.256	0.550–2.872	0.588			
Age	1.043	1.006–1.081	0.022			
Viral liver disease	0.153	0.063–0.370	<0.001	0.032	0.003–0.415	0.008
MELD	1.166	0.977–1.392	0.089			
cGMP	1.076	1.047–1.106	<0.001	1.151	1.058–1.252	0.001
PC/SD ratio	0.999	0.998–0.999	<0.001			
Lok index	1.706	1.230–2.366	0.001	1.650	1.006–2.708	0.047
APRI	2.842	1.202–6.716	0.017			

APRI, aspartate-aminotransferase to platelet ratio index; cGMP, cyclic guanosine monophosphate; MELD, Model of End Stage Liver Disease; PC/SD ratio, platelet count/spleen diameter ratio.

patients without CSPH ($p < 0.001$). Logistic regression analyses adjusting for factors such as liver function confirmed that plasma cGMP was an independent predictor of CSPH in the patient collective investigated. These results are in conformity with previous reports of elevated plasma cGMP in patients with cirrhosis and CSPH in smaller patient collectives (18–21). In contrast to previous studies, we systematically investigated patients in different clinical states of portal hypertension. Here, we observed that plasma cGMP was not linked to the manifestation of portal hypertension, as there was no significant difference between patients with varices and patients with ascites. Further sub-analyses revealed that the group of patients with varices detected during screening endoscopy and no prior manifestations of portal hypertension also displayed significantly elevated cGMP levels in comparison to cirrhotic patients without portal hypertension ($p < 0.001$). Naturally, prediction of CSPH by non-invasive markers is most relevant in this early stadium of portal hypertension in which the condition has not yet been unmasked by variceal hemorrhage or the development of ascites. Hence, this finding suggests that cGMP could be a valuable parameter in screening for esophageal varices. Another important aspect of our study is that we also incorporated patients with chronic liver disease without liver fibrosis and healthy controls. Notably, their plasma cGMP levels were not significantly different from those of cirrhotic patients without portal hypertension. This finding supports the conclusion that elevated plasma cGMP is indeed primarily related to the development of portal hypertension and not to liver cirrhosis alone. Further, we studied the effects of TIPS placement on plasma cGMP in a subset of cirrhotic patients. Here, we observed no significant decrease in plasma cGMP after TIPS implantation. Prior studies have shown that while the portosystemic shunt offers effective portal decompression, it does not resolve the state of systemic vasodilatation characteristic of cirrhotic portal hypertension (22–24). Considering this aspect, the persistent elevation of plasma cGMP after TIPS insertion suggests that altered plasma cGMP in cirrhotic portal hypertension is mainly a correlate of systemic vasodilatation. In any case, this finding argues against a usefulness of cGMP for monitoring the absence or recurrence of portal hypertension after TIPS implantation on a pathophysiological basis. However, future studies in larger patient collectives should investigate the relation between response of plasma cGMP and clinical response following TIPS placement. Furthermore, the effect of treatment with NSBBs on plasma cGMP was studied in patients with a history of variceal bleeding. Comparison of patients who received NSBBs for secondary prophylaxis of variceal hemorrhage to patients without a NSBB medication revealed no significant difference in plasma cGMP between the patient groups. Importantly, in patients with NSBB treatment no plasma cGMP measurements prior to commencement of NSBB therapy were available as reference in the present study, so an impact of NSBBs on plasma cGMP cannot be excluded on the basis of the present results. Another aspect that needs to be addressed is the impact of co-morbidities on plasma cGMP. For example, elevated plasma cGMP levels have been described in patients with congestive heart failure (25). To minimize bias regarding this aspect we only included patients who had no severe internistic co-morbidities. However, future studies will have to consider the influence of co-morbidities when evaluating the specificity of elevated plasma cGMP for the prediction of CSPH in patients with cirrhosis.

Our study has some limitations that need to be discussed: We incorporated 110 patients and controls in our analysis, which was a sufficiently high number to detect significant differences in plasma cGMP between patients in different stages of chronic liver disease and portal hypertension. Still, it is important to keep in mind that our results are derived from a limited number of patients in each subgroup. Another limitation of our study are inhomogeneities between patient groups. This aspect showed especially with respect to etiology of liver disease: While alcoholic liver disease was the leading etiology in patients with CSPH, patients without CSPH mostly had viral liver disease. To adjust for this fact, we applied logistic regression models. Importantly, plasma cGMP prevailed as independent predictor of CSPH in multivariable regression. Still, further studies in larger, more homogeneous patient collectives are necessary to confirm the findings of the present study and to systematically investigate if plasma cGMP levels are affected by different etiologies of liver disease.

In conclusion, the present study demonstrates that plasma cGMP homeostasis is significantly altered in cirrhotic patients with CSPH. Our results propose that cGMP could serve as a blood-derived biomarker of CSPH, especially with respect to screening for esophageal varices. Follow-up studies are necessary to evaluate plasma cGMP's diagnostic performance in the prediction of CSPH in comparison to other non-invasive parameters and scoring systems.

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 Ethics Committee University of Freiburg, Germany. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LStu, DB, and MS: study concept, design, and drafting of the manuscript. LStu, DB, LR, KZ, LSto, CG, and MS: acquisition of data. LStu, LR, DB, and MS: analyses and interpretation of data. TB, JH, MR, RK, WK, and RT: critical revision of the manuscript for important intellectual content. MS: supervision. All authors contributed to the article and approved the submitted version.

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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.2021.803119/full#supplementary-material

Supplementary Figure 1 | Plasma cGMP and portosystemic pressure gradient. The graph shows the relation of plasma cGMP levels and portosystemic pressure gradient (PSG) measurements in 13 cirrhotic patients with implantation of a transjugular intrahepatic portosystemic shunt (TIPS). For better visualization the cGMP measurement of two patients with very high plasma cGMP measurements (249.6 pmol/l and 320.1 pmol/l) are plotted at 150 pmol/l.

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DIC Score Combined With CLIF-C OF Score Is More Effective in Predicting Prognosis in Patients With Hepatitis B Virus Acute-on-Chronic Liver Failure

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Coagulation and fibrinolysis disorders are major prognostic factors in hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF) patients. Here, we aimed to clarify the role of disseminated intravascular coagulation (DIC) scores in predicting HBV-ACLF patient prognosis. We assessed the DIC score from HBV-ACLF patients at Huashan Hospital in Shanghai, China from June 2013 to May 2021 and evaluated it in relation to short-term mortality, clinical course, and infection. A novel prognostic scoring model was proposed based on DIC scores. A total of 163 transplant-free HBV-ACLF patients were enrolled. DIC scores were higher in non-survivors than survivors (6 vs. 4, $P = 0.000$) and were independently associated with short-term mortality [hazard ratio (HR): 1.397, 95% confidence interval (95% CI): 1.040–1.875, $P = 0.026$]. DIC scores were associated with ACLF grade, clinical course, and infection. Moreover, they were correlated with model for end-stage liver disease (MELD) scores ($r = 0.521$, $P < 0.001$). The area under the receiver operating curve (auROC) of CLIF-C OF-DICs [a novel prognostic score based on age, DIC score, and Chronic liver failure-consortium organ function score (CLIF-C OFs)] for 90-day mortality was 0.936, which was higher than six other generic prognostic scoring models. These results were confirmed in a validation cohort ($n = 82$). In conclusion, elevated DIC score is associated with poor prognosis in HBV-ACLF patients, and can be used jointly with CLIF-C OFs to improve the accuracy of prognosis prediction.

Keywords: prognosis, acute-on-chronic liver failure, DIC score, coagulation, prognostic score

INTRODUCTION

Acute-on-chronic liver failure (ACLF) is a syndrome characterized by acute deterioration of pre-existing chronic liver disease and associated with substantial short-term mortality (1), with an overall 28-day mortality of 30–50% and a 90-day mortality of 50–80%. In the Asian-Pacific and African regions, hepatitis B virus (HBV) infection is the main cause of ACLF, resulting

in HBV-related ACLF (HBV-ACLF). Its common precipitating factor is hepatitis B flare (2), and it presents with higher severity and mortality as well as a higher prevalence of liver and coagulation failure.

Coagulation disorders are common in ACLF, especially in HBV-ACLF, and they play a significant role in the prognosis evaluation of liver disease (3). The coagulation system consists of a coagulation promoting system and an anticoagulation system. The former mainly involves platelets and clotting factors, while the latter includes anticoagulants and the fibrinolytic system (4). In ACLF, the patient's perturbed hemostatic system is often precariously rebalanced by off-setting factors, with declines in thrombocytes, liver-derived procoagulant factors (such as factors V, VII, and X), and anticoagulant factors (especially protein C) concurrent with increases in endothelial-derived von Willebrand factor (VWF) and factor VIII. The net result is a thrombin-generating capacity comparable to or even increased relative to healthy individuals (5, 6). The international normalized ratio (INR) is the most widely used indicator of ACLF prognosis (1, 2, 7–9). It mathematically standardizes prothrombin time (PT) to allow PT results from different laboratories to be compared. However, it is accurate only for values within 1.5–4.5, and it only reflects the extrinsic coagulation pathway and does not include liver-derived anticoagulant factors (4). The disseminated intravascular coagulation (DIC) score is a classical diagnostic scoring system; it includes platelet counts, fibrin-related markers, fibrinogen, and PT (10). Consequently, it more comprehensively reflects the coagulation system than any single standard coagulation test. DIC is a syndrome characterized by widespread intravascular activation of coagulation leading to substantial fibrin deposition. It can be induced by infection (such as sepsis) or non-infection (such as severe hepatic failure) (11). DIC score has been demonstrated to be an independent predictor of organ failure and mortality in sepsis (12). The effects of sepsis on coagulation are complex and are similar to those of ACLF on hemostasis, especially when sepsis-induced DIC occurred (6, 13). However, no study has yet explored the relationship between DIC score and ACLF.

The purpose of this retrospective, single-center study was to assess the association between DIC score and HBV-ACLF patient prognosis, as well as to build a novel prognostic scoring model based on DIC score that can help with treatment decisions for HBV-ACLF patients in the clinic.

METHODS

Patients

Two cohorts were enrolled in this study. For the derivation cohort, 240 patients at Huashan Hospital, Fudan University, a tertiary hospital in Shanghai, China, from June 2013 to May 2021 who met the Asian Pacific Association for the Study for the Liver (APASL) HBV-ACLF criteria were consecutively enrolled; 77 were excluded. Sixteen patients withdrew during the third month of follow-up. The validation cohort included 82 patients treated at the Shanghai Public Health Clinical Center from May 2019 to May 2021 (detailed inclusion and exclusion criteria are described in **Figure 1** and **Supplementary Method 1**). This study was conducted in accordance with the Helsinki Declaration and was approved by the Ethical Committee of Huashan Hospital of Fudan University and the Ethical Committee of the Shanghai Public Health Clinical Center. Written informed consent was obtained from each patient or their legal representative.

Data Collection

Detailed clinical characteristics were collected from medical records or the hospital database, and these included blood parameters (coagulative function, routine blood tests, biochemical examination, alpha fetoprotein, and HBV index), history of chronic disease, potential precipitating events, history of antiviral therapy, ascites, infection, organ failure, and treatments received. Chinese Group on the Study of Severe Hepatitis B-ACLF (COSSH-ACLF) grades, European Association for the Study of the Liver-ACLF (EASL-ACLF) grades and DIC score were calculated at baseline/initial diagnosis (baseline was the first day of admission for patients who met HBV-ACLF criteria at admission; for those who didn't, baseline was the day of diagnosis) and on the final day (defined as the last day of the 28-day follow-up period or the last examination before death or discharge from the hospital). Prognostic scores, including model for end-stage liver disease (MELD) score (14), the chronic liver failure-sequential organ failure assessment (CLIF-SOFA) (1), CLIF Consortium Organ Failure score (CLIF-C OFs), CLIF Consortium ACLF score (CLIF-C ACLFs) (7), COSSH-ACLFs (2), and COSSH-ACLF IIs were calculated at diagnosis. Survival information at days 28 and 90 was collected via medical records, telephone interviews, or outpatient visits after discharge.

Definitions

The APASL criteria for ACLF are an acute hepatic insult manifesting as jaundice (serum bilirubin $\geq$ 5 mg/dL) and coagulopathy (INR $\geq$ 1.5 or prothrombin activity $<$ 40%) complicated with clinical ascites and/or hepatic encephalopathy (HE) within 4 weeks in a patient with previously diagnosed or undiagnosed chronic liver disease/cirrhosis (8). According to the updated proposals (15, 16), when the above criteria were met, patients with a history of decompensated cirrhosis were also included.

The definitions of DIC score, organ failure, infection, ascites grade, cirrhosis, COSSH-ACLF grades, EASL-ACLF grades, clinical course, chronic hepatitis B, HBV reactivation, as well as MELD, CLIF-C OFs, CLIF-C ACLFs, COSSH-ACLFs, and CLIF-SOFA score are described in **Supplementary Method 2**.

All patients received standard medical treatment (detailed information is given in **Supplementary Method 3**).

Statistical Analyses

All statistical analyses were performed using Graphpad 8.0 (Graphpad Software, San Diego, CA) and SPSS version 23 (SPSS, Chicago, IL). Categorical variables were expressed as percentages (frequencies), and continuous variables were expressed as medians (interquartile ranges). Categorical variables were compared using the Chi-squared test or Fisher's test. Continuous variables were analyzed using the Mann-Whitney *U*-test, the Kruskal-Wallis test, or the Wilcoxon matched-pairs

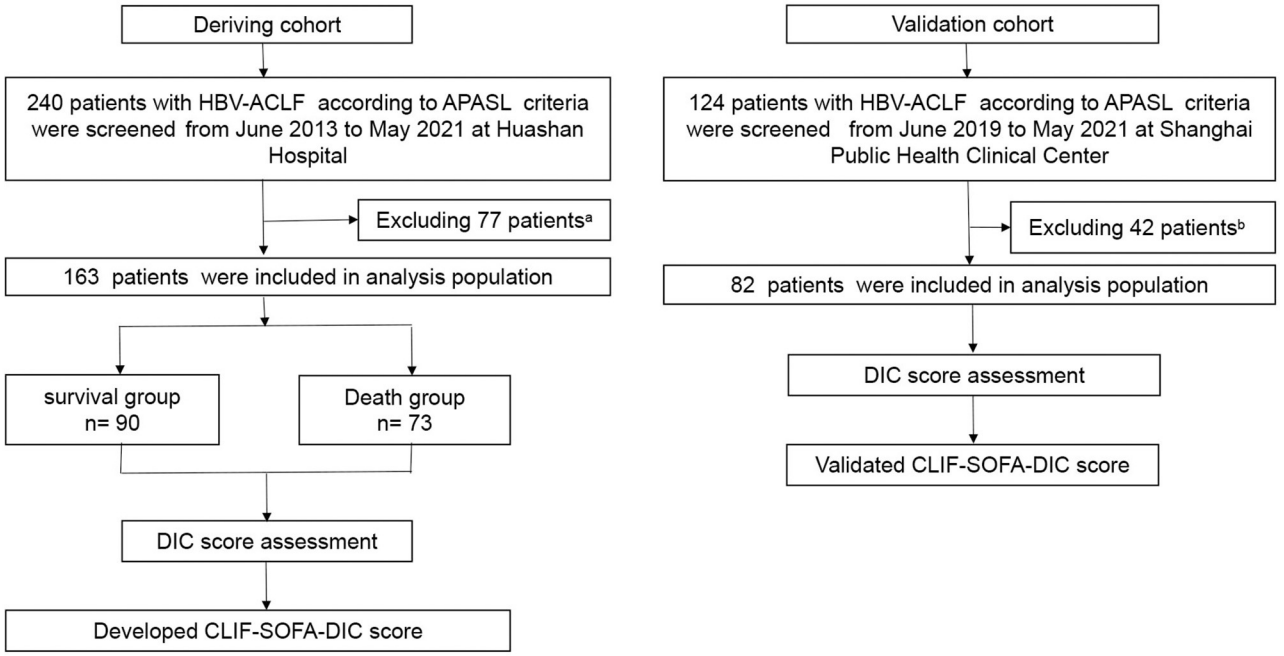

FIGURE 1 | Flow chart of the study design. [a]A total of 77 patients from the derivation cohort were excluded for one or more of the following reasons: 29 had hepatocellular carcinoma or other tumors; 12 had severe extra-hepatic diseases; 6 were receiving immunosuppressive drugs for reasons other than chronic liver diseases; 6 complicated with alcoholic hepatitis; 1 complicated with schistosomiasis cirrhosis; 2 complicated with Wilson disease; 14 had received liver transplants within 28 days; and 7 were lost to follow-up within 28 days. [b]A total of 42 patients in validation cohort were excluded for one or more of the following reasons: 13 had hepatocellular carcinoma or other tumors; 8 had severe extra-hepatic diseases; 2 were receiving immunosuppressive drugs for reasons other than chronic liver diseases; 12 complicated with alcoholic hepatitis; 1 complicated with schistosomiasis cirrhosis; 3 patients received liver transplants within 28 days; and 3 were lost to follow-up within 28 days. HBV-ACLF, hepatitis B virus-related acute-on-chronic liver failure; APASL, Asian Pacific Association for the Study of the Liver.

signed rank test, as appropriate. The association between DIC score system and MELD score was assessed using Pearson correlation coefficient. Survival probabilities based on DIC scores and CLIF-C OF-DIC scores at diagnosis were estimated using Kaplan-Meier survival curves and compared using the log-rank test. The auROC and Z-test (Delong's method) were used to compare the predictive value of different prognostic scoring models. A multivariate Cox regression analysis was performed to identify independent prognostic factors for HBV-ACLF according to the enter method. Two-tailed *P*-values were calculated, and the significance level was set at $P < 0.05$.

RESULTS

Patient Characteristics

This study included 163 patients diagnosed with HBV-ACLF according to the APASL criteria, of which 73 died within 90 days (**Figure 1**). The clinical characteristics of all enrolled patients according to their 90-day survival states are summarized in **Table 1**. The most common precipitating event was hepatitis B relapse ($n = 78$, 47.9%), followed by bacterial infection ($n = 9$, 5.5%). Moreover, liver failure was the most frequent type of organ failure ($n = 136$, 83.4%), followed by coagulation failure ($n = 61$, 37.4%). Compared to survivors, patients who died within 90 days had more complications, of which 61 (83.6%) presented with bacterial infection, 61 (83.6%) suffered from ascites, and 11 (15.1%) had complications with gastrointestinal (GI) hemorrhage. Survivors had higher alanine aminotransferase, sodium, platelet, and hemoglobin levels but had significantly lower DIC scores, INR, and occurrence of organ failure (except liver failure) and were younger.

We graded patients according to EASL-ACLF criteria and COSSH-ACLF criteria. According to EASL-ACLF criteria, 103 patients had ACLF grade 0 (63.19%), 3 patients ACLF grade 1 (1.84%), 47 patients ACLF grade 2 (28.83%), and 10 patients ACLF grade 3 (6.13%) at baseline. In Europe, the first two causes of ACLF were alcohol and hepatitis C; alternately, in the Asian-Pacific and African regions, HBV infection was the main cause of ACLF. Patients with different etiologies vary in clinical features. COSSH-ACLF criteria were proposed based on a prospective multicenter cohort of HBV-ACLF patients (2). According to COSSH-ACLF criteria, 21 patients was ACLF grade 0 (12.88%), 85 patients ACLF grade 1 (52.15%), 47 patients ACLF grade 2 (28.83%), and 10 patients ACLF grade 3 (6.13%) (**Supplementary Figure 1**).

TABLE 1 | Clinical characteristics of patients with HBV-ACLF.

	Total cohort ($n = 163$)	Survivors ($n = 90$)	No-survivors ($n = 73$)	*P*-value
Clinical data				
Age (yr)	46 (37–56)	43 (33–53)	51 (42–62)	0.000
Male sex, % (no.)	91.4 (149)	92.2 (83)	90.4 (66)	0.682
Underlying liver disease, % (no.)				0.101
Chronic hepatitis B	49.1 (80)	55.6 (50)	41.1 (30)	
Compensated cirrhosis	31.3 (51)	30.0 (27)	32.9 (24)	
Decompensated cirrhosis	19.6 (32)	14.4 (13)	26.0 (19)	
Precipitating events				0.135
HBV reactivation, % (no.)	47.9 (78)	56.6 (51)	37.0 (27)	
Bacterial infection, % (no.)	5.5 (9)	3.33 (3)	8.2 (6)	
Superimposed HAV or HEV infection, % (no.)	3.7 (6)	4.4 (4)	2.7(2)	
Hepatotoxic drugs, % (No.)	4.9 (8)	4.4 (4)	5.5 (4)	
Active drinking, % (No.)	4.3 (7)	4.4 (4)	4.1 (3)	
Unknown, % (No.)	33.7 (55)	26.6 (24)	42.5 (31)	
Complications, % (no.)				
Ascites	69.3 (113)	57.8 (52)	83.6 (61)	0.000
GI hemorrhage	7.4 (12)	1.1 (1)	15.1 (11)	0.001
Bacterial infection	62.0 (101)	44.4 (40)	83.6 (61)	0.000
Artificial liver, % (no.)	28.2 (46)	20.0 (18)	38.4 (28)	0.010
Laboratory data				
Alanine aminotransferase (U/L)	157 (72–431)	184 (92–467)	150 (57–341)	0.059
Albumin (g/L)	32 (29–36)	32 (29–36)	32 (29–37)	0.507
Total bilirubin (μmol/L)	307.1 (72.0–431.0)	284.5 (220.5–358.0)	354.0 (257.1–503.8)	0.000
Creatinine (μmol/L)	69 (58–86)	68 (57–75)	75 (59–109)	0.006
Sodium (mmol/L)	136 (132–139)	137 (134–140)	135 (130–138)	0.004
White blood cell count (10^9/L)	6.76 (5.04–10.36)	6.24 (4.51–8.37)	8.29 (5.79–10.74)	0.005
Hemoglobin (g/L)	120 (104–136)	123 (109–139)	117.5 (94–130.5)	0.024
Platelet count (10^9/L)	90 (62–121)	103 (79–133)	76 (47–101)	0.000
INR	2.11 (1.80–2.64)	1.85 (1.71–2.11)	2.65 (2.25–3.19)	0.000
DIC score	5 (4–6)	4 (4–5)	6 (5–7)	0.000
Fibrinogen (g/L)	1.17 (0.8–1.5)	1.3 (1–1.7)	0.91 (0.62–1.30)	0.000
D-dimer (mg/L FEU)	3.05 (1.42–4.92)	1.73 (0.95–3.66)	4.25 (2.91–9.54)	0.000
FDPs (mg/L)	7.5 (3.6–14.6)	4.1 (2.6–9.6)	12.9 (7.4–32.6)	0.000
Organ failure, % (no.)				
Liver	83.4 (136)	78.9 (71)	89.0 (65)	0.083
Coagulation	37.4 (61)	8.9 (8)	72.6 (53)	0.000
Kidney	14.1 (23)	4.4 (4)	26.0 (19)	0.000
Cerebral	19.6 (32)	0 (0)	43.8 (32)	0.000
Lung	8.6 (14)	0 (0)	19.2 (14)	0.000
Circulation	14.61(23)	0 (0)	31.5 (23)	0.000

The information about ascites, laboratory data was acquired at baseline while GI hemorrhage, bacterial infection, artificial liver treatment, organ failure assessment was gathered throughout the whole course within 90 days.

ACLF, acute-on-chronic liver failure; HBV, hepatitis B virus; INR, international normalized ratio; COSSH-ACLF, Chinese Group on the Study of Severe Hepatitis B-ACLF; GI, gastrointestinal; DIC, disseminated intravascular coagulation; FDPs, fibrinogen degradation products; HAV, hepatitis A virus; HEV, hepatitis E virus.

Data are expressed as the median (interquartile range) or percent (number).

All Indicators in the DIC Score System Were Correlated With Poor Prognosis

The DIC scoring system includes platelet count, a fibrin-related marker, fibrinogen, and PT. We assessed these parameters at baseline and on the final day. Patients with infection at baseline had worse DIC score system than those without infection or those that developed infection after admission (**Supplementary Figure 2**). More importantly, however, was that in both bacteria-infected and uninfected patients, PT, D-dimer, and fibrinogen degradation products (FDPs) levels were significantly higher in non-survivors than survivors.

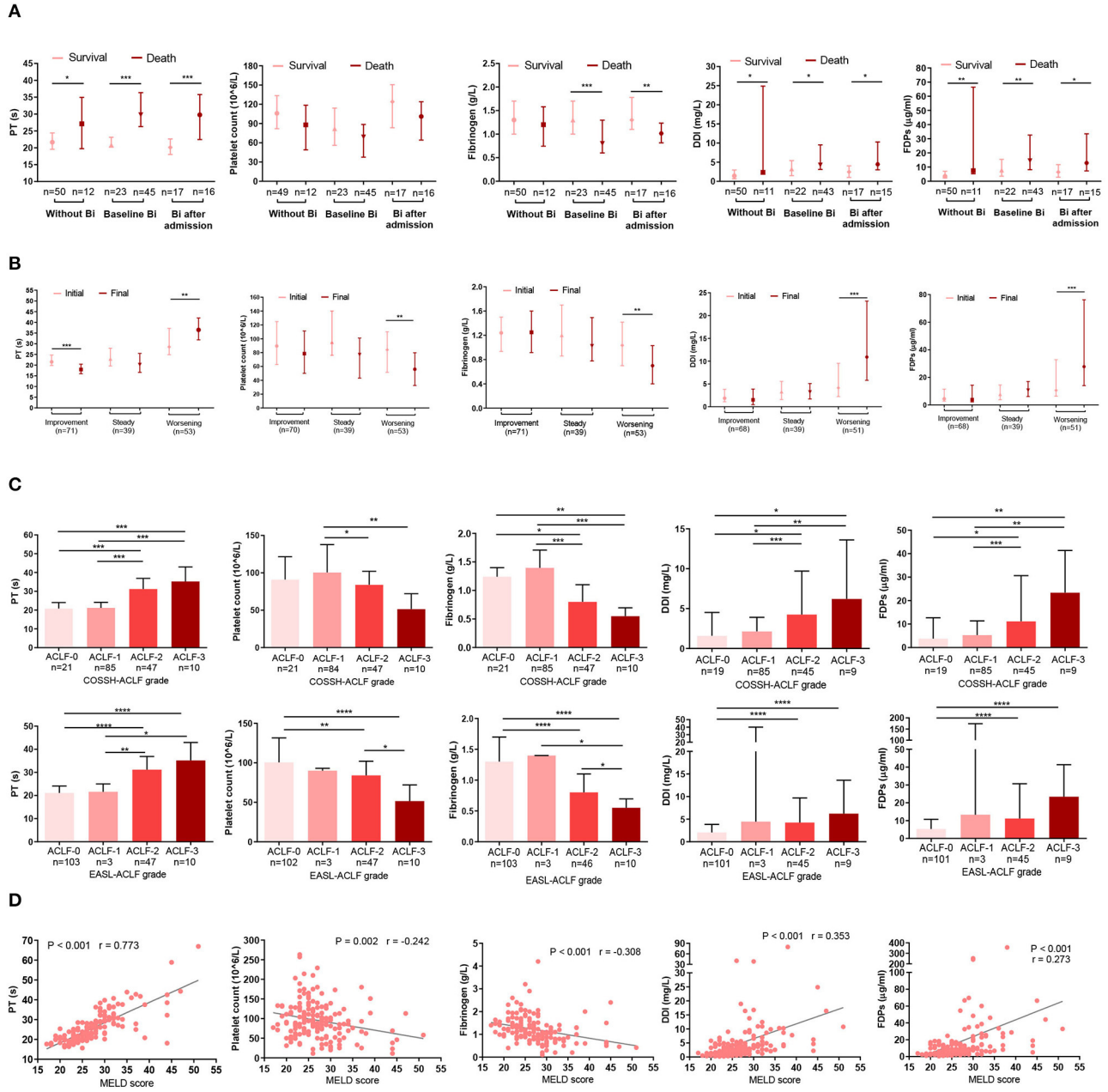

FIGURE 2 | Association between indicators in the DIC score system and prognosis. **(A)** The bar indicates the mode of DIC score indicators (platelet count, PT, DDI, FDPs, and fibrinogen) in infected or non-infected HBV-ACLF patients who survived or died. Without Bi: patients who never had infection during the whole course within 90 days; baseline Bi: patients who had infection at baseline; Bi after admission: patients without infection at baseline and developed bacterial infection within 90 days. **(B)** Changes in DIC score indicators (platelet count, PT, DDI, FDPs, and fibrinogen) according to the clinical course. Initial: the parameters were detected at baseline; final: the parameters were detected at last within 28 days of diagnosis or before death or discharge from the hospital. **(C)** Association between DIC score indicators (platelet count, PT, DDI, FDPs, and fibrinogen) and COSSH-ACLF/EASL-ACLF grade. **(D)** Correlations between DIC score indicators (platelet count, PT, DDI, FDPs and fibrinogen) and MELD score. Platelet count was missing in one patient, and five patients lacked D-dimer and FDPs information. Some patients had been treated in other hospitals before being admitted to Huashan Hospital, so some had complications with Bi upon admission. DIC, disseminated intravascular coagulation; PT, prothrombin time; DDI, D-dimer; FDPs: fibrinogen degradation products; HBV-ACLF, hepatitis B virus-related acute-on-chronic liver failure; Bi, bacterial infection; COSSH-ACLF, Chinese Group on the Study of Severe Hepatitis B-acute-on-chronic liver failure; EASL-ACLF, European Association for the Study of the Liver-ACLF; MELD, Model for end-stage liver disease. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

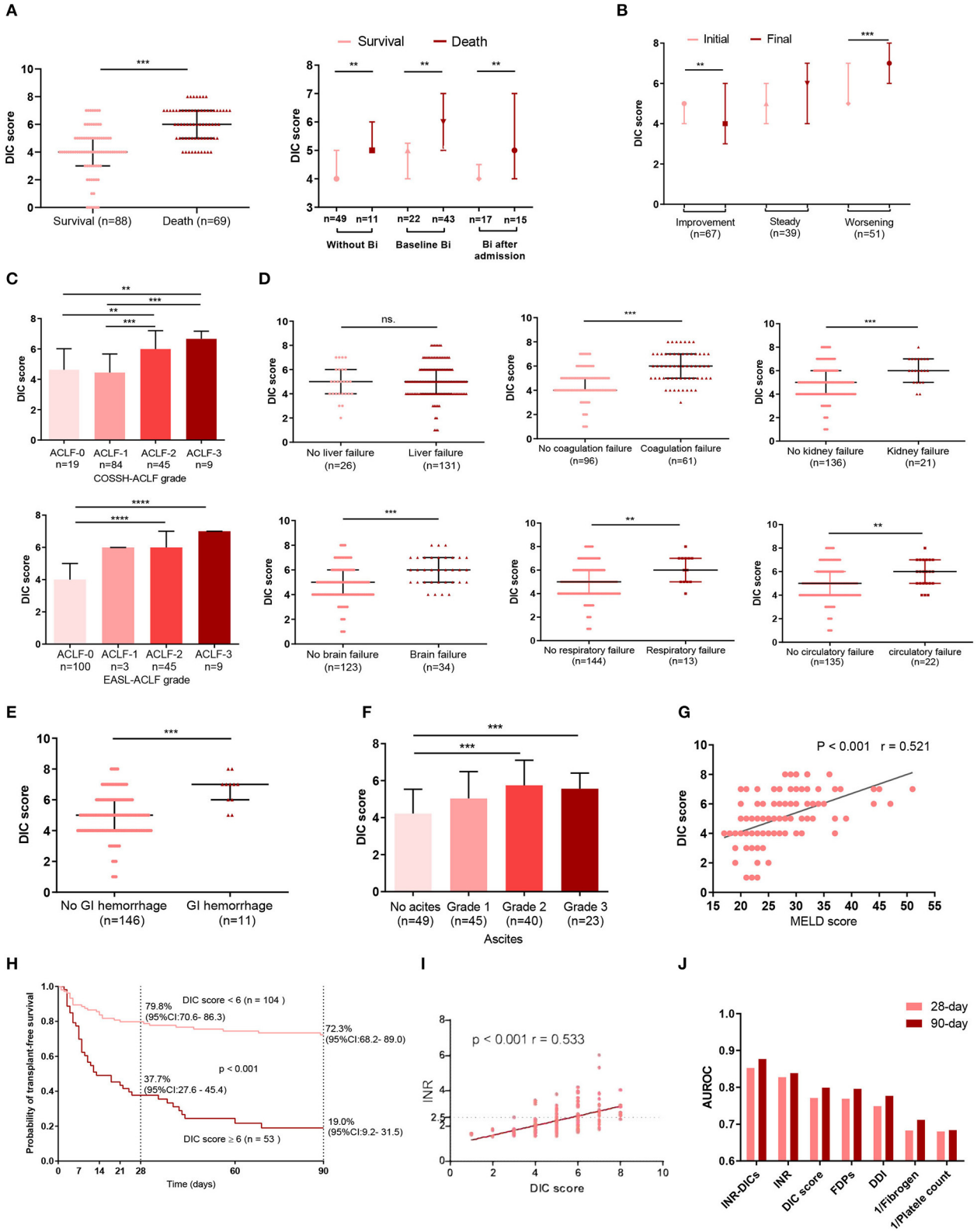

FIGURE 3 | The relationship between complications, organ failure, prognosis, INR and DIC score. **(A)** The DIC scores of ACLF patients with or without bacterial infection were sorted by survival states. Without Bi: patients who never had infection during the whole course within 90 days; Baseline Bi: patients who had infection at baseline; Bi after admission: patients without infection at baseline and developed bacterial infection within 90 days. **(B)** Dynamic changes in DIC score from initial to

(Continued)

FIGURE 3 | final assessment according to clinical course. Initial: the DIC score was assessed at baseline; Final: the last DIC score assessed within 28 days of diagnosis or before death or discharge from the hospital. **(C)** Comparisons of DIC scores among subgroups of ACLF patients according to COSSH-ACLF, and EASL-ACLF grade. The association between DIC scores and single organ failure is illustrated in **(D)**. **(E)** Comparison of DIC scores between patients with or without GI. **(F)** The relationship between ascites and DIC scores. **(G)** Correlations between DIC score and MELD score. **(H)** 28- and 90-day transplant-free survival rates of HBV-ACLF patients based on the cutoff DIC score at diagnosis. **(I)** The correlation between DIC score and INR. **(J)** The area under the receiver operating curve of the DIC score system at diagnosis for predicting the 28-day and 90-day mortality of HBV-ACLF patients. For 28-day mortality: INR-DICs, 0.853; INR, 0.828; DIC score, 0.771; FDPs, 0.769; DDI, 0.749; Fibrogen, 0.683; platelet, 0.680. For 90-day mortality: INR-DICs, 0.877; INR, 0.839; DIC score, 0.799; FDPs, 0.796; DDI, 0.777; 1/Fibrogen, 0.712; 1/platelet, 0.684. DIC, disseminated intravascular coagulation; ACLF, acute-on-chronic liver failure; BI, bacterial infection; COSSH-ACLF, Chinese Group on the Study of Severe Hepatitis B-acute-on-chronic liver failure score; GI, gastrointestinal hemorrhage; MELD, Model for End-stage Liver Disease; INR, the international normalized ratio; PT, prothrombin time; DDI, D-dimer; FDPs, fibrinogen degradation products; INR-DICs, INR combined with DIC score; HBV-ACLF, hepatitis B virus-related acute-on-chronic liver failure; EASL-ACLF, European Association for the Study of the Liver-ACLF. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

Conversely, fibrinogen and platelet count were higher in survivors (**Figure 2A**).

The dynamic changes in DIC indicators were consistent with the clinical course. Among the HBV-ACLF patients, the clinical course improved in 71, worsened in 53, and was steady in 39. Compared with the initial day, patients with a worsening clinical course on the final day had longer PTs ($P = 0.004$) and higher D-dimer ($P < 0.001$) and FDPs ($P < 0.001$) levels. However, their platelet counts ($P = 0.005$) and fibrinogen ($P = 0.004$) levels were lower (**Figure 2B**). Similarly, ACLF-3/-2 patients had prolonged PT and increased D-dimer and FDPs levels, as well as reduced fibrinogen and platelet counts than ACLF-1/-0 patients (**Figure 2C**). Finally, we analyzed the relationship between index in the DIC scoring system and MELD score. MELD scores were positively associated with D-dimers ($r = 0.353$, $P < 0.001$), FDPs ($r = 0.273$, $P < 0.001$), and PT ($r = 0.773$, $P < 0.001$) but were inversely correlated with fibrinogen ($r = -0.308$, $P < 0.001$) and platelet counts ($r = -0.242$, $P = 0.002$) (**Figure 2D**). Note that platelet count was missing in one patient, and five patients lacked information on D-dimer and FDPs.

DIC Score Were Correlated With Poor Prognosis

Considering that all DIC indicators were significantly correlated with survival, clinical course, and disease severity, we further evaluated the prognostic value of the DIC score in HBV-ACLF.

In both bacteria-infected and uninfected groups, DIC score was significantly higher in non-survivors than survivors (**Figure 3A**), and the DIC scores of patients with infection were higher than those without (**Supplementary Figure 2**). Also, patients with a worsening clinical course on the final day had higher DIC scores ($P < 0.001$) than on the initial day, and patients with an improving clinical course presented with lower DIC scores on the final day ($P = 0.008$; **Figure 3B**). Patients with more organ failures (ACLF-3/-2) had higher DIC scores (**Figure 3C**), as did those with complications such as organ failure (coagulation, kidney, cerebral, lungs, circulatotion) (**Figure 3D**), or GI hemorrhage (**Figure 3E**). Previous studies have reported that ascites contains large amounts of fibrinolytic products (17); accordingly, we found that patients with grade 2 or 3 ascites had larger DIC scores (**Figure 3F**). MELD scores were also positively associated with DIC scores ($r = 0.521$, $P < 0.001$; **Figure 3G**).

The auROC of the DIC scores for 90-day mortality was 0.799 with a sensitivity of 0.594 and specificity of 0.864 at a cut-off value of 6. Patients with DIC score< 6 at diagnosis had significantly improved short- and mid-term survival than those with DIC scores ≥ 6 (28 day: 79.8 vs. 37.7%, 90-day: 72.3 vs. 19.0%, $P < 0.001$) (**Figure 3H**).

Although DIC score performs well in predicting prognosis in HBV-ACLF, the majority of ACLF patients ($n = 142$, 87.1%) had PT extensions of more than 6 sec (PT > 6 sec are scored two points in the DIC score), so their DIC scores were mainly affected by the other three indicators (platelet counts, D-dimer and fibrinogen levels). INR, as the most widely used indicator of ACLF prognosis, is mathematically standardized PT. Thus, combining INR with DIC score may be more accurate in reflecting the status of the coagulation system in HBV-ACLF patients. Besides, r value of Pearson correlation between the INR and DIC scores was 0.533 (**Figure 3I**). To assess the prognosis predictive value of the combination of INR and DIC scores, we put DIC score and INR into a multivariate Cox regression (**Supplementary Table 1**), and obtained a new coagulation score for HBV-ACLF patients (INR-DIC score) as calculated by the following formula: INR-DICs $= 0.404 \times$ DIC score $+ 0.916 \times$ INR. Then we calulated the auROC of INR-DIC and individual makers of DIC score, and found that combined INR with DIC score had the largest auROC (28-day prognosis: 0.853; 90-day prognosis: 0.877) (**Figure 3J**).

Development of a Prognostic Score for HBV-ACLF

We performed a multivariate Cox regression to identify the most significant factors related to survival (**Supplementary Table 2**). We found age (hazard ratio [HR]: 1.031, 95% confidence interval [CI]: 1.005–1.056, $P = 0.017$), INR (HR: 2.524, 95% CI: 1.628–3.914, $P = 0.000$), HE grade (HR: 1.494, 95% CI: 1.117–1.999, $P = 0.007$), total bilirubin (HR: 1.001, 95% CI: 1.001–1.002, $P = 0.000$), and DIC scores (HR: 1.397, 95% CI: 1.040–1.875, $P = 0.026$) to be independent risk factors.

Among the identified independent predictors of death, INR, total bilirubin, and hepatic encephalopathy are components of CLIF-C OFs that are used to assess organ failure. CLIF-C OFs is a classic prognosis model widely used in ACLF. Therefore, to improve the prognostic value of CLIF-C OFs, a multivariate Cox regression including CLIF-C OFs, DIC scores and age was analyzed again (**Table 2**). We obtained a new prognostic score for HBV-ACLF patients (CLIF-C OF-DIC score) as calculated by the following formula: CLIF-C OF-DICs $= 0.679 \times$ CLIF-C OFs

TABLE 2 | Risk factors associated with transplant-free 90-day mortality in patients with HBV-ACLF according to a multivariate Cox PH model.

	Regression coefficient	HR	95%CI	*P*-value
Age (yr)	0.039	1.039	1.018–1.061	0.000
DIC score	0.344	1.410	1.148–1.733	0.000
CLIF-C OFs	0.679	1.971	1.680–2.312	0.000

ACLF, acute-on-chronic liver failure; HBV, hepatitis B virus; CLIF-C OFs, Chronic liver failure-consortium organ function score; DIC, disseminated intravascular coagulation.

$+ 0.344 \times$ DIC score $+ 0.039 \times$ Age. The auROC of the CLIF-C OF-DICs for 90-day mortality was 0.936 with a sensitivity of 84.06% and specificity of 88.64% at a cut-off value of 10.03.

We compared the prognostic value of CLIF-C OF-DICs for 28- and 90-day mortality with MELD, CLIF-SOFA, CLIF-C ACLFs, CLIF-C OFs, COSSH-ACLFs, and COSSH-ACLF-IIs and found the CLIF-C OF-DICs had the highest auROC (28-day: 0.924, 90-day: 0.936; **Figure 4A** and **Supplementary Table 3**). Using the cut-off value, patients in the derivation cohort were categorized into the high CLIF-C OF-DICs (CLIF-C OF-DICs $>$ 10.03) and low CLIF-C OF-DICs ($<$ 10.03) groups. The cumulative 28- and 90-day transplant-free survival rates of the low CLIF-C OF-DICs group were significantly higher than the high CLIF-C OF-DICs group (28-day: 93.2 vs. 30.4%, 90-day: 86.7 vs. 14.1%; $P < 0.0001$; **Figure 4B**). Besides, patients with organ failures (liver, coagulation, kidney, cerebral, lungs, circulation) had higher CLIF-C OF-DICs (**Figure 4C**), as did those with higher ACLF grade (**Figure 4D**). CLIF-C ACLFs scores were also positively associated with CLIF-C OF-DICs ($r = 0.853$, $P < 0.001$; **Figure 4E**).

External Validation of DIC Score Performance

We recruited an external cohort to validate the prognostic value of DIC score and CLIF-C OF-DICs. The clinical and laboratory characteristics of the derivation and validation cohorts are listed in **Supplementary Table 4**. The two cohorts differed on 28-day mortality, their distributions of underlying liver diseases, and baseline laboratory data, which included total bilirubin, creatinine, fibrinogen, and D-dimer levels. DIC score demonstrated similar prognostic value in an external validation group of 82 HBV-ACLF patients.

Compared with survivors, non-survivors had significantly higher DIC scores ($P = 0.006$, **Figure 5A**). The association between DIC scores and clinical course is illustrated in **Figure 5B**. DIC scores declined in HBV-ACLF patients with an improving clinical course ($P = 0.002$), remained nearly constant in patients with a steady clinical course ($P = 0.364$), and significantly increased in patients with a worsening clinical course ($P = 0.001$).

The prognostic value of CLIF-C OF-DICs in predicting 28-day mortality was comparable to six other generic prognostic score models (auROC: 0.791) and was superior in predicting 90-day mortality (auROC: 0.812) in HBV-ACLF patients, although this increase was not statistically significant (**Figure 5C**). The auROC of all scores are listed in **Supplementary Table 5**. The cumulative survival rates of the low CLIF-C OF-DICs group ($<$ 10.03) were significantly higher than the high CLIF-C OF-DICs group ($>$ 10.03) with 28-day survival rates of 90.7% and 59.3% and 90-day survival rates of 70.9% and 25.9%, respectively ($P < 0.0001$, 90-day mortality: HR: 0.255, 28-day mortality: HR: 0.184; **Figure 5D**).

DISCUSSION

This retrospective study evaluated the DIC score system in patients with HBV-ACLF and demonstrated that these subjects frequently displayed an abnormal coagulation state similar to DIC. They had prolonged PT, reduced fibrinogen, decreased platelet counts, elevated D-dimers and FDPs, and increased overall DIC scores. DIC scores system deteriorates as ACLF grade increases, and the results were consistent under different diagnostic criteria (EASL or COSSH ACLF criteria). A prolonged PT, a result of impaired liver synthesis function, has been widely recognized in ACLF patients (2). Thrombocytopenia is common in patients with advanced cirrhosis, and it is related to portal hypertension and hepatic decompensation (18). Fibrinogen, a key component of blood clots and a modest acute-phase reactant, is primarily synthesized in hepatocytes and has a shorter half-life in cirrhosis (3, 19). Fisher et al. discovered that compared to healthy controls, patients with acute decompensated cirrhosis and ACLF have lower fibrinogen levels, which in stable cirrhosis patients are a bit higher (5). The abnormally elevated D-dimer and FDPs levels in ACLF patients reflect activated coagulation and fibrinolysis, which is consistent with other studies in patients with cirrhosis and ACLF (6, 20–22). The origin of the elevated D-dimer and FDP levels is not clear. Ascites may be a source of fibrinolytic products (17), which was common in our cohort accounting for 69.3% of patients and was associated with DIC score. However, at present, no evidence of pathological microthrombosis in ACLF has been reported.

The widely activated coagulation system in HBV-ACLF patients may result from infection or a high-inflammatory state (23). Infection and sepsis are prevalent in ACLF patients and are related to their prognosis (24, 25). Up to 62.0% of patients in our cohort had bacterial infections. Hemostatic changes in ACLF and sepsis greatly overlap: they are both associated with elevated levels of VWF, D-dimer, factor VIII, thrombin-antithrombin and decreased levels of the VWF-regulating protease ADAMTS13 and coagulation factors (6). Hypercoagulable profiles and hypofibrinolysis states result in microvascular thrombosis or even DIC; they play essential roles in the pathological process of multiple organ failure in sepsis (26). Therefore, changes in the hemostasis system of ACLF patients result from multiple complex factors, which include the impairment of liver synthesis function, the consumption of coagulation factors in intravascular coagulation, and the accumulation of secondary fibrinolytic products. Furthermore, infection and systemic inflammatory response syndrome may aggravate coagulation disorders (23, 27). These results emphasize the importance of coagulation and fibrinolysis in the progression of ACLF and provide ideas for follow-up studies in their mechanisms.

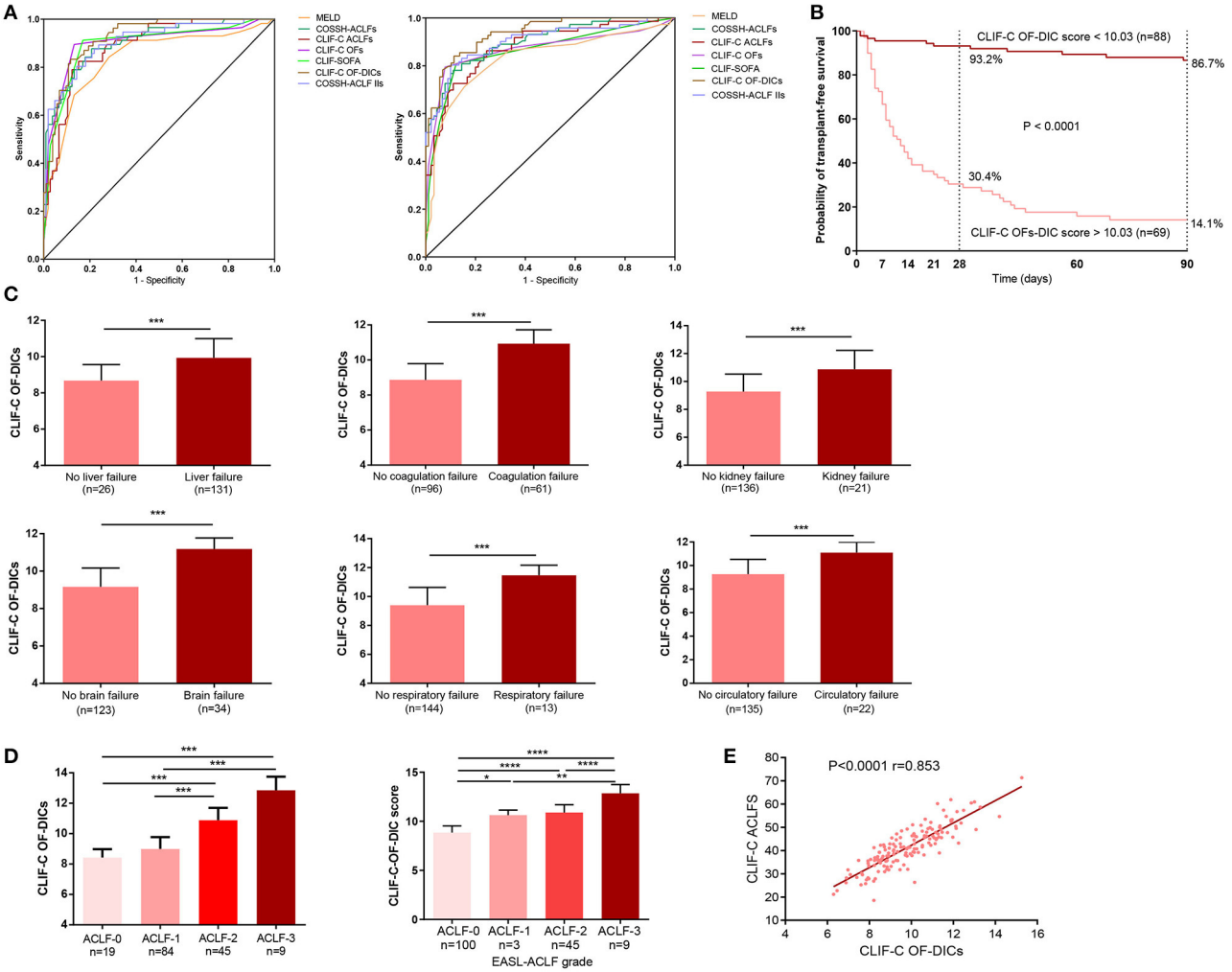

FIGURE 4 | Seven prognostic models used to predict the 28- and 90-day mortality of patients and the correlation between CLIF-C OF DICs and disease severity. **(A)** Accuracy of the CLIF-OF C-DICs as compared to MELDs, COSSH-ACLFs, CLIF-C ACLFs, CLIF-C OFs, CLIF-SOFA, and COSSH-ACLF-IIs in predicting 28-day (left) and 90-day (right) mortality of ACLF patients. The areas under the receiver operating curve were as follows. For 28-day mortality: CLI-C OF-DICs, 0.924; CLIF C-OFs, 0.900; CLIF-C ACLFs, 0.888; CLIF-SOFA, 0.895; MELD, 0.836; COSSH-ACLFs, 0.904; COSSH-ACLF-IIs, 0.905. For 90-day mortality: CLI-C OF-DICs: 0.936; CLIF C-OFs, 0.870; CLIF-C ACLFs, 0.879; CLIF-SOFA, 0.863; MELD, 0.830; COSSH-ACLFs, 0.903; COSSH-ACLF-IIs, 0.901. **(B)** Probability of 28- and 90-day transplant-free survival in ACLF patients based on the CLIF-C OF-DICs cutoff value (10.03). Kaplan-Meier curves were compared using the log-rank test. **(C)** The CLIF-C OF-DICs between patients with organ failure or not. **(D)** The CLIF-C OF-DICs in patients with different ACLF grades. **(E)** The correlation between CLIF-C OF-DICs and CLIF-C ACLFs. ACLF, acute-on-chronic liver failure; DIC, disseminated intravascular coagulation; CLIF, Chronic Liver Failure; COSSH-ACLF, Chinese Group on the Study of Severe Hepatitis B-ACLF; EASL-ACLF, European Association for the Study of the Liver-ACLF; CLIF-SOFA score, CLIF-sequential organ failure assessment score; CLIF-C OF-DICs, a novel prognostic score based on age, DIC score, and CLIF-C OFs; MELD, Model for end-stage liver disease; COSSH-ACLFs, Chinese Group on the Study of Severe Hepatitis B-ACLF score; CLIF-C OFs, CLIF-Consortium Organ Failure score; CLIF-C ACLFs, CLIF-Consortium ACLF score; COSSH-ACLF IIs, Chinese Group on the Study of Severe Hepatitis B-ACLF II score. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

The DIC score was proposed by the ISTH in 2001 to standardize DIC criteria (10). It has been applied in predicting the prognosis of sepsis, post-trauma multiple organ dysfunction syndrome, and thrombosis in acute myeloid leukemia (12, 28, 29). Here, we demonstrated that the DIC score, as an independent predictor of 90-day mortality, forms part of a novel prognostic tool in patients with HBV-ACLF. The DIC score is more comprehensive in the evaluation of coagulation, but in the DIC score, the upper limit for evaluating the PT is extended by 6 s, and most ACLF patients have a PT extension of more than 6 s. INR is the mathematical standardization of PT. Therefore, DIC score combined with INR is more effective in assessing coagulation dysfunction and prognosis in hepatitis B virus acute-on-chronic liver failure patients.

Because HBV-ACLF can deteriorate rapidly and lead to death, an accurate prognostic score can help liver transplantation decision making. CLIF-C OFs is a summation of organ failure severity that is used for ACLF diagnosis and prognosis prediction (1). To improve CLIF-C OFs, we combined DIC score with age and CLIF-C OFs to generate a new prognostic

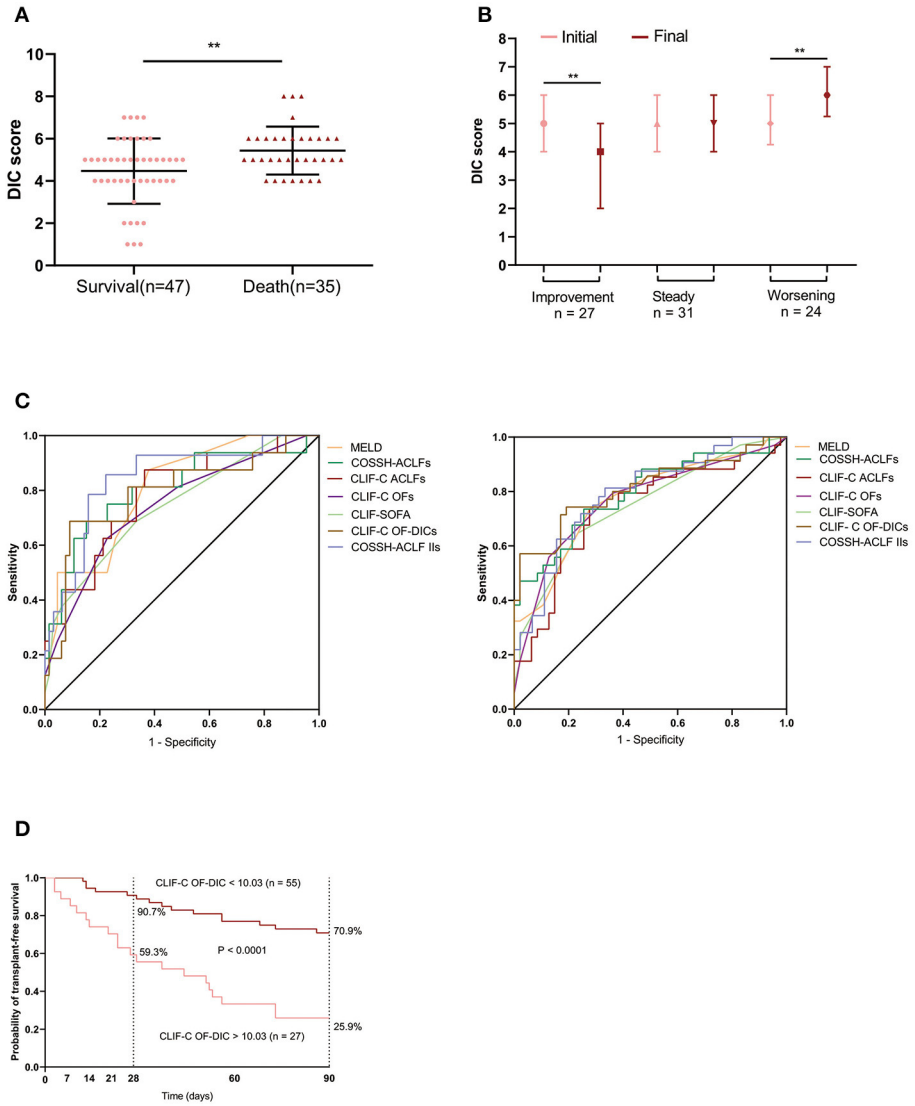

FIGURE 5 | DIC scores in external HBV-ACLF validation cohort. **(A)** The bars indicate DIC scores between patients who survived or died within 90 days. **(B)** Changes in DIC scores from initial to final assessments according to clinical course. Initial: the DIC score was assessed at baseline; final: the last DIC score assessed within 28 days of diagnosis, or before death or discharge from the hospital. **(C)** Comparison of CLIF-C OF-DICs with MELD, COSSH-ACLFs, CLIF-C ACLFs, CLIF-C OFs, CLIF-SOFA and COSSH-ACLF-IIs in predicting 28-day and 90-day mortality of ACLF patients. The areas under the receiver operating curve were as follows. For 28-day mortality: CLI-C OF-DICs, 0.791; CLIF C-OFs, 0.744; CLIF-C ACLFs, 0.784; CLIF-SOFA, 0.744; MELD, 0.804; COSSH-ACLFs, 0.804; COSSH-ACLF-IIs, 0.845. For 90-day mortality: CLI-C OF-DICs: 0.812; CLIF C-OFs, 0.767; CLIF-C ACLFs, 0.737; CLIF-SOFA, 0.741; MELD, 0.774; COSSH-ACLFs, 0.797; COSSH-ACLF-IIs, 0.790. **(D)** Probability of 28- and 90-day transplant-free survival in ACLF patients based on the CLIF-C OF-DICs cutoff value (10.03) acquired in the derivation cohort. DIC, disseminated intravascular coagulation; ACLF, acute-on-chronic liver failure; HBV-ACLF, hepatitis B virus-related ACLF; CLIF, Chronic Liver Failure; CLIF-SOFA, CLIF-sequential organ failure assessment score; CLIF-C OF-DICs, a novel prognostic score based on age, DIC score, and CLIF-C OFs; MELD, Model for end-stage liver disease; CLIF-C ACLFs, CLIF-Consortium ACLF score; CLIF-C OFs, CLIF-Consortium Organ Failure score; COSSH-ACLFs, Chinese Group on the Study of Severe Hepatitis B-ACLF score; COSSH-ACLF IIs, Chinese Group on the Study of Severe Hepatitis B-acute-on-chronic liver failure II score. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

model, CLIF-C OF-DICs, and we confirmed its predictive power in derivation and validation cohorts. These two cohorts differed in clinical characteristic and auROC, which may be related to the different patient sources and treatment strategies between the two hospitals, as well as the patients' economic situations.

This study had several limitations. The DIC score analysis and the CLIF-C OF-DICs prognostic model were based on a retrospective analysis, and the sample size was relatively low. Moreover, the patients in the current cohort were HBV-related, and the prognosis value of DIC scores in ACLF patients associated with other etiologies need to be determined. In

the future, additional prospective studies with larger patient populations should be conducted to clarify the DIC score system in ACLF patients and to explore the mechanisms underlying coagulation and fibrinolysis disorders.

In conclusion, this study demonstrated that DIC scores were correlated with short-term prognosis in HBV-ACLF. Patients with elevated DIC scores (≥6) at admission had increased risks of 28- and 90-day mortality. Monitoring the DIC score will help in predicting short-term prognosis in HBV-ACLF patients.

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/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee of Huashan Hospital of Fudan University and the Ethical Committee of the Shanghai Public Health Clinical Center. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YH and XZ made the study concept and design, statistical analysis and drafting of manuscript was done by JH and XZ. The collection of data and statistics for patient recruitment in the verification cohort was completed by XQ. The data collection of the deriving cohort by YZ and XL. Enrolling participants for deriving cohort was done by YY and PZ. Critical revision of manuscript done for important intellectual content was done by JZ and WZ. All authors contributed to the article and approved the submitted version.

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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.2022.815580/full#supplementary-material

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Quantitative Assessment of Portal Hypertension by Two-Dimensional Shear Wave Elastography in Rat Models of Nonalcoholic Fatty Liver Disease: Comparison With Four Composite Scores

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Background: Measurement of hepatic venous pressure gradients is the gold standard for assessing portal hypertension (PH) but is invasive with potential complications. We aimed to assess the performance in liver and spleen stiffness measurement (LSM and SSM, respectively) by two-dimensional shear wave elastography (2D-SWE) and composite scores including liver stiffness-spleen diameter to platelet ratio score (LSPS), platelet (PLT) count/spleen diameter ratio (PSR), aspartate aminotransferase (AST)/alanine aminotransferase ratio (AAR), and AST-to-PLT ratio index (APRI) for diagnosing PH in nonalcoholic fatty liver disease (NAFLD) rat models.

Methods: Animal models with PH in NAFLD were established in 65 rats, which then underwent 2D-SWE measurements. Morphological and biological parameters were collected for calculation of four composite scores. Correlations of noninvasive methods with portal venous pressure were evaluated by Spearman correlation analysis. The area under the receiver operating characteristic curve (AUC) was used to assess the performance of noninvasive methods in predicting PH.

Results: LSM and SSM were significantly associated with portal venous pressure ($r = 0.636$ and 0.602, respectively; all $P < 0.001$). The AUCs of LSM and SSM in the diagnosis of PH were 0.906 (95% confidence interval [CI]:0.841–0.97) and 0.87 (95% CI:0.776–0.964), respectively, and were significantly higher than those in composite scores. The AUCs for LSPS, PSR, AAR, and APRI were 0.793, 0.52, 0.668, and 0.533, respectively, for diagnosing PH. The AUCs of the combined models of LSM and SSM, LSM and PLT, SSM and PLT, and LSM, SSM and PLT were 0.923, 0.913, 0.872, and 0.923, respectively. The four combined models showed no statistical differences compared to LSM and SSM in evaluating PH (all $P > 0.05$).

Conclusions: LSM and SSM by 2D-SWE can be used as promising noninvasive parameters for diagnosing PH in NAFLD and have higher accuracy than composite scores. The combined models, compared to LSM and SSM, did not significantly improve the performance in diagnosing PH.

Keywords: nonalcoholic fatty liver disease, portal hypertension, diagnosis, noninvasive method, two-dimensional shear wave elastography

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease, with an estimated prevalence of 25% worldwide (1, 2). Notably, approximately one-quarter of NAFLD cases progress into cirrhosis in 10 years and are at an increased risk of developing portal hypertension (PH) (3). PH is defined as pathologically elevated pressure in the portal venous system (4). It can lead to several serious complications associated with advanced NAFLD, including bleeding from gastroesophageal varices, and has high morbidity and mortality (5, 6). Therefore, accurate and timely assessment of PH is crucial to improve prognosis and clinical decision-making.

Traditionally, measurement of hepatic venous pressure gradient (HVPG) remains the gold standard for diagnosing PH (7). It is, however, an invasive and costly procedure that requires a specialized angiographic interventional center as well as skillful measurement by an experienced operator, which greatly hampers its routine use in clinical practice (8). Given the drawbacks of HVPG measurement, considerable effort has been devoted to develop a noninvasive tool that can evaluate and monitor PH (9, 10).

In this regard, development of a noninvasive assessment method by elastography may offer a valuable alternative. Elastography is an imaging method that objectively evaluates tissue stiffness, and has recently been developed for assessment of liver fibrosis stage and PH (11, 12). Two-dimensional shear wave elastography (2D-SWE), a promising novel ultrasound-based elastography technique for quantitatively real-time imaging of tissue stiffness (13), has lower cost, is readily available, and simple to utilize compared with invasive methods (e.g., liver biopsy and HVPG measurement). 2D-SWE combines elastogram with conventional B-mode ultrasonography, so operators can directly visualize the liver for high-quality measurements while performing elastography (14). Importantly, 2D-SWE has high applicability in clinics, and measurements can be performed on patients with ascites (8). As such, this technique is suitable for advanced liver diseases, where PH is the main driver of prognosis (15). In particular, liver stiffness measurement (LSM) obtained by 2D-SWE has recently been demonstrated to predict the degree of fibrosis with good diagnostic performance (16–18). Our previous meta-analysis has shown that 2D-SWE has better diagnostic performance than serum fibrosis biomarkers in predicting liver fibrosis induced by chronic hepatitis B (CHB) (19). In recent years, composite scores combining LSM with other parameters, such as liver stiffness-spleen diameter to platelet ratio score (LSPS), have been developed to evaluate esophageal varices and PH (20). Platelet count (PLT)/spleen diameter ratio (PSR) has also been introduced for diagnosing esophageal varices (21).

Previously, several studies have reported that spleen stiffness measurement (SSM) by transient elastography (TE) can be used for noninvasive assessment of PH (22, 23). However, this technique has some technical limitations; for example, it cannot be used on patients with ascites, which limit its clinical application in advanced liver diseases (8). For patients with obesity or a narrow intercostal space, the applicability of TE may also be limited (15). At present, there are insufficient studies on the diagnostic efficiency of SSM obtained by 2D-SWE in the prediction of PH. Moreover, to the best of our knowledge, the value of 2D-SWE in the evaluation of PH in NAFLD has not yet been investigated and compared to the performance of composite scores.

Thus, in this study, we investigated the diagnostic performance of LSM and SSM obtained by 2D-SWE in predicting PH in NAFLD rat models and compared it with that of the four composite scores. In addition, we also studied four combined models, namely, the LSM and SSM combined model, the LSM and PLT combined model, the SSM and PLT combined model, and the LSM, SSM and PLT combined model, for the diagnosis of PH.

MATERIALS AND METHODS

Animal Model

All the experiments were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of The Second Affiliated Hospital of Fujian Medical University.

Male Sprague-Dawley rats (200–250 g) were purchased from Shanghai Laboratory Animal Center (SLAC, Shanghai, China) and housed in sterile isolated cages with a 12:12 light-dark cycle at room temperature (20–25 °C) and relative humidity of 40–60%. Eighty rats were divided into 2 groups. In total, 65 rats were randomly included in the first group and used for the experimental model of NAFLD, and provided a methionine- and choline-deficient (MCD) diet for 12 weeks (24). The MCD diet was obtained from the branch of Dyets Inc. in China (#519580; Wuxi, China). The second group (control group) consisted of 15 rats, which were provided a standard diet with sterilized food and water. NAFLD severity was histologically confirmed.

Liver and Spleen Stiffness Measured by 2D-SWE

The rats were fasted overnight before 2D-SWE measurements. LSM and SSM by 2D-SWE were performed using an Aixplorer

(Supersonic Imagine, Aix-en-Provence, France) ultrasound imaging system with an SL15-4 transducer. For the rat study, this ultrasound imaging system was set to the superficial (thyroid) imaging mode. 2D-SWE analysis was conducted by an experienced radiologist who was blinded to the results of other diagnostic tests. For 2D-SWE measurements, (1) the sampling frame was set to a 1.5 cm × 1.5 cm which was placed ∼1 cm under the liver capsule, avoiding large bile and vessels; and (2) the region of interest had a diameter of 2 mm for LSM and SSM, which was placed in the position of the homogeneous elastographic image signal for quantitative analysis (**Figure 1**). For each rat, LSM and SSM were considered reliable if the inter-quartile range (IQR)/median value was <30% (25). Five LSMs and SSMs per rat were performed on a defined site, and the median value of five readings was recorded as LSM/SSM expressed in kilopascal (kPa).

Morphological and Biological Parameters

At the time of ultrasound examination, spleen diameter was determined. Spleen diameter was defined as the maximum spleen bipolar diameter at the level of the splenic hilum (20). Biological parameters, such as PLT, red cell distribution width, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT), were also collected on the day of ultrasound examination. Four composite scores were calculated as follows: (1) LSPS = LSM (kPa) × spleen diameter (in cm)/PLT ($\times 10^9$/L); (2) PSR = PLT ($\times 10^9$/L)/spleen diameter (in cm); (3)AST/ALT ratio (AAR) = AST (IU/L)/ALT (IU/L); and (4) AST-to-PLT ratio index (APRI) = (AST/upper limit of normal for AST) × 100/PLT ($\times 10^9$/L). Of note, LSPS was calculated in our study according to the same formula described by Kim et al. (20) but by 2D-SWE instead.

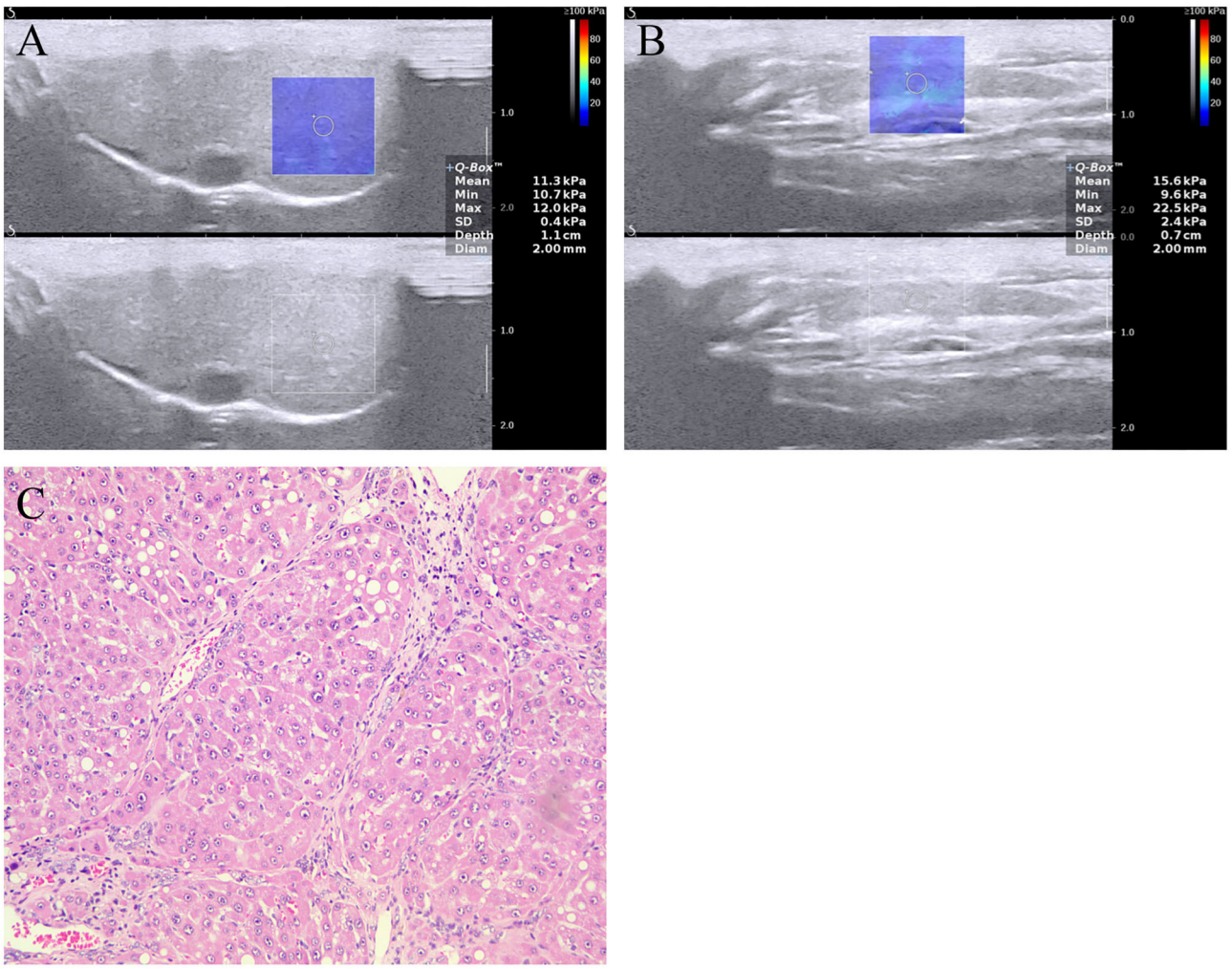

FIGURE 1 | Two-dimensional shear wave elastography (2D-SWE) measurement of the **(A)** liver and **(B)** spleen, and **(C)** image of liver section stained with hematoxylin and eosin in a rat model with NAFLD. The mean LSM and SSM were 11.3 ± 0.4 and 15.6 ± 0.7 kPa, respectively. The image of liver section revealing fibrosis stage F4 (cirrhosis). LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; SSM, spleen stiffness measurement.

Portal Venous Pressure Measurement

After the rats were fasted overnight, portal venous pressure measurement was performed immediately after 2D-SWE scan. Portal venous pressure was measured using a digital blood pressure analyzer (BL-420F; Techman Software, Chengdu, China) with computer interface. A pressure transducer module (PT-120; Techman Software, Chengdu, China) was connected to the digital blood pressure analyzer (channel 1). Before the portal venous pressure measurement, an anticoagulant citrate dextrose (ACD) solution was used to perfuse this entire setup. The ACD solution was purchased from Macklin (#A885470; Shanghai, China). Calibration of the analyzer was carried out before each reading according to the manufacturer's instructions. Then, a 12-gauge needle was used and inserted into the exposed portal vein of the rats. Finally, we continuously monitored real-time portal venous pressure and recorded it as an average reading.

Tissue Analyses

After the portal venous pressure measurement, the animals were sacrificed immediately, and liver tissues were harvested, fixed in 10% buffered formalin, and then sliced to a thickness of 5 um for staining with hematoxylin and eosin and Masson's trichrome. In this study, the sections were assessed for severity of lipid infiltration, lobular inflammation, ballooning degeneration, and fibrosis using the semiquantitative scoring system of steatosis, activity, and fibrosis (SAF) (26).

Statistical Analysis

Statistical analyses were conducted using SPSS version 26.0 (IBM, Armonk, NY, United States) and GraphPad Prism (GraphPad Prism 7.0, United States). For comparison between groups *t*-test or Mann-Whitney U test was performed when appropriate. The intra-operator reliability of 2D-SWE was assessed with intra-class correlation coefficient (ICC), with a value of >0.75 indicating excellent reliability. Moreover, the coefficient of variation (CV) was also calculated. A CV value of 10% or less was considered to indicate good reproducibility. Absolute ICC was used to test the concordance among LSM values calculated as a median of three or five measurements. Similarly, the absolute ICC was calculated to test the concordance among SSM values. Spearman correlation test was conducted in this study to evaluate the correlation between noninvasive methods and portal venous pressure. PH-positive was defined as portal venous pressure ≥5 mmHg, while PH-negative was defined as portal venous pressure <5 mmHg (4). The diagnostic performance of different noninvasive methods in predicting PH was assessed by receiver operating characteristic (ROC) curves. In addition, the four combined models, namely, the LSM and SSM combined model (combined model 1), the LSM and PLT combined model (combined model 2), the SSM and PLT combined model (combined model 3), and the LSM, SSM, and PLT combined model (combined model 4), were also explored by multivariate logistic analysis. Cutoff values were defined using the Youden index. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Comparisons of the area under the ROC curves (AUC) were performed using the DeLong test. P <0.05 was considered statistically significant.

TABLE 1 | Biological, morphological, and elastography characteristics of controls and rats with NAFLD.

Characteristic	Control	NAFLD	*P* value
n	15	51	
Platelet count, 10^9/L	787.5 ± 32.18	828.9 ± 23.41	0.419
Red cell distribution width (%)	14.7 ± 0.12	15.8 ± 0.16	0.041
ALT, IU/L	60.7 ± 2.58	145.5 ± 15.73	0.009
AST, IU/L	208.5 ± 12.34	245.8 ± 18.82	0.333
GGT, IU/L	0.90 ± 0.18	1.91 ± 0.48	0.441
Spleen diameter, cm	3.58 ± 0.01	3.81 ± 0.03	<0.001
PVP measurement, mmHg	4.80 ± 0.03	12.03 ± 0.28	<0.001
LSM, kPa	7.1 (6.6–7.4)	9.1 (7.9–11.0)	<0.001
SSM, kPa	12.8 (12.3–13.3)	15.7 (14.1–17.9)	<0.001

Data are mean ± standard deviation or median (interquartile range), or number of rats, when appropriate.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; PVP, portal venous pressure; SSM, spleen stiffness measurement.

RESULTS

Baseline Characteristics

Of all the 80 rats, 1 (1.3%) with NAFLD was excluded because of death after anesthesia before laparotomy. Then, the initial study samples included 79 rats. There are 15 controls and a total of 64 rats had NAFLD. The severity of NAFLD was histologically confirmed. The portal venous pressure measurement was performed after modeling, and all the rats with NAFLD had a portal venous pressure value >5 mmHg, indicating successful modeling. For that control rats that were provided a standard diet with sterilized food and water, all portal venous pressure values were <5 mmHg.

Table 1 shows in detail the biological, morphological, and elastography characteristic parameters observed in the rats. When compared with controls, rats with NAFLD had significantly elevated red cell distribution width ($P = 0.041$), ALT ($P = 0.009$), spleen diameter ($P < 0.001$), portal venous pressure ($P < 0.001$), LSM ($P < 0.001$), and SSM ($P < 0.001$). Between the two groups, there were no significant differences in PLT ($P = 0.419$), AST ($P = 0.333$), and GGT ($P = 0.441$).

Technical Success and Reliability of 2D-SWE for LSM and SSM

A total of 64 rats with NAFLD underwent liver and spleen 2D-SWE measurements. LSMs were successfully performed on the 64 rats (100%), and all were considered reliable (100%). However, it was successful for SSM in 51 rats (79.7%); SSM obtained by 2D-SWE failed in 8 rats (12.5%) and 5 rats were considered unreliable (7.8%) (**Table 2**). The success rate of LSM by 2D-SWE was higher than that of SSM ($P < 0.001$).

TABLE 2 | Technical success and reliability of LSM and SSM by 2D-SWE in rats with NAFLD.

Parameter	Successful	Unsuccessful	
		Failure	Nonreliable
LSM	64 (100%)*	0	0
SSM	51 (79.7%)	8 (12.5%)	5 (7.8%)

Data are expressed as number of rats, with percentages in parentheses.
**P < 0001; the success rate of LSM by 2D-SWE was higher than that of SSM.*
LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; SSM, spleen stiffness measurement; 2D-SWE, two-dimensional shear wave elastography.

There was no difference among the median LSM values in rats with NAFLD if they were calculated using three or five measurements: 9.52 (95% confidence interval [CI]: 8.77–10.27) kPa vs. 9.58 (95% CI: 9–10.17) kPa ($P = 0.904$). Similarly, no significant difference was detected among the median SSM values: 15.98 (95% CI: 14.77–17.18) kPa vs. 16.29 (95% CI: 15.32–17.27) kPa ($P = 0.68$). Between the two values calculated using three or five measurements, the concordance was perfect with an ICC of 0.941 (95% CI:0.904–0.964, $P < 0.001$) for LSM and 0.92 (95% CI:0.843–0.958, $P < 0.001$) for SSM.

The intra-operator reliability of LSM and SSM by 2D-SWE was assessed in the 64 and 51 rats with NAFLD and showed technical success. The intra-operator reliability of the five measurements for LSM was excellent, with an ICC of 0.923 (95% CI:0.889–0.949, $P < 0.001$) and a CV of 9.5% (95% CI: 6.7–12.2). The ICC and the CV of the five measurements for SSM were 0.913 (95% CI:0.854–0.95, $P < 0.001$) and 14% (95% CI: 5.2–22.9), respectively, which suggested that the stability of LSM was better than that of SSM.

Based on the above results, the median LSM and SSM values of five 2D-SWE measurements were calculated for further analysis.

Correlation of Noninvasive Methods With Portal Venous Pressure

LSM and SSM values increase with increase in portal venous pressure of the rats with NAFLD (**Figure 2**). Among all the noninvasive methods, LSM had the strongest correlation with portal venous pressure values ($r = 0.636$, $P < 0.001$), followed by SSM ($r = 0.602$, $P < 0.001$). At the same time, LSM displayed a positive correlation with SSM in the rats with NAFLD ($r = 0.539$, $P < 0.001$). However, the correlation between the four composite scores (LSPS, PAR, AAR, and APRI) and portal venous pressure was limited.

The LSM values were significantly higher in rats with PH than in those without: 9.6 (95% CI: 9–10.2) kPa vs. 6.9 (95% CI: 6.6–7.2) kPa, respectively, ($P < 0.001$). Similarly, the SSM values were also significantly higher in rats with PH than in those without: 16.3 (95% CI: 15.3–7.3) kPa vs. 12.8 (95% CI: 12.1–13.6) kPa, respectively, ($P < 0.001$). The results are shown in **Figure 3**. Furthermore, the LSM values were significantly higher in the rats with NAFLD, with a portal venous pressure of 10 mmHg or higher, that in those without (10 vs.8 kPa, $P < 0.001$). The same trend was observed for SSM (16.6 vs.14.1 kPa, $P < 0.05$).

Diagnostic Performance of LSM, SSM, and Composite Scores in Predicting PH

The AUCs, cutoff values, sensitivity, specificity, PPV, and NPV for the prediction of PH using LSM, SSM, and composite scores are presented in **Table 3**. In addition, we also investigated four combined models by multivariate logistic analysis. The AUCs of LSM and SSM were 0.906 (95% CI:0.841–0.97) and 0.87 (95% CI:0.776–0.964), respectively, for the diagnosis of PH. Using the Youden index, the cutoff LSM for predicting PH was 7.7 kPa (sensitivity 79.7%, specificity 100%), and the cutoff SSM was 13.4 kPa (sensitivity 86.3%, specificity 80%) (**Figure 4**). Furthermore, in descending order, the AUCs of LSPS, AAR, APRI, and PSR for predicting PH were 0.793 (95% CI:0.688–0.898),0.668 (95% CI:0.550–0.772),0.533 (95% CI:0.414–0.649), and 0.52 (95% CI:0.366–0.673), respectively. The AUCs of combined models 1 to 4 for the diagnosis of PH were 0.923 (95% CI:0.858–0.988),0.913 (95% CI:0.851–0.974),0.872 (95% CI:0.779–0.965), and 0.923 (95% CI:0.858–0.988), respectively.

When comparing the AUCs, the performance of LSM in the diagnosis of PH was significantly higher than that of LSPS ($P = 0.047$), AAR ($P < 0.001$), APRI ($P < 0.001$), and PSR ($P < 0.001$). However, there was no significant difference between LSM and SSM in evaluating PH ($P = 0.618$). Among the four composite scores, LSPS had higher performance than APRI and PSR in assessing PH (all $P < 0.001$), and no significant difference between LSPS and AAR was found ($P = 0.167$). The AUCs of combined models 1 to 4 for the assessment of PH were more than 085, with no significant differences among the combined models (all $P > 0.05$). The AUCs of combined models 1, 2, and 4 in the assessment of PH were >0.9, and no significant differences were found among the three combined models (all $P > 0.05$). Our results also showed that the AUCs of combined models 1, 2, and 4 were slightly higher than those of LSM, but that the differences were not statistically significant (all $P > 0.05$). Furthermore, the four combined models had higher AUC values than SSM (AUC = 0.872–0.923 vs. AUC = 0.87, all $P > 0.05$).

DISCUSSION

In this study, we revealed the value of LSM and SSM obtained by 2D-SWE, the four composite scores (LSPS, PSR, AAR, and APRI), and the four combined models, namely, combined model 1 (LSM and SSM), combined model 2 (LSM and PLT), combined model 3 (SSM and PLT), and combined model 4 (LSM, SSM and PLT), for predicting PH in rat models with NAFLD. Our study demonstrated that both LSM and SSM obtained by 2D-SWE showed a positive correlation with portal venous pressure and exhibited higher diagnostic accuracy for assessing PH in NAFLD compared with the four composite scores. In addition, the diagnostic performance of combined models 1, 2, and 4 were similar and slightly higher than that of LSM, but the differences were not significant.

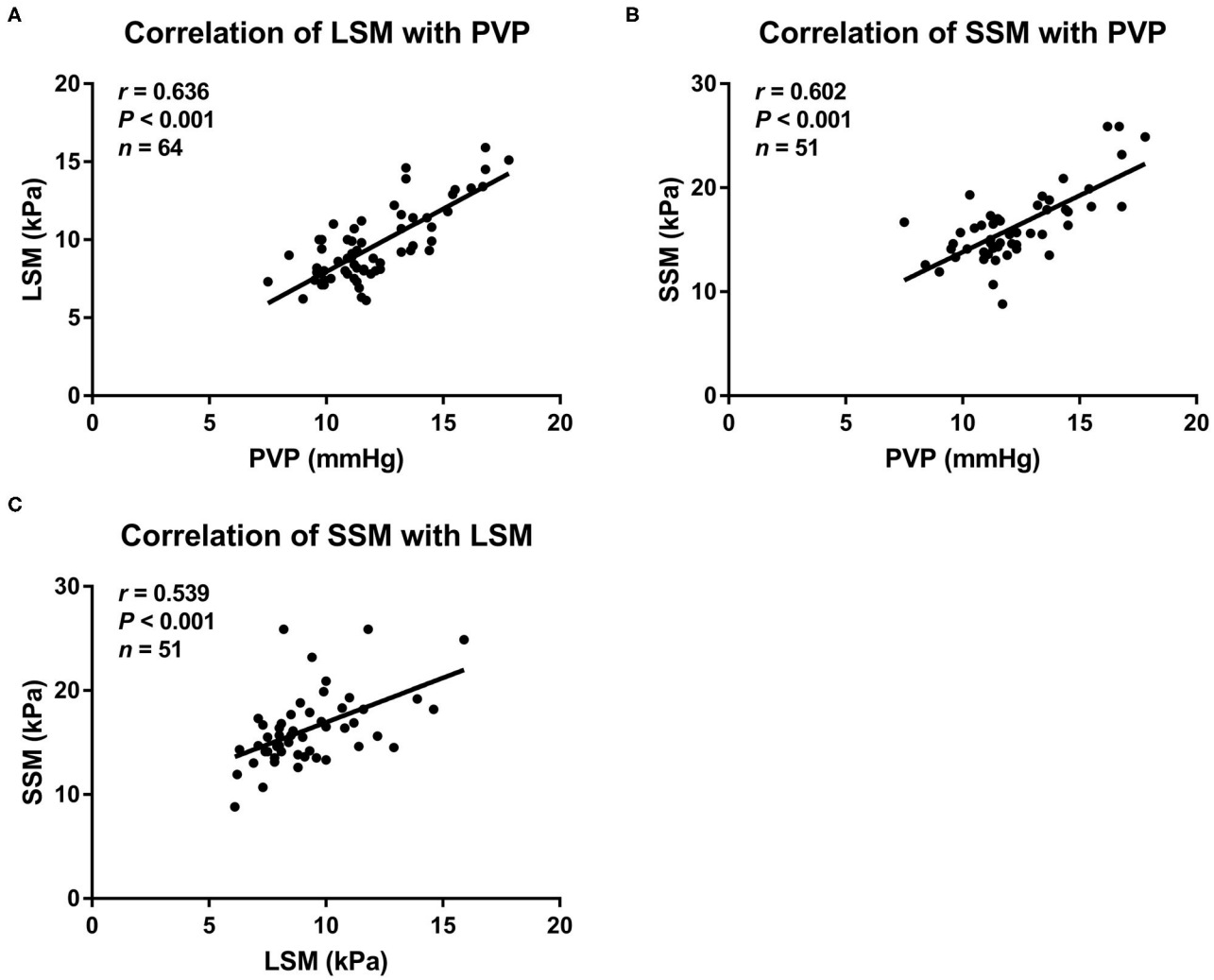

FIGURE 2 | Scatterplots showing correlations between **(A)** LSM, **(B)** SSM, and portal venous pressure, as well as **(C)** LSM with SSM in rat models with NAFLD. LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; PVP, portal venous pressure; SSM, spleen stiffness measurement.

There is an urgent need to develop alternative, noninvasive methods for detecting PH, and as such, it will be very important for early diagnosis and predictive significance (27). Some studies have explored the value of LSM obtained by 2D-SWE in diagnosing PH in recent years. However, only few previous studies have involved patients with NAFLD. A prospective study by Jeon et al. (28) demonstrated that the AUC of LSM was 0.818 for the diagnosis of clinically significant PH in patients with hepatitis B-related liver disease. Another study reported that the AUCs of LSM were 0.72 and 0.77 for diagnosing clinically significant PH and severe PH, respectively, in patients with hepatitis B-related cirrhosis (8). In our study, LSM was significantly increased in the rat models with NAFLD when compared to those in controls and positively correlated with portal venous pressure, with a correlation coefficient of 0.636 ($P < 0.001$), which was relatively higher than that of a previous study ($r = 0.607$) (8). Moreover, LSM showed a good diagnostic value for evaluating PH, with an AUC of more than 0.9. Our results were higher than those of previous studies (8, 28). This discrepancy may be due to the development of PH influenced by a pattern of fibrosis in the liver specific to the etiology of chronic liver disease. According to the etiology, PH is likely to have a different onset (12), for instance, in patients with nonalcoholic steatohepatitis, which may develop PH even in pre-cirrhotic stages (29).

The hemodynamics and morphologic characteristics of the spleen are subsequently changed when chronic liver disease progresses (30). PH can cause splenic congestion, which increases the stiffness of splenic tissue (31). Our study showed that SSM had a moderately strong correlation with portal venous pressure values ($r = 0.602$, $P < 0.001$), and exhibited a good

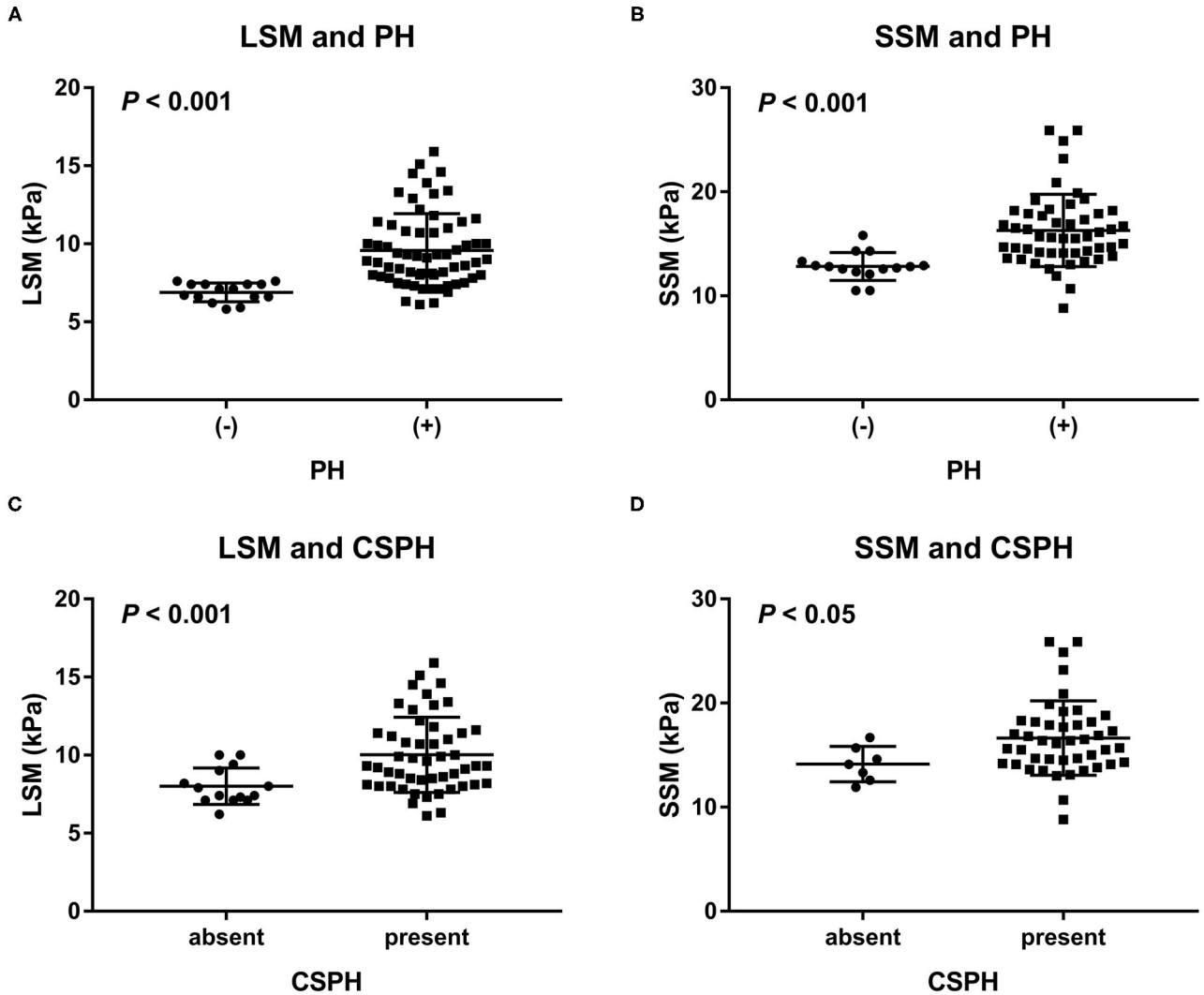

FIGURE 3 | Distribution of **(A)** LSM and **(B)** SSM in rats with and those without PH, as well as distribution of **(C)** LSM and **(D)** SSM in NAFLD rats with and those without CSPH (a portal venous pressure of 10 mmHg or higher). LSM and SSM were significantly higher in the rats with PH than in the rats without PH. Similarly, LSM and SSM were significantly higher in the rats with NAFLD and CSPH than in those with NAFLD but without CSPH. CSPH, clinically significant portal hypertension; LSM, liver stiffness measurement; PH, portal hypertension; SSM, spleen stiffness measurement.

performance that was comparable to that of LSM in terms of detecting PH (AUC = 0.87 vs. AUC = 0.906, $P > 0.05$). Furthermore, compared to LSM, SSM displayed a relatively higher sensitivity for evaluation of PH. Altogether, both LSM and SSM obtained by 2D-SWE can be used as promising noninvasive parameters for the diagnosis of PH in NAFLD. However, we found that the success and stability rates of SSM were relatively lower than those of LSM. In our study, we could not obtain satisfactory SSM results (including failed and unreliable) in 13 rats with NAFLD, accounting for 20.3% (13/64) of all the rats with NAFLD. This was mainly due to two factors: small spleen size and colonic gas, which affect the visualization of the spleen and the clarity of its image, and result in poor sonic window of the spleen. The stability of SSM may also be influenced by cardiac beat-induced modifiable movements. Similarly, a recent study by Jeon et al. (28) reported that the failure and unreliable results of SSM were more frequent than those of LSM. These results, therefore, indicated that LSM seemed to be more reliable and useful for the evaluation of PH in NAFLD than SSM given the technical success and stability results.

Previously, Elkrief et al. (32) have reported that SSM did not achieve satisfactory results in the diagnosis of clinically significant PH (AUC = 0.64). Of note, the population in this study was composed of patients with predominantly decompensated cirrhosis (Child-Pugh C 44%) and severe PH (median HVPG = 17 mmHg). Sharma et al. (22) found that SSM did not show a correlation with HVPG in twenty-four

TABLE 3 | Predictive value of noninvasive methods and combined models for assessing portal venous pressure.

Noninvasive parameter	Portal hypertension					
	Cutoff	AUC (95% CI)	Sensitivity (95% CI, %)	Specificity (95% CI, %)	PPV (%)	NPV (%)
LSM, kPa	7.7	0.906 (0.841–0.970)	79.7 (67.8–88.7)	100 (78.2–100)	100	53.6
SSM, kPa	13.4	0.870 (0.776–0.964)	86.3 (73.7–94.3)	80.0 (51.9–95.7)	93.6	63.2
LSPS	0.04	0.793 (0.688–0.898)	73.4 (60.9–83.7)	73.3 (44.9–92.2)	92.2	39.3
PSR	197.8	0.520 (0.366–0.673)	37.5 (25.7–50.5)	86.7 (59.5–98.3)	92.3	24.5
AAR	3.18	0.668 (0.550–0.772)	76.7 (64.0–86.6)	60.0 (32.3–83.7)	88.5	39.1
APRI	0.23	0.533 (0.414–0.649)	50.0 (36.8–63.2)	73.3 (44.9–92.2)	88.2	26.8
Combined model 1†	0.73	0.923 (0.858–0.988)	86.3 (73.7–94.3)	100 (78.2–100)	100	58.2
Combined model 2‡	0.74	0.913 (0.851–0.974)	84.4 (73.1–92.2)	100 (78.2–100)	100	60.0
Combined model 3§	0.65	0.872 (0.779–0.965)	88.2 (76.1–95.6)	80.0 (51.9–95.7)	93.8	66.6
Combined model 4‡	0.71	0.923 (0.858–0.988)	84.3 (71.4–92.3)	100 (78.2–100)	100	65.2

†, combined model 1 represents LSM and SSM combined model; ‡, combined model 2 represents LSM and platelet count combined model; §, combined model 3 represents SSM and platelet count combined model; ‡, combined model 4 represents LSM and SSM, platelet count combined model.

AAR, aspartate aminotransferase/alanine aminotransferase ratio; APRI, aspartate aminotransferase-to-platelet count ratio index; AUC, area under the receiver operating characteristic curve; CI, confidence interval; LSM, liver stiffness measurement; LSPS, liver stiffness-spleen diameter to platelet ratio score; NPV, negative predictive value; PPV, positive predictive value; PSR, platelet count/spleen diameter ratio; SSM, spleen stiffness measurement.

patients with more severe PH (HVPG $\geq$ 19 mmHg). In these previous studies, the unsatisfactory results of SSM may be due to the effect of various shunts arising during PH progression (33). Further studies focusing on the diagnostic superiority of SSM over LSM obtained by 2D-SWE for evaluation of PH in patients with compensated chronic liver disease are warranted.

LSPS, PSR, AAR, and APRI are common composite scores. Zhu et al. (8) showed that the AUC of LSPS was 0.76 for assessing clinically significant PH and 0.8 for assessing severe PH. In Elkrief's study (32), the AUC of LSPS (by 2D-SWE) was 0.76 for diagnosis of clinically significant PH. In our study, we found that the correlation between the four composite scores and portal venous pressure was limited. Among these, LSPS had better diagnostic performance, with an AUC of 0.793, which was slightly higher than that reported in previous studies. LSPS combines LSM, spleen diameter, and PLT; however, its value was not better than that of LSM alone for diagnosing PH. In contrast, the performance of LSM was superior to that of LSPS in our study. Besides, PSR and APRI could not display satisfactory results in the diagnosis of PH. Initially, PSR was proposed as a noninvasive parameter for predicting esophageal varices (21). Nevertheless, a previous study has confirmed that PSR was unable to distinguish between patients with large esophageal varices and those with small ones, and that its accuracy in diagnosing the presence of esophageal varices was also lower than that of LSM (obtained by TE) (22). Blood parameters, such as PLT, AST, and ALT, may be influenced by extrahepatic lesions (30); AAR, combining AST and ALT, was not reliable enough to accurately evaluate PH. Therefore, 2D-SWE measurements including LSM and SSM may be more advantageous for diagnosing PH in NAFLD than composite scores.

Furthermore, to improve the accuracy of 2D-SWE measurements, we attempted to study four combined diagnostic models and compared LSM and SSM with the combined diagnostic models. In our study, combined models 1, 2, and 4 showed a similar diagnostic value that was slightly higher than that of combined model 3, although the difference was not significant. In addition, between the four combined models and the single-measurement methods (LSM and SSM), no significant differences were found. The combined models may be too complex in clinical practice. Hence, it appears that LSM is both a convenient and dependable noninvasive diagnostic tool for evaluation of PH.

Some limitations are worth considering in this study. First, the unsuccessful result of SSM was explicitly higher than that of LSM in our rat models as well as in clinical samples reported in previous studies (8, 28). It is principally because of small spleen size and colonic gas. To improve the technical success results of SSM, further studies are required. It is worth mentioning that 2D-SWE as a novel elastography technique has a significantly higher rate of success and reliability for the measurement of SSM than TE (32). Second, portal venous pressure was measured under aseptic conditions in an operation room by experienced researchers after the rats were anesthetized. There may be a difference in the portal venous pressure measurement between animals in the conscious state and those in the anesthetized state. Third, the optimal cutoff values of LSM and SSM obtained by 2D-SWE for evaluating PH in subjects with NAFLD should be determined. Of course, it needs further studies. Finally, our study used rat models of NAFLD. Additional studies with a population of patients with NAFLD are required to confirm our results. Moreover, only male animals were used in our study. Future efforts are required to address gender disparities.

In conclusion, LSM and SSM obtained by 2D-SWE had notably better diagnostic performance in evaluating PH in the rat models with NAFLD than the composite scores such as LSPS, PSR, AAR, and APRI. Considering the technical success and stability results, LSM seemed to be more reliable and useful than SSM. The four combined models, compared to LSM

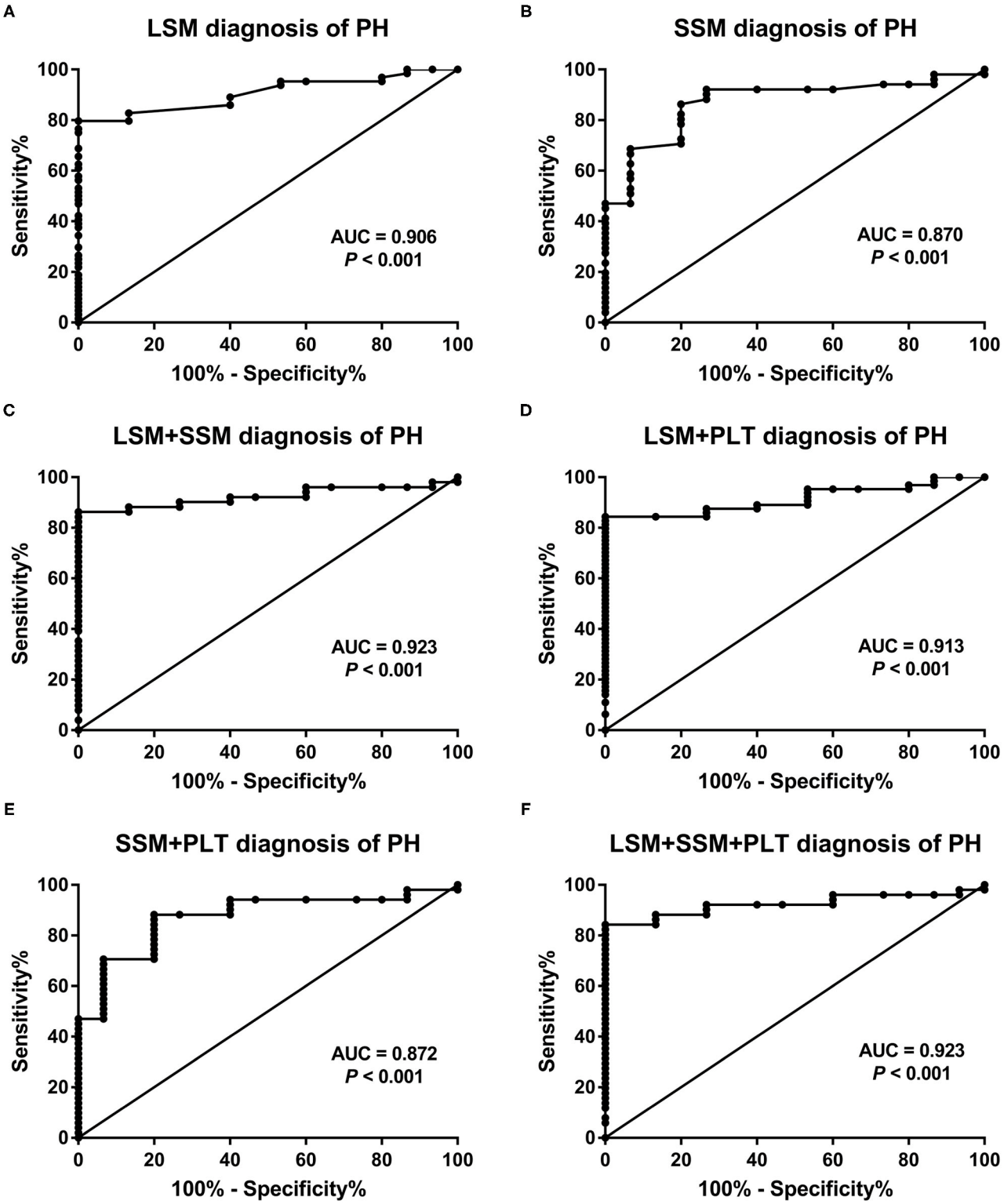

FIGURE 4 | Receiver operating characteristic curves of **(A)** LSM, **(B)** SSM, and **(C–F)** the four combined models for predicting portal venous pressure. AUC, area under the receiver operating characteristic curve; LSM, liver stiffness measurement; PH, portal hypertension; PLT, platelet count; SSM, spleen stiffness measurement.

and SSM, did not significantly improve diagnostic accuracy in evaluating PH.

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 animal study was reviewed and approved by Institutional Animal Care and Use Committee (IACUC) of The Second Affiliated Hospital of Fujian Medical University.

AUTHOR CONTRIBUTIONS

BD collected the data and wrote the first draft of the manuscript. YPC provided the pathological scores of liver tissues. BD, YJC, and RQ performed the statistical analysis. GL conceived of and supervised the study. All the authors edited the manuscript, provided intellectual input, contributed to the article and approved the submitted version.

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Computed Tomography-Based Texture Features for the Risk Stratification of Portal Hypertension and Prediction of Survival in Patients With Cirrhosis: A Preliminary Study

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Objective: Clinical evidence suggests that the risk stratification of portal hypertension (PH) plays a vital role in disease progression and patient outcomes. However, the gold standard for stratifying PH [portal vein pressure (PVP) measurement] is invasive and therefore not suitable for routine clinical practice. This study is aimed to stratify PH and predict patient outcomes using liver or spleen texture features based on computed tomography (CT) images non-invasively.

Methods: A total of 114 patients with PH were included in this retrospective study and divided into high-risk PH (PVP $\geq$ 20 mm Hg, n = 57) or low-risk PH (PVP $<$ 20 mm Hg, n = 57), a progression-free survival (PFS) group (n = 14), or a non-PFS group (n = 51) based on patients with rebleeding or death after the transjugular intrahepatic portosystemic shunt (TIPS) procedure. All patients underwent contrast-enhanced CT, and the laboratory data were recorded. Texture features of the liver or spleen were obtained by a manual drawing of the region of interest (ROI) and were performed in the portal venous phase. Logistic regression analysis was applied to select the significant features related to high-risk PH, and PFS-related features were determined by the Cox proportional hazards model and Kaplan-Meier analysis. Receiver operating characteristic (ROC) curves were used to test the diagnostic capacity of each feature.

Results: Five texture features (one first-order feature from the liver and four wavelet features from the spleen) and the international normalized ratio (INR) were identified as statistically significant for stratifying PH (p $<$ 0.05). The best performance was achieved by the spleen-derived feature of wavelet.LLH_ngtdm_Busyness, with an AUC of 0.72. The only log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation feature from the liver was associated with PFS with a C-index of 0.72 (95% CI 0.566–0.885), which could stratify patients with PH into high- or low-risk groups. The 1-, 2-, and 3-year

survival probabilities were 66.7, 50, and 33.3% for the high-risk group and 93.2, 91.5, and 84.4% for the low-risk group, respectively ($p < 0.05$).

Conclusion: CT-based texture features from the liver or spleen may have the potential to stratify PH and predict patient survival.

Keywords: risk stratification, survival, computed tomography, texture features, portal hypertension

INTRODUCTION

Portal hypertension (PH) is the initial and main consequence of cirrhosis and is responsible for the majority of its complications (1), which, by definition, is an increase in the pressure in the portal vein and its territory (2). The direct measurement of portal vein pressure (PVP) is the most accurate technique for reflexing PH, but it is extremely invasive (2). Thus, the indirect and less invasive measurement of the hepatic venous pressure gradient (HVPG), widely accepted as the PVP equivalent, has been applied in clinical practice (2–4).

In recent years, clinically significant portal hypertension (CSPH) has been recognized in patients with liver cirrhosis and is defined by an HVPG of at least 10 mm Hg, which is associated with an increased risk of variceal bleeding, hepatic encephalopathy (HE), post-surgical decompensation (5), and hepatocellular carcinoma (HCC) (6). Patients at this stage may have different prognoses based on the level of HVPG (7); notably, an HVPG of at least 20 mm Hg is considered a strong predictor of early rebleeding and death (8, 9), which would put patients at higher risk of decompensation and poor clinical outcome. The findings of these studies revealed the clinical significance of identifying severe PH. Previous studies also indicated that recurrent variceal bleeding occurs in 60% of patients after variceal rupture, if untreated, usually within 1–2 years of index hemorrhage (1, 10). Herein, the risk stratification of PH and individualizing care for patients are warranted in clinical decision-making.

Despite the crucial role of PVP or HVPG measurements for the assessment and prognostic evaluation of PH (11), the invasive nature and high-cost effectiveness of these techniques have limited their clinical application as ideal surveillance tools for monitoring disease progression (12). Currently, liver stiffness (LS) by transient elastography (TE; Fibro-Scan) is recognized as the backbone of the non-invasive diagnosis of PH (1, 13); however, controversy still exists regarding its application in patients with obesity, non-alcoholic fatty liver disease, or severe ascites (1). In the past few years, imaging modalities have shown potential in the assessment of PH as non-invasive and effective procedures (12). The literature has demonstrated that computed tomography (CT) has shown promising results for diagnosing PH based on morphological measurements or computational algorithms (9, 14, 15), however, non-invasive stratification of PH on images has not been specified and remains challenging.

Most patients with PH asking for medical help present overt clinical manifestations, such as varices or variceal hemorrhage (12), which, by definition, with CSPH. Abraldes et al. indicated that an HVPG $\geq$ 20 mm Hg is an independent factor that predicts failure to control bleeding in patients with PH (16), and another study demonstrated that HVPG is the only variable associated with patient outcome and that an HVPG $\geq$ 20 mm Hg predicts poor evolution when compared with HVPG $<$ 20 mm Hg, specifically, longer intensive care unit stay, longer hospital stay, and greater transfusion requirements. Thus, stratifying PH and further predicting patients' clinical outcomes with non-invasive tests are urgently needed for patient management (1, 7).

Texture analysis can non-invasively extract digital information from images that naked eyes cannot with a high throughput and can thus explore more characteristics and provide more quantitative information from images (17). This imaging-based technique has been applied to tumor characterization, differential diagnosis, and prediction of prognosis (17–20). A landmark report indicated that the radiomics signature extracted from CT could achieve significant clinical benefits in the detection of CSPH (14). Another study found that CT-based radiomics features may predict PVP (21); however, the specified stratification of PH has not yet been investigated. To the best of our knowledge, there is still a lack of reports on CT-based texture features for the stratification of PH and the prediction of survival conditions in patients with PH.

In this study, we aimed to assess whether CT-based liver or spleen texture signatures could be used to predict high-risk PH and patients' long-term clinical outcomes.

Abbreviations: PH, portal hypertension; PVP, portal vein pressure; HVPG, hepatic venous pressure gradient; CSPH, clinically significant portal hypertension; HE, hepatic encephalopathy; HCC, hepatocellular carcinoma; LS, liver stiffness; TE, transient elastography; CT, computed tomography; TIPS, transjugular intrahepatic portosystemic shunt; PFS, progression-free survival; FHVP, free hepatic venous pressure; WHVP, wedged hepatic venous pressure; ROIs, regions of interest; GLCM, gray-level cooccurrence matrix; GLSZM, gray-level size zone matrix; GLRLM, gray-level run length matrix; NGTDM, neighboring gray-tone difference matrix; GLDM, gray-level dependence matrix; ICC, intraclass correlation coefficient; ROC, receiver operating characteristic; AUC, curve and the area under the curve; INR, international normalized ratio; OR, odds ratio; SD, standard deviation; IQR, interquartile range; HR, hazard ratio; PT, prothrombin time; CTP, Child-Turcotte-Pugh.

MATERIALS AND METHODS

Patients

This retrospective study was approved by the West China Hospital Ethics Committee and had a waiver of patients' written informed consent. This study was conducted following the Declaration of Helsinki. From January 2016 to October 2020, patients with PH admitted to our medical center for a transjugular intrahepatic portosystemic shunt (TIPS) procedure were eligible for study participation. The inclusion

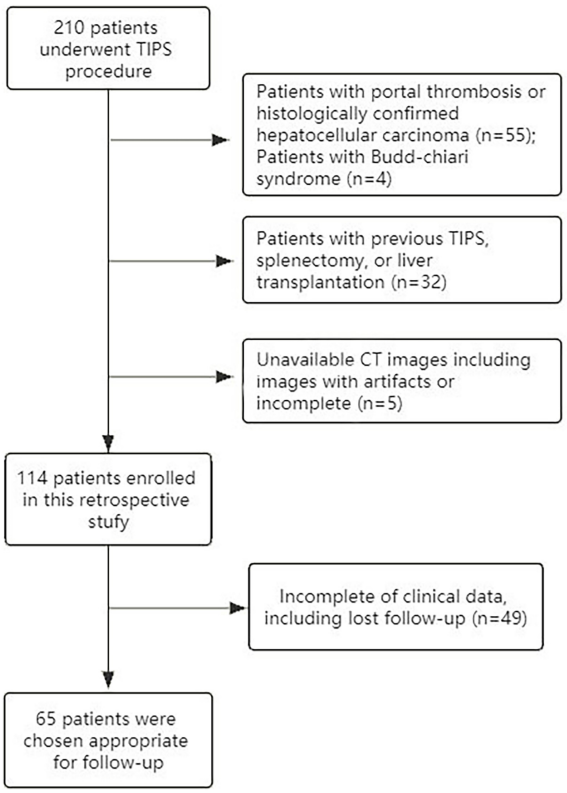

FIGURE 1 | The flowchart of patient enrollment.

criteria were as follows: (1) patients who were diagnosed with liver cirrhosis; (2) patients with available intraoperative direct measurements of PVP and abdominal contrast-enhanced CT scans; and (3) adult patients (age $\geq$ 18 years). The exclusion criteria were as follows: (1) patients who previously underwent one of the following surgical procedures: TIPS, splenectomy, partial splenic embolization, balloon-occluded retrograde, transvenous obliteration, or liver transplantation; (2) patients with portal thrombosis or histologically confirmed HCC; and (3) patients with non-sinusoidal PH (e.g., hepatic cavernoma, Budd-Chiari syndrome). All patients received the TIPS procedure with direct PVP measurement during this hospitalization and underwent contrast-enhanced CT within 4 weeks prior to the TIPS procedure. The patients' laboratory assessments were also recorded, and the patients were divided into a high-risk PH group (PVP $\geq$ 20 mm Hg) and a low-risk PH group (PVP $<$ 20 mm Hg) according to the PVP levels. The flowchart of patient enrollment is shown in **Figure 1**.

Transjugular Intrahepatic Portosystemic Shunt Procedure

The TIPS procedure was performed using a previously described standard process (22). The jugular vein was accessed and a TIPS set (Cook Medical Co., Bloomington, IN, United States) was introduced into the right hepatic vein. The metal cannula was bent by the operator according to the anatomical relationship between the hepatic vein and the targeted puncture site along the portal vein branch. A 3D roadmap was used for portal vein puncture guidance, and access to the portal vein was confirmed by injecting the contrast using a 5-ml syringe under fluoroscopy. Subsequently, direct portography was performed, and PVP measurements were made. The intrahepatic parenchymal tract was then dilated with an 8-mm balloon (Powerflex; Cordis, Roden, Netherlands) and an 8-mm stent graft (Fluency; C.R. Bard, Murray Hill, NJ, United States) was placed. The direct PVP was measured again, and the targeted threshold after stent deployment was 12 mm Hg (23, 24).

Computed Tomography Image Acquisition

The investigated individuals underwent contrast-enhanced CT imaging with one of the following systems: Sensation 64 CT (Siemens), Sensation 16 CT (Siemens), or 64 LightSpeed VCT (GE Healthcare). Triple-phase CT examinations were conducted, i.e., non-enhanced, arterial, and portal vein phases. Abdominal scouts were acquired from the dome of the diaphragm to the iliac crests. The arterial phase of the same region was started at approximately 20–30 s after contrast agent administration and was followed by the portal phase (30–40 s). The reconstructions were conducted on a GE Advantage Windows 3D workstation (GE Healthcare, Waukesha, WI, United States), and the reconstitution thickness was set at 1–2 mm. The detailed scanning parameters are listed as follows: tube voltage, 120 or 100 kVp; tube current, 150–600 mA; slice thickness, 1.25 mm; and pitch, 1.375. All patients received an intravenous, non-ionic contrast agent (iodine concentration, 370 mg/ml; volume, 1.5–2.0 ml/kg of body weight; contrast type, Omnipaque 300, GE Healthcare, Ireland) at a rate of 3–5 ml/s. A volume of 20 ml saline was injected after the injection of the contrast.

Follow-Up

Patients were consistently followed up after the TIPS procedure by periodic re-examinations of CT scans in the outpatient clinics at intervals of 3–6 months or by telephone verification. The time of disease-specific progression (rebleeding) or death was recorded, and patients were censored on October 30, 2021. Patients for follow-up were divided into a progression-free survival (PFS) group or a non-PFS group based on patients with rebleeding or death after the TIPS procedure.

Texture Feature Extraction

Portal venous phase CT images were used for texture feature extraction (14, 25). Regions of interest (ROIs) were drawn around the liver at the porta hepatis level and around the spleen at the splenic hilum level using ITK-SNAP 3·6 (ITK-SNAP 3·X TEAM) (14). Then, Artificial Intelligence Kit software (A.K. software; GE Healthcare, Life Sciences, Beijing, China) was used to extract feature parameters for each ROI, which was based on the image biomarker standardization initiative (IBSI). **Figure 2** shows the delineation of the ROI of the liver and spleen. Before feature extraction, image normalization was performed by remapping the histogram to fit $\mu \pm 3\sigma$: (μ, average grayscale

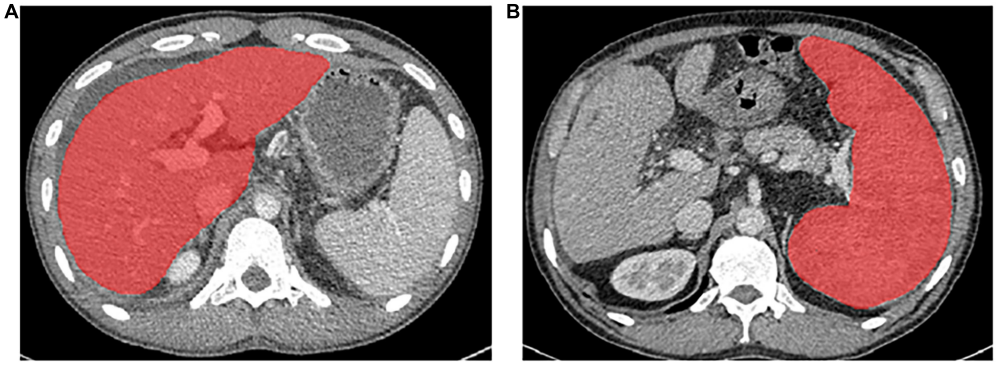

FIGURE 2 | The delineation of the region of interest of the liver **(A)** and spleen **(B)** on CT images.

within ROI; σ, grayscale SD) (26). Texture features were also extracted from images conducted with the Laplacian of Gaussian filter (Log) and wavelet filter. All scans were analyzed by two senior residents independently (CWY, 5 years of experience in abdominal imaging analysis, and YW, 8 years of experience in abdominal imaging analysis) and were supervised by a senior radiologist (FY, 13 years of experience) to handle the non-consensus.

Texture Analysis and Statistical Analysis

A total of 1,037 radiomics features were calculated for original images and filtered images from liver or spleen segmentation that include first-order features of 18 intensity statistics and 14 3D shape features, 24 gray-level co-occurrence matrix (GLCM), 16 gray-level size zone matrix (GLSZM), 16 gray-level run length matrix (GLRLM), 5 neighboring gray-tone difference matrix (NGTDM), and 14 gray-level dependence matrix (GLDM) features and features with two filters that include 744 wavelet features and 186 LoG filtered features.

The intraclass correlation coefficient (ICC) was considered to evaluate the interobserver agreement, and ICC values of > 0.85 represent an almost perfect agreement between observers. The Mann-Whitney *U*-test was used to compare continuous variables, and the chi-square test was used to compare categorical variables. Univariate and multivariate logistic regression analyses were used to screen the independent risk factors for discriminating the high-risk or low-risk PH group. Univariate analyses were performed first, and only parameters found to have statistical significance were used for further stepwise multivariate logistic regression.

The diagnostic performance of each texture feature for discriminating the high-risk or low-risk PH group was quantified by the receiver operating characteristic (ROC) curve and the area under the curve (AUC), and the accuracies, sensitivities, and specificities were also calculated. Additionally, univariate analyses with Cox proportional hazards regression identified the predictors of disease progression of variceal bleeding recurrence and death. The Cox proportional hazards model was used to assess the PFS-associated texture features that predicted the probabilities of 1-, 2-, and 3-year PFS in the followed up patients. The risk probability of followed up patients was stratified into high-risk and low-risk groups using the optimal cutoff point determined by X-tile software (27). Survival curves were generated with the Kaplan-Meier method and compared by a 2-sided log-rank test. The C-index was used to determine the diagnostic capabilities of risk factors associated with PFS.

Categorical variables are reported as frequencies and proportions. Continuous variables are reported as the means (SD) and medians (interquartile ranges, IQR). Statistical analysis was performed using R software (version 3.5.3). A values of p of less than 0.05 was defined as significant in two-tailed analyses.

RESULTS

Patient Characteristics

The characteristics of the patients are summarized in **Table 1**. Out of 114 patients included, there were 57 cases in the high-risk PH group (PVP $\geq$ 20 mm Hg) and 57 cases in the low-risk PH group (PVP $<$ 20 mm Hg). A total of 65 patients were finally followed that include 14 cases in the PFS group and 51 cases in the non-PFS group. In the low- and high-risk PH groups, the value of the international normalized ratio (INR) was found to be statistically significant between these two groups ($p < 0.05$), except that the remaining clinical parameters were not statistically significant between the two groups (**Table 1**).

Clinical Variables and Texture Features for Portal Hypertension Stratification

Texture features that had greater ICCs considering a threshold of 0.85 were robust and adopted for later analysis. Of all the clinical factors or CT-based texture features, six significant features, i.e., 1 clinical variable [INR, odds ratio (OR) 7.76, 95% confidence interval (CI) 1.14–52.88], and 5 texture features, were identified as independent predictors by univariate analysis. Out of the 5 CT-based texture features, log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation was identified from the liver (OR: 0.71, 95% CI: 0.54–0.94), and wavelet.LLH_ngtdm_Busyness (OR 3.74, 95% CI 1.28–10.9), wavelet.HLL_glrlm_RunLengthNonUniformity (OR 2.08,

TABLE 1 | Demographics and clinical characteristics of the study population.

Variables	*N*	Low-risk PH	High-risk PH	Statistics	*P*
Age, years[a]	114	48.64 ± 11.45	52.04 ± 8.87	−1.711	0.091
Gender[b]				1.671	0.196
Male	78	29 (61.70%)	49 (73.13%)		
Female	36	18 (38.30%)	18 (26.87%)		
Etiology[b]				−0.066	0.947
Post-hepatic cirrhosis	73	32 (68.09%)	41 (61.19%)		
Alcoholic cirrhosis	14	2 (4.26%)	12 (17.91%)		
Combined cirrhosis	12	4 (8.51%)	8 (11.94%)		
Primary biliary cirrhosis	7	3 (6.38%)	4 (5.97%)		
Others	8	6 (12.77%)	2 (2.99%)		
Child–Pugh class[b]				−0.253	0.8
Child–Pugh class A	35	15 (31.91%)	20 (29.85%)		
Child–Pugh class B	61	25 (53.19%)	36 (53.73%)		
Child–Pugh class C	18	7 (14.89%)	11 (16.42%)		
PVP (mm Hg)[c]	114	17.00 (15.20, 18.00)	22.00 (21.00, 27.00)	−8.805	<0.001
EVB history[b]				1.131	0.288
Absent	17	9 (19.15%)	8 (11.94%)		
Present	97	38 (80.85%)	59 (88.06%)		
Ascites[b]				0.77	0.38
Absent	20	10 (21.28%)	10 (14.93%)		
Present	94	37 (78.72%)	57 (85.07%)		
Hypersplenism[b]				0.337	0.561
Absent	88	35 (74.47%)	53 (79.10%)		
Present	26	12 (25.53%)	14 (20.90%)		
Hepatic encephalopathy[b]				2.433	0.119
Absent	105	46 (97.87%)	59 (88.06%)		
Present	9	1 (2.13%)	8 (11.94%)		
Total bilirubin[c]	114	21.50 (13.30, 30.72)	26.30 (16.34, 32.46)	−1.361	0.173
Albumin[a]	114	34.40 ± 6.50	33.09 ± 6.12	1.096	0.275
Globulin[c]	114	27.60 (23.68, 31.82)	26.90(22.62, 32.06)	0.653	0.514
ALT[c]	114	21.00 (13.20, 31.80)	21.00 (14.00, 43.40)	−0.458	0.647
AST[c]	114	32.00 (22.00, 43.80)	31.00 (21.20, 55.60)	−0.622	0.534
INR[c]	114	1.24 (1.15, 1.39)	1.35 (1.23, 1.48)	−2.331	0.02
PLT[c]	114	62.00 (51.20, 89.20)	63.00 (40.20, 100.40)	0.636	0.525

PH, portal hypertension; PVP, portal vein pressure; EVB, esophageal variceal bleeding; ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; PLT, platelet count.

[a]*Data were compared by using Student's t-test and are presented as the means ± deviation.*

[b]*Data were compared using chi-square test and are presented as numbers (%).*

[c]*Data were compared using the Mann-Whitney test and are presented as medians (interquartile range).*

95% CI 1.1–3.95), avelet.HLH_glcm_MCC (OR 0.57, 95% CI 0.34–0.95), and wavelet.LLL_glrlm_RunLengthNonUniformity (OR 2.1, 95% CI 1.09–4.05) were identified in the spleen (**Table 2**). Clinical variable of INR and spleen-derived feature of wavelet.LLH_ngtdm_Busyness showed the most significant association with the high-risk PH group (OR 7.76, 95% CI 1.14–52.88, $p = 0.036$ vs. OR 3.74, 95% CI 1.28–10.9, $p = 0.016$).

Receiver operating characteristic analysis showed that the above texture features had moderate capabilities to distinguish between the high- and low-risk PH groups, of which the best performance was achieved by the spleen-derived feature of wavelet.LLH_ngtdm_Busyness, with an AUC of 0.72, an accuracy of 0.746, a specificity of 0.681, and a sensitivity of 0.791 when using a cutoff value of 0.517 (**Table 3** and **Figure 3**). The clinical feature of INR also showed a moderate performance for stratifying PH, with an AUC of 0.629, an accuracy of 0.649, a specificity of 0.468, and a sensitivity of 0.776 when using a cutoff value of 0.528 (**Figure 3**).

Texture Features for Progression-Free Survival

As of October 30, 2021, a total of 65 of 114 (57.0%) patients had completed the PFS follow-up, the overall recurrence rate of bleeding was 12.3% (8/65), and the overall death rate was 9.2% (6/65). **Table 4** shows the results of univariate Cox proportional hazard regression analysis for PFS, of which only log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation had a statistically significant difference for the PFS stratification

TABLE 2 | Univariate analysis for stratifying portal hypertension.

Variables	Prediction of high-risk PH	
	OR (95% CI)	*P*
log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation	0.71 (0.54–0.94)	0.017
wavelet.LLH_ngtdm_Busyness	3.74 (1.28–10.9)	0.016
wavelet.HLL_glrlm_RunLengthNonUniformity	2.08 (1.1–3.95)	0.025
wavelet.HLH_glcm_MCC	0.57 (0.34–0.95)	0.03
wavelet.LLL_glrlm_RunLengthNonUniformity	2.1 (1.09–4.05)	
INR	7.76 (1.14–52.88)	0.036

PH, portal hypertension; OR, odds ratio; CI: confidence interval; INR, international normalized ratio.

TABLE 3 | The performance of texture features and INR for stratifying portal hypertension.

Variables	AUC (95% CI)	Accuracy	Specificity	Sensitivity	Cutoff
log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation	0.605 (0.49–0.710)	0.658	0.333	0.899	0.493
wavelet.LLH_ngtdm_Busyness	0.72 (0.622–0.817)	0.746	0.681	0.791	0.517
wavelet.HLL_glrlm_RunLengthNonUniformity	0.594 (0.509–0.717)	0.55	0.843	0.333	0.618
wavelet.HLH_glcm_MCC	0.593 (0.489–0.705)	0.65	0.275	0.928	0.5
wavelet.LLL_glrlm_RunLengthNonUniformity	0.604 (0.518–0.726)	0.592	0.588	0.594	0.553
INR	0.629 (0.525–0.733)	0.649	0.468	0.776	0.528

AUC, the area under the ROC curve; CI, confidence interval; INR, international normalized ratio.

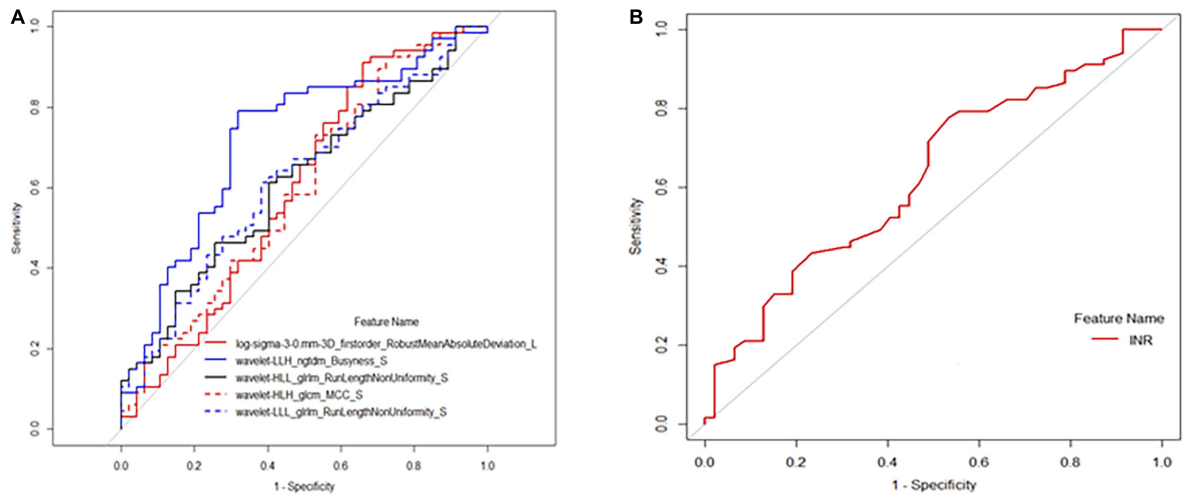

FIGURE 3 | Receiver operating characteristic (ROC) analysis of selected texture .features and international normalized ratio (INR) for predicting high-risk portal hypertension (PH). **(A)** The ROC curve calculated by texture features of log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation, wavelet.LLH_ngtdm_Busyness, wavelet.HLL_glrlm_RunLengthNonUniformity, wavelet.HLH_glcm_MC, and wavelet.LLL_glrlm_RunLengthNonUniformity. **(B)** The ROC curve of INR for predicting high-risk PH.

and could divide the followed up patients into high- or low-risk groups (log-rank test, $p < 0.05$; **Figure 4**). It was lower in the high-risk group (medium 3.839; IQR 3.465–4.027) than in the low-risk group (medium 5.868; IQR 5.166–6.942) [hazard ratio (HR) 0.529, 95% CI 0.322–0.869, $p = 0.012$], and the remaining texture features were not found to be associated with PFS. The feature of log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation presented a moderate prognostic performance for predicting the high-risk group with a C-index of 0.72 (95% CI 0.566–0.885) when using a cutoff value of 4.15. We also evaluated clinical characteristics for survival using univariate Cox proportional hazard regression. We found that the variables of hypersplenism and HE had statistical significance for survival analysis for PFS ($p < 0.05$; **Supplementary Table 1**), with HRs of 3.80 (95% CI 1.31–10.99) and 4.27 (95% CI 1.33–13.64), respectively. **Supplementary Figures 1**, **2** show the survival curves of hypersplenism and HE, respectively. Clinical manifestations

of hypersplenism presented a C-index of 0.643 (95% CI 0.514–0.772), and HE presented a C-index of 0.614 (95% CI 0.494–0.734).

The median survival time was 20.5 (IQR 10.25–39.75) months for high-risk patients and 37 (IQR 32.5–41.5) months for low-risk patients. The 1-, 2-, and 3-year survival probabilities were 66.7, 50, and 33.3% for the high-risk group and 93.2, 91.5, and 84.4% for the low-risk group, respectively (log-rank test, $p = 0.0014$). Representative cases were given to show the discriminative performance of the features for stratifying PH and predicting PFS (**Figure 5**).

DISCUSSION

Non-invasive stratification of PH and prediction of high-risk PH in patients with cirrhosis have been highlighted in recent years due to the lack of widespread application of invasive PVP or HVPG measurements. In this study, we assessed the texture features based on CT and clinical data non-invasively for predicting high-risk PH patients. In addition, we evaluated patient outcomes using the extracted features, with the aim of aiding clinical decision-making.

Our results suggested that texture features from the liver or spleen were significantly different between the high-risk PH and low-risk PH groups in cirrhotic patients that include log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation from the liver, wavelet.LLH_ngtdm_Busyness, wavelet.HLL_glrlm_RunLengthNonUniformity, wavelet.HLH_glcm_ MCC, and wavelet.LLL_glrlm_RunLengthNonUniformity from the spleen. Out of these features, the feature of wavelet.LLH_ngtdm_Busyness from the spleen demonstrated the best diagnostic performance, with an AUC of 0.72. Furthermore, we found that only the feature of log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation was associated with patient outcomes, and it also showed a moderate prognostic capability for discriminating the high-risk group from the low-risk group, with a C-index of 0.726 based on a cutoff value of 0.415.

A previous study demonstrated that the non-invasive radiomics signature based on a machine-learning method, which they termed *r*HVPG, could accurately facilitate the diagnosis of PH in patients with cirrhosis (14). Their findings underlined the significance of the detection of CSPH in clinical treatment and inspired more investigation using the advanced machine-learning algorithm for the evaluation of PH. However, current guidelines indicate that different levels of portal pressure are a strong predictor for patient outcomes (1). As mentioned previously, an HVPG $\geq$ 20 mm Hg predicts poor patient long-term survival and a higher incidence of rebleeding (8, 16); thus, stratification of PH and identification of severe PH should be more emphasized. Therefore, we conducted a further investigation based on a previous report (14), and we evaluated the performance of the texture signature from the machine-learning method for the stratification of PH.

We found that out of 5 texture features associated with high-risk PH, four were derived from the spleen, which might refer to previous literature. They found that non-invasive spleen-related parameters have the potential to predict the grade of PH and the presence of varices (28–30). For example, spleen stiffness measurement by Fibro-Scan has been found to be more closely related to PH than LS measurement (31, 32). The following reason might explain the spleen-related finding of the present study. We all know that patients with severe PH generally present spleen enlargement in the natural history of disease progression. It is relevant to note that splenomegaly in cirrhosis is characterized by enlargement and hyperactivation of the splenic lymphoid tissue and increased angiogenesis and fibrogenesis, in addition to passive congestion due to increased portal pressure (33, 34). Briefly, the pathogenetic changes leading to spleen enlargement can be reflected in the spleen tissue that includes the outer splenic morphological features and the inner compartment; thus, the measurement of spleen stiffness could reveal the physical property of spleen tissue consequent to the hyperactivation condition of PH, by which satisfactory results were obtained to be closely correlated with the degree of PH, at least not inferior to that of LS, particularly, in a more advanced stage of PH (32); therefore, spleen stiffness showed a close relationship with PH. Likely, as an advanced imaging-based technique, texture analysis can extract more valuable data of the tissue component that traditional methods cannot detect, partially, such as spleen stiffness measurement (17), it might be able to reveal more inner pathologic characteristics of the spleen and can thus have the ability to correlate with PH, especially in a more advanced stage of PH as mentioned previously, for example, the stage of the high-risk PH.

Tseng et al. indicated that a radiomics model based on the spleen signature can yield superior performance for predicting portal pressure when compared with the model of the liver signature (AUC 0.832 vs. 0.789, respectively) (21), which highlighted the spleen-derived signature on images. However, they only evaluated the association between portal pressure and the radiomics model and failed to further investigate the risk stratification of PH. In this study, similar results were observed in the diagnostic performance of spleen-derived texture features (AUC, wavelet.LLH_ngtdm_Busyness of 0.72 from spleen vs. log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation of 0.605 from liver). The spleen-derived texture outperformed that of the liver and seemed more suitable to evaluate PH. We speculated that the splenic-dominated result may be associated with the complex vascular branch, particularly, the opening of portosystemic shunts in the late stage of PH, which may have significant implications on the liver tissue (35, 36). As a result, texture features from the liver might not be able to reflect the complex hemodynamic changes of severe PH and may not correlate well with severe PH. However, as a relatively isolated organ, the spleen may not be influenced as much as the liver by the collateral circulation in the late stage of PH (37, 38); thus, the spleen-derived features seem more stable and reliable. The findings of this study suggest the potential of splenic texture features for the prediction of high-risk PH; however, due to the lack of relevant literature regarding the stratification of

TABLE 4 | Univariate Cox proportional hazard regression for survival.

Variables	Survival analysis for PFS	
	HR (95% CI)	*P*
log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation	0.529 (0.322–0.869)	0.012
wavelet.LLH_ngtdm_Busyness	0.727 (0.266–1.991)	0.535
wavelet.HLL_glrlm_RunLengthNonUniformity	1.053 (0.671–1.653)	0.821
wavelet.HLH_glcm_MCC	1.352 (0.775–2.361)	0.288
wavelet.LLL_glrlm_RunLengthNonUniformity	1.03 (0.651–1.63)	0.9

HR, hazard ratio; CI, confidence interval; PFS, progression-free survival.

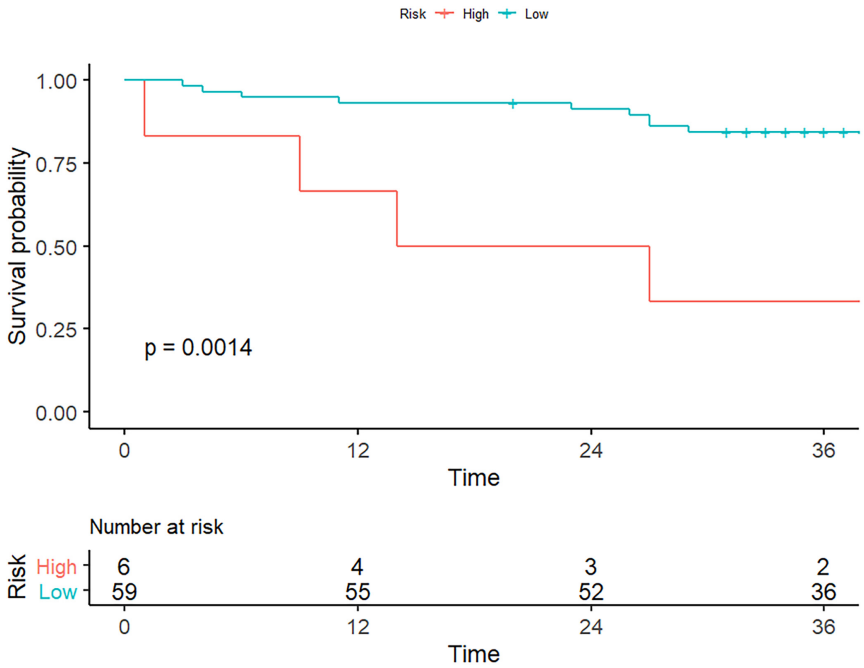

FIGURE 4 | Texture features for the evaluation of patient survival. As the picture depicts, only the liver-derived feature of log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation could stratify patients with portal hypertension into high- or low-risk group (log-rank test, $p < 0.05$).

PH and the relatively limited diagnostic capability, the results of this study should be interpreted cautiously and need to be corroborated in further prospective studies.

The clinical value of INR in the evaluation of liver cirrhosis and the prognostic condition of patients still remains controversial (1, 39). Malinchoc et al. have previously reported that INR for prothrombin time (PT) could be used as a predictor for survival conditions in patients with liver cirrhosis undergoing the TIPS procedure (40). Zhang et al. reported that the INR and PT in the bleeding group were higher than those in the non-bleeding group in patients with cirrhosis (41). They indicated that the liver is an important site for coagulation factor synthesis, and INR represents the deficiency in procoagulant proteins in cirrhosis (42). The changes in PT and INR can accurately reflect the degree of liver function impairment, and a longer PT or INR usually suggests a worse liver function (41, 43). However, the association between INR and portal pressure has not been described, and our results suggested a higher level of INR in the high-risk PH group than in the low-risk PH group (medium 1.35 vs. 1.24), with an OR of 7.76. As we mentioned above, patients with a higher INR are often accompanied by poor liver function; theoretically, patients with poor liver function [Child-Turcotte-Pugh (CTP) B or C] are usually decompensated or at an advanced stage of PH (1), given that a higher INR value may be associated with severe PH, such as high-risk PH. Regarding this, the findings of this study with INR may provide valuable information for clinicians for the stratification of PH.

To the best of our knowledge, this is the first report that predicts patient outcomes consequent to PH using texture signatures from the liver or spleen. Based on the significant features for diagnosing high-risk PH patients, we found that only the feature of log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation was associated with patient outcomes. A previous study applied the radiomics technique in the assessment of portal pressure along with the outcome (21); however, they only evaluated

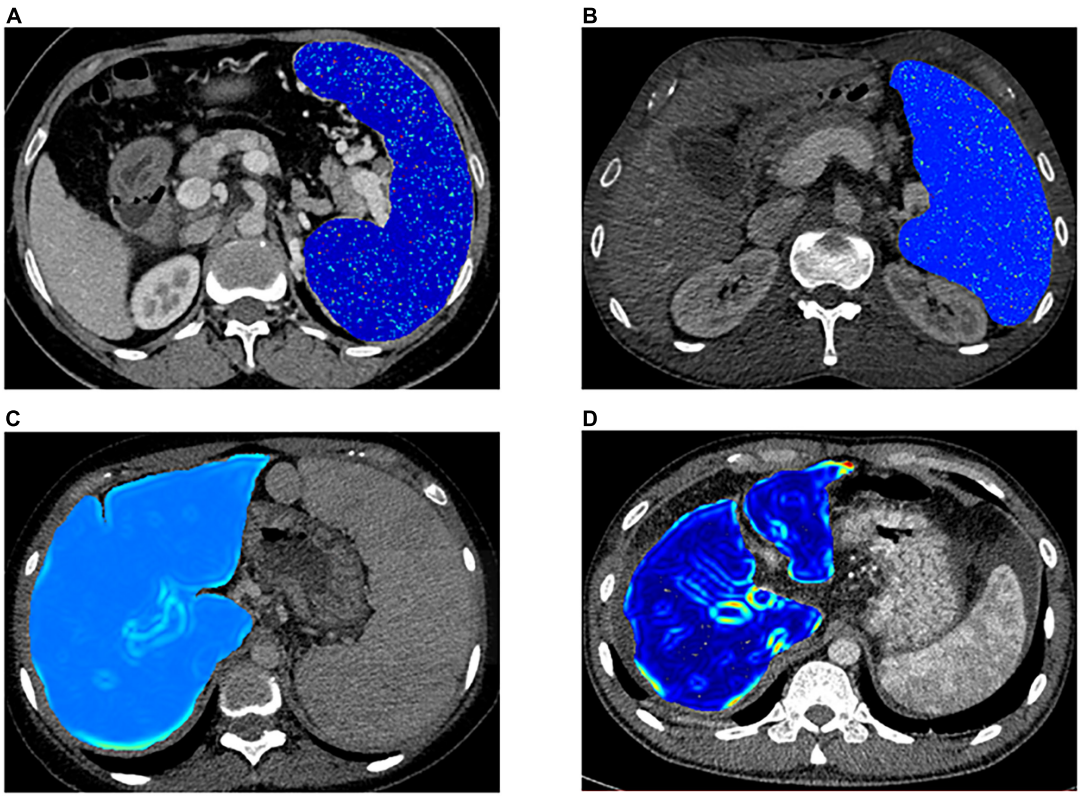

FIGURE 5 | Representative cases for stratifying portal hypertension (PH) and patients' survival condition. **(A,B)** Visualized spleen-derived texture feature of wavelet.LLH_ngtdm_Busyness in high-risk PH patients [portal vein pressure (PVP) ≥ 20 mm Hg] and low-risk PH patients (PVP < 20 mm Hg) respectively. **(C,D)** Visualized liver-derived texture feature of log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation in the high-risk patients and low-risk patients, respectively.

patients' outcome of variceal recurrence after initial endoscopic therapy (suggesting a high portal pressure) (44), by which the association between the portal pressure and the radiomics was obtained. Unlike the previous study, in this study, we evaluated patients' survival condition more directly by collecting the data of recurrence of bleeding or death, which can be more clinically relevant and may provide more helpful information for disease progression, and we found that the feature can yield a moderate capability for discriminating the high-risk group from the low-risk group when using a cutoff value of 4.15 in this preliminary study, obtaining a C-index of 0.726. We know that texture signatures can quantify image features by extracting the distribution and relation of pixel or voxel grayscale in images (17), and the 3D feature (log.sigma.3.0.mm.3D_firstorder_RobustMeanAbsoluteDeviation) indicates the rate of intensity change of the images. In this study, we found a lower value of that in the high-risk group than that in the low-risk group (medium 3.839 vs. 5.868), suggesting a higher homogeneity of images in the high-risk group. Furthermore, the only significant feature was derived from the liver; we assumed that the difference between the high- and low-risk groups may correlate with the inner alteration of liver tissue consequent to cirrhosis. Additionally, we found that clinical variables of hypersplenism and HE were also associated with PFS, and this finding is consistent with the evolution of cirrhosis and PH (1). Since PH is not an isolated complication, it should be considered in the context of advances in the staging of cirrhosis and in the context of other complications of cirrhosis (1, 45). Whether these variables can be used as independent prognostic factors for PFS should be validated with more studies.

Several limitations of our study should be noted. First, due to the retrospective nature of this study, a large number of patients with portal thrombosis were excluded for its high prevalence in patients with PH, which might lead to potential selection bias and may impair the reproducibility of the results. Second, the sample size of patients for follow-up and those with disease progression was limited, and the interpretation of the results should be carried out with caution and still needs further validation with a large-scale sample size. Third, the findings of this preliminary study are less than ideal, which is the main limitation of this research. We are now collecting more eligible patients, and we plan to conduct consecutive research with a large sample size to improve the predictive performance. We hope that the preliminary results could be suggestive for researchers. Finally, this study is retrospective and was carried out in a single-center institution, and more prospective multicenter investigations are needed to better validate the results in clinical practice.

In conclusion, our study demonstrated that CT-based texture signatures from the liver or spleen may have the potential to stratify PH and predict patient survival. The results still need to be corroborated by further multicenter prospective studies.

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/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the West China Hospital Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SW and YW contributed to the conception and design of the study. SW and CY organized the database. XZ carried out data statistics and analysis. SW wrote the manuscript. FH and BS revised the manuscript. All authors have read and approved the final manuscript.

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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.2022.863596/full#supplementary-material

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ORIGINAL RESEARCH
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Combination of Fat-Free Muscle Index and Total Spontaneous Portosystemic Shunt Area Identifies High-Risk Cirrhosis Patients

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Background: Sarcopenia and spontaneous portosystemic shunts (SPSSs) are common complications of liver cirrhosis, and both are associated with higher rates of hepatic encephalopathy (HE) development in these patients. This study aimed to evaluate the simultaneous impact of skeletal muscle mass and spontaneous portosystemic shunting, measured from routine diagnostic CT on outcomes in patients with liver cirrhosis.

Methods: Retrospective analysis of patients with cirrhosis. Skeletal muscle mass [including fat-free muscle index (FFMI) as a surrogate for sarcopenia] and total cross-sectional spontaneous portosystemic shunt area (TSA) were quantified from CT scans. The primary endpoint was the development of HE, while the secondary endpoint was 1-year mortality.

Results: One hundred fifty-six patients with liver cirrhosis were included. Patients with low (L-) FFMI and large (L-)TSA showed higher rates of HE development. In multivariable analysis, L-FFMI and L-TSA were independent predictors of HE development (L-FFMI HR = 2.69, CI 1.22–5.93; L-TSA, HR = 2.50, CI = 1.24–4.72) and 1-year mortality (L-FFMI, HR = 7.68, CI 1.75–33.74; L-TSA, HR = 3.05, CI 1.32–7.04). The simultaneous presence of L-FFMI and L-TSA exponentially increased the risk of HE development (HR 12.79, CI 2.93–55.86) and 1-year mortality (HR 13.66, CI 1.75–106.50). An easy sequential algorithm including FFMI and TSA identified patients with good, intermediate, and poor prognoses.

Conclusion: This study indicates synergy between low skeletal muscle mass and large TSA to predict exponentially increased risk of HE development and mortality in

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liver cirrhosis. Simultaneous screening for sarcopenia and TSA from routine diagnostic CT may help to improve the identification of high-risk patients using an easy-to-apply algorithm.

Clinical Trial registration: [ClinicalTrials.gov], identifier [NCT03584204].

Keywords: sarcopenia, cirrhosis, spontaneous portosystemic shunt, fat-free muscle index, hepatic encephalopathy, acute decompensation, acute-on-chronic liver failure, ACLF

INTRODUCTION

Liver cirrhosis is a major health care burden, particularly due to its variety of severe complications caused by portal hypertension, such as variceal bleeding, ascites, and hepatic encephalopathy (HE), leading to high hospitalization rates and increased morbidity and mortality in these patients (1).

Portal hypertension is known to precipitate the development of spontaneous portosystemic shunts (SPSSs), which is frequently found in advanced stages of liver cirrhosis. Interestingly, a recently large international study showed that, although the prevalence of portosystemic shunts increased with deteriorating liver function, the presence of portosystemic shunts was associated with an increased risk for complications and also death in patients with preserved liver function (2). Accordingly, in another report, the TSA as a quantitative measure of portosystemic shunting was shown to predict HE and mortality development, independent of liver function (3).

Another increasingly recognized complication of liver cirrhosis is a continuous decline of skeletal muscle mass and function, commonly termed sarcopenia, which was shown to be frequent among patients with decompensated stages of disease (4, 5). Recently, an increasing number of studies demonstrated its negative impact on the outcome, especially with respect to the development of HE, waitlist mortality, and overall survival (5–11). This has led to the inclusion of sarcopenia in the current nutrition guidelines of the European Association of the Study of the Liver (EASL) (12).

In this context, it has been suggested that portosystemic shunting may directly contribute to muscle wasting (12, 13). Circulating blood can bypass the hepatic perfusion *via* collaterals, which may lead to increased ammonia levels in skeletal muscles and has been suggested to mediate myocyte autophagy. Moreover, muscular ammonia metabolism is known to deplete amino acids, which are crucial for the maintenance of muscle cells (14). Both skeletal muscles and portosystemic shunts can be reliably quantified from routine cross-sectional imaging and, hence, may be used to determine sarcopenia and the amount of portosystemic shunting, respectively (15).

However, the clinical interplay of these conditions, as well as their joint impact on the outcome in patients with liver cirrhosis, is not fully understood yet. Hence, this study aimed to explore (I) the synergetic impact of sarcopenia and portosystemic shunting on outcomes in patients with liver cirrhosis and (II) to determine whether quantification of these parameters from routine diagnostic imaging may help to improve the risk stratification for deleterious outcomes in these patients.

MATERIALS AND METHODS

Study Population

For this study, consecutive patients, who presented to our centre from 2010 through 2015 due to liver cirrhosis, were retrospectively evaluated (**Figure 1A**). The included patients were at least 18 years old. Diagnosis of liver cirrhosis was made by clinical, histological, or imaging criteria. Patients were excluded if no diagnostic CT scan was available or if the image quality precluded an adequate assessment of portosystemic shunts and skeletal muscle mass. The baseline was set at the time of the CT scan. The clinical data and laboratory parameters were reviewed for baseline and a follow-up period of 1 year.

The primary endpoint was the development of HE (assessed by West-Haven criteria and neuropsychometric tests) and the secondary endpoint was 1-year survival. The study was performed in accordance with the Declaration of Helsinki. The study was approved by the institutional review board, and the necessity for written informed consent was waived due to its retrospective and monocentric character (ClinicalTrials.gov Identifier: NCT03584204).

Assessment of TSA

Radiologists with an expertise in abdominal diagnostic imaging screened available CT scans [all with an indication for Hepatocellular Carcinoma (HCC) screening] for the presence of SPSS. Portosystemic shunts were identified as additional vessels originating from the superior and inferior mesenteric vein, the splenic vein, the portal vein, the renal veins, and the inferior vena cava and were verified from sagittal and coronal reformations. As it was reported previously, the largest short-axis diameter of the relevant shunt vessel was measured to obtain the maximal vessel diameter and to calculate the TSA (3).

Abbreviations: (O)HE, (overt) hepatic encephalopathy; HRS, hepatorenal syndrome; ACLF, acute-on-chronic liver failure; CLIF-C, European Foundation for the study of chronic liver failure consortium;, AD, acute decompensation; TPMT, transversal psoas muscle thickness; MRI, magnetic resonance imaging; CT, computed tomography; ROC, receiver operating characteristics; AUC, area under the curve; HU, Hounsfield unit; MELD, model of end-stage liver disease; INR, international normalized ratio; WBC, white blood cell count; HR, hazard ratio; 95% CI, 95% confidence interval; EASL, European Association of the Study of the Liver; SMI, skeletal muscle index; LT, liver transplantation; FFMI, Fat-free muscle index; SPSS, spontaneous portosystemic shunt.

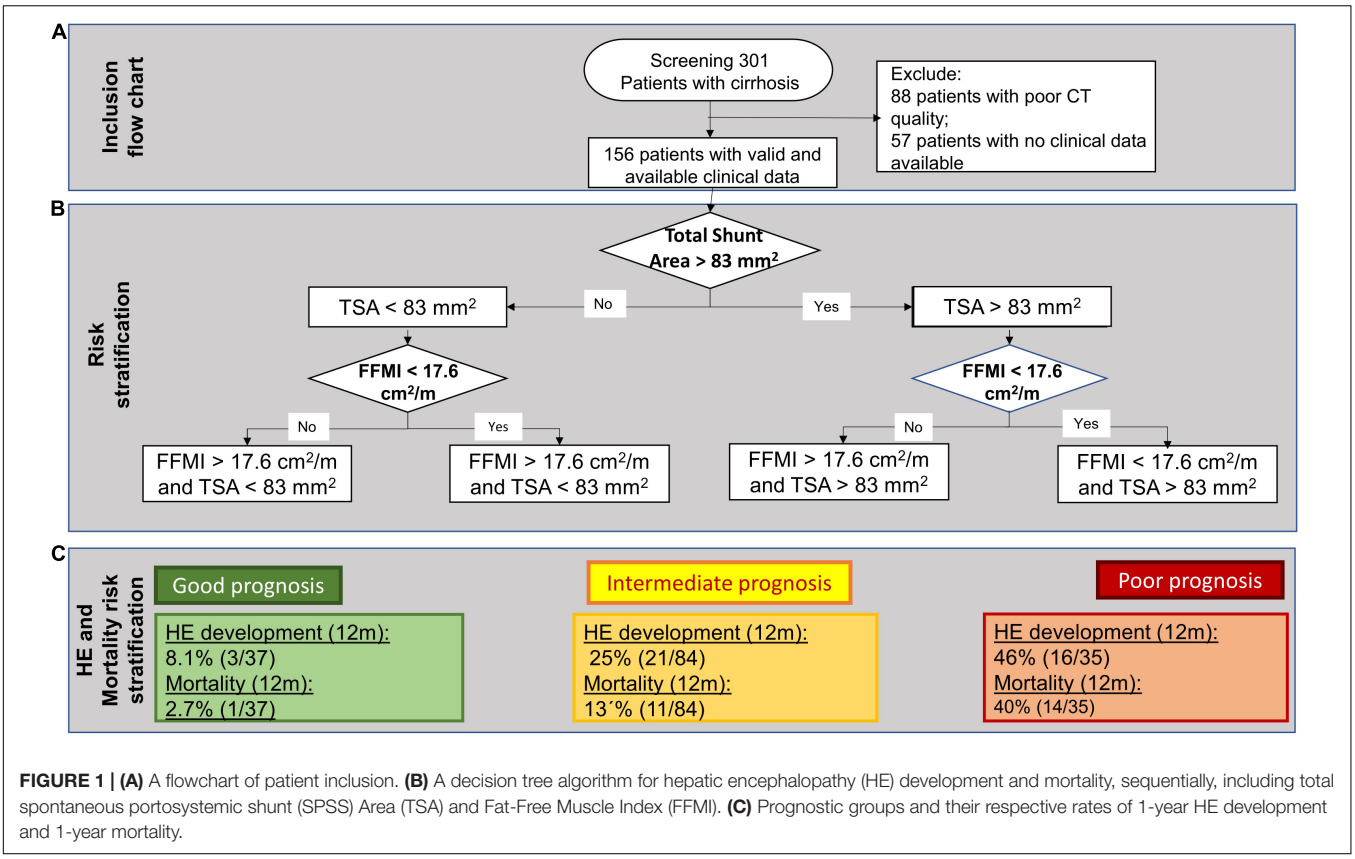

FIGURE 1 | (A) A flowchart of patient inclusion. **(B)** A decision tree algorithm for hepatic encephalopathy (HE) development and mortality, sequentially, including total spontaneous portosystemic shunt (SPSS) Area (TSA) and Fat-Free Muscle Index (FFMI). **(C)** Prognostic groups and their respective rates of 1-year HE development and 1-year mortality.

As it was done in previous studies, gastric, esophageal, and anal varices were excluded from the calculation of TSA, as these are rather considered vessel networks and, therefore, do not allow for exact determination of vessel diameter and, thereby, vessel area (16).

Assessment of Fat-Free Muscle Index

All patients underwent routine diagnostic multislice CT imaging of the abdomen in a supine position with the administration of iodinated contrast on a clinical CT-scanner (iCT, Philips Healthcare, Amsterdam, Netherlands). The typical imaging parameters were slice thickness of 1 or 2 mm, tube current (exposure time product) of 100 mAs, and tube voltage of 120 kVp.

Skeletal muscle areas of the paraspinal skeletal muscles at the intervertebral disc space level, between the third and fourth lumbar vertebra, were previously demonstrated to be highly correlated with total compartment volume and, therefore, were used for the estimation of skeletal muscle mass in this study (15).

To determine muscle quality, the skeletal muscle area was separated into areas of fatty and lean muscles based on densitometric thresholds. Fatty and lean muscle tissues were identified by ranges of low [−30 to 29 Hounsfield units (HU)] and high attenuation (30–100 HU), respectively. Skeletal muscle index (SMI) was measured as proposed in a previous study (12). Moreover, fat-free muscle area (FFMA) was calculated for each patient and was normalized for the patient's height to obtain fat-free muscle index (FFMI) using the equation:

$$\text{FFMI} = \text{FFMA}\left[\text{cm}^2\right] / \text{height}\ [\text{m}]\,. \quad (1)$$

Statistical Analysis

We performed descriptive statistics for all variables. A non-parametric testing was used to compare different groups when suitable. The correlation of metric variables was performed using Spearman's rank correlation coefficient. For the selection of cut-off values to determine low and high FFMI, a receiver-operating characteristics (ROC) analysis with the development of HE within a 1-year follow-up was calculated using the Youden index. The cut-off for TSA was used as previously reported in a large multicentre cohort (3).

The Kaplan–Meier analysis with the log-rank test was used to determine the impact of TSA and FFMI/SMI on the development of HE and mortality. Univariate and multivariate risk analyses were performed, including factors with the potential impact of outcome [age, baseline laboratory values, history of HE episodes, Chronic-liver-failure Consortium Acute Decompensation score (CLIF-C AD), as well as measurements of portosystemic shunt area and sarcopenia] with the Cox regression for 1-year mortality and occurrence of HE. A multivariate analysis included all values with $p < 0.05$ from univariate Cox regression. Prognostic scores with overlapping parameters (CLIF-C AD, MELD, and Child-Pugh score) were not entered simultaneously in multivariate

regression analyses due to collinearity. The number of liver transplantation (LT) as competing events was low (8%). Thus, LT was censored, and competing risk analysis was not performed.

Continuous variables are presented as median (range). Categorical variables are presented as absolute cases or percentages. All data were analysed using statistics software SPSS (version 25, IBM, Armonk, NY, United States). The *p*-value < 0.05 was considered a statistically significant difference.

RESULTS

General Patient Characteristics

Of the 301 evaluated patients, automated muscle measurement was not possible in 88 patients, which were therefore excluded. Of the remaining 213 patients, a clinical follow-up was available in 156 patients (**Figure 1A**). In this cohort, the median age at baseline was 58 (31–85) years and 92 (59%) patients were male. The majority had alcoholic cirrhosis (82, 53%). Thirty-one (21%) patients had viral liver cirrhosis and 43 (28%) had other causes of cirrhosis.

Seventy-eight patients (53%) had a history of ascites, 47 (30%) had gastrointestinal bleeding, and 21 (14%) had reported prior episodes of HE. At baseline, 92 (59%) patients exhibited ascites and 36 (23%) had an episode of HE. Seventeen patients (11%) were diagnosed with hepatic cellular carcinoma (within Milan criteria) at baseline.

At baseline, most patients had decompensated liver cirrhosis according to the Child-Pugh classification (55% with Child-Pugh class B/C). The median MELD and CLIF-C-AD scores were 12 (6–40) and 47 (31–78), respectively. Further general characteristics are detailed in **Table 1**.

Median follow-up was 19 (0–97) months. Within 1-year follow-up, 40 patients (26%) developed at least one episode of HE. These patients were significantly older, were predominantly female, and showed worse prognostic scores (**Table 1**). Compared to the 116 patients who did not develop HE in the 1-year follow-up, they were more likely to have ascites in their prior clinical history (ascites: 72 vs. 42%, $p = 0.001$) and HE at baseline (HE: 35 vs. 19%; $p = 0.002$). Moreover, baseline serum albumin levels were significantly lower in patients who experienced episodes of HE (29 g/l vs. 32 g/l, $p = 0.014$, **Table 1**).

Sarcopenia and TSA Classification

The mean FFMI was significantly lower in patients who developed episodes of HE compared to patients without episodes of HE within the follow-up period (24.8 vs. 32.1 cm^2/m, $p = 0.042$). With the receiver operating characteristics (ROC) analysis, with an HE development within 1-year follow-up as an outcome, an area under the curve (AUC) of 0.623 ($p = 0.023$, CI 0.522–0.724) was observed for FFMI. The optimal cut-off value was found at 17.6 cm^2/m (sensitivity 78%, specificity 47%) *via* the Youden index. Analysing sex-specific cut-offs did not improve performance. Therefore, sarcopenia was defined by a cut-off value of 17.6 cm^2/m with patients having a lower FFMI classified as being sarcopenic (L-FFMI). In total, 96 (62%) patients were defined as sarcopenic and 60 (38%) as not sarcopenic.

To quantify the amount of portosystemic shunting, a previously validated cut-off value was used with patients having a TSA above 83 mm^2 defined as having large TSA (L-TSA) (3).

Association of Sarcopenia and TSA With HE and Mortality

The Kaplan-Meier analysis showed that patients with sarcopenia exhibited significantly higher rates of HE development (32 vs. 15%, $p = 0.004$) and a higher 1-year mortality (24 vs. 5%, $p = 0.002$) (**Figures 2A,B**). Also, the patients with L-TSA were more likely to develop episodes of HE (45 vs. 30%, $p = 0.003$) and showed significantly higher 1-year mortality (31 vs. 20%, $p = 0.003$) (**Figures 2C,D**).

Acute-on-chronic liver failure (ACLF) was the most common cause of death (89%). Only three patients (11%) died of other causes (all malignancy) within 12 months follow-up (**Supplementary Table 1**). Of note, a patient stratification by L3-SMI (gender-specific cut-off values of 38.5 kg/m^2 for women and 52.4 kg/m^2 as validated in previous studies) did not show a significant difference for the development of HE and a 1-year mortality in this cohort (**Supplementary Figures 1A,B**). Therefore, L3-SMI was not used for further stratification.

To identify risk factors for the occurrence of HE, risk factor stratifications using Cox-regression analyses were performed. In a multivariate analysis, including all factors that were significantly associated with HE and 1-year mortality on the respective univariate analysis, large TSA, low FFMI, history of HE episodes, and CLIF-C AD remained as independent predictors for developments of HE within 1-year follow-up (**Table 2**). Similarly, large TSA, low FFMI, and CLIF-C AD were independent predictors of 1-year mortality (**Table 3**). Patients with sarcopenia (low FFMI) exhibited a distinctly higher risk to develop episodes of HE [hazard ratio (HR) = 2.685, 95% CI 1.215–5.932] and showed markedly increased risk of 1-year mortality (HR = 7.683, 95% CI 1.749–33.743). Similarly, individuals with L-TSA exhibited a higher risk to develop HE (HR 2.500, 95% CI 1.324–4.718) and to die within this time period (HR 3.050, 95% CI 1.323–7.035).

Combination of FFMI and TSA for the Prediction of HE Development and Mortality

To assess dependency between muscle mass (FFMI) and TSA, we performed a correlation analysis. This showed no significant correlation of FFMI with TSA (**Supplementary Figure 2**).

Using a decision tree algorithm, sequentially including FFMI and TSA, an easy to assess prognostic algorithm was developed (**Figure 1B**). First, the CT scans are assessed for the presence of L-TSA and, then, further stratified by the presence of L-FFMI. With this algorithm, patients were stratified into three risk groups: good, intermediate, and poor prognoses (**Figure 1C**).

TABLE 1 | General characteristics stratified for 1-year hepatic encephalopathy (HE) development.

	Parameter median (range) or absolute (%)	All (n = 156)	Patients without 1-year HE development (n = 116)	Patients with 1-year HE development (n = 40)
Baseline General	Age (in years)	58 (31–85)	57 (31–85)	62 (39–79)**
	Sex (male/-female)	92/64 (59/41%)	70/40 (64/36%)	22/24 (48/52%)*
	Etiology of cirrhosis (alcoholic/viral/others)	82/31/43 (53/20/28%)	65/22/29 (56/19/25%)	17/9/14 (43/23/35%)
	Height (in m)	1.72 (1.5–1.92)	1.73 (1.52–1.9)	1.70 (1.5–1.92)
	Weight (in kg)	77 (39–147)	78 (49–147)	79 (39–110)
Historical Clinical Events	Ascites	78 (53%)	49 (42%)	29 (72%)**
	Hepatocellular carcinoma	17 (11%)	14 (12%)	3 (8%)
	Hepatic encephalopathy	21 (14%)	12 (10%)	9 (23%)
	Spontaneous bacterial peritonitis	9 (6%)	6 (6%)	3 (8%)
	Hepatorenal syndrome	19 (12%)	12 (10%)	7 (18%)
	Gastrointestinal bleeding	47 (30%)	39 (34%)	8 (21%)
Baseline Clinical Events	Ascites	92 (59%)	64 (55%)	28 (70%)
	Hepatic encephalopathy	36 (23%)	22 (19%)	14 (35%)**
	Spontaneous bacterial peritonitis	16 (10%)	10 (9%)	6 (15%)
	Hepatorenal syndrome	22 (14%)	14 (12%)	8 (20%)
	Gastrointestinal bleeding	18 (12%)	16 (14%)	2 (7%)
Baseline Scores	MELD	12 (6–40)	11 (6–33)	13 (6–24)*
	MELD-Na	13 (6–40)	12 (6–33)	14 (7–28)
	Child-Pugh score	7 (5–13)	6 (5–13)	7 (5–10)**
	Child-Pugh (class A/B/C)	65/69/12 (45/47/8%)	54/45/9 (50/42/8%)	11/24/3 (29/63/8%)**
	CLIF-C-AD	47 (31–78)	45 (31–78)	49 (37–71)**
Baseline Laboratory	Sodium (mmol/l)	138 (122–147)	138 (122–147)	138 (127–144)
	Creatinine (mg/dl)	0.9 (0.3–5.1)	0.9 (0.3–3.3)	1.1 (0.6–5.1)
	Bilirubin (mg/dl)	1.7 (0.2–34.8)	1.5 (0.2–12)	1.8 (0.2–13.1)
	AST (U/l)	50 (12–387)	48 (12–300)	56 (14–190)
	ALT (U/l)	32 (8–282)	33 (8–187)	32 (11–282)
	Albumin (g/l)	31 (3–49)	32 (3–45)	29 (3–44)*
	INR	1.2 (0.9–3)	1.2 (0.9–3)	1.2 (1–2.4)
	WBC ($10^3/\mu$l)	5.7 (1–35.1)	5.2 (1.6–35.1)	5.7 (1.5–18.8)
	CRP (mg/dl)	9.5 (0.2–172)	7.9 (0.2–172)	11.4 (0.9–148)
	Platelets ($\times 10^9$/L)	111 (24–440)	109 (29–440)	126 (36–272)

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
MELD(-Na) Score, Model of End-Stage Liver Disease (Natrium) Score; CLIF-C-AD, chronic-liver-failure Consortium Acute Decompensation Score; AST, aspartat transaminase; ALT, alanine transaminase; INR, internationale normalized ratio (of prothrombin time); WBC, white blood cells; CRP, C-reactive protein.

Accordingly, the cohort was subdivided into these three subsets [Good prognosis: high FFMI and small TSA, n = 37 (24%); Intermediate prognosis: high FFMI and large TSA, n = 23 (15%) plus low FFMI and small TSA, n = 61 (39%); Poor prognosis: low FFMI and large TSA, n = 35 (22%); **Figure 3A**]. In the Kaplan–Meier analysis, comparing these subcohorts, the highest and lowest rates of HE development was observed for poor and good prognoses groups, respectively (**Figure 3B**). This result was confirmed in competing risk analysis for HE, with death and LT as competing events (**Supplementary Figure 3**). Similarly, the poor prognosis group had the highest 1-year mortality, while the good prognosis group had the lowest (**Figure 3C**).

According to these data, additional risk factor analyses for the development of HE and mortality were performed, comparing good and poor prognoses groups. In a multivariate Cox regression analysis, the simultaneous presence of L-FFMI and L-TSA (poor prognosis group), alongside the history of HE episodes, was the only independent predictor for the development of HE within 1-year follow-up (HR 12.790, 95% CI 2.928–55.864, $p = 0.001$, **Table 4**). Regarding 1-year mortality, the simultaneous presence of L-FFMI and L-TSA (poor prognosis group, HR 13.660, 95% CI 1.752–106.495, $p = 0.013$), CLIF-C-AD (HR 1.085, 95% CI 1.019–1.155, $p = 0.011$) were independent predictors (**Table 5** and **Figure 3D**). These data suggest an exponentially increased risk of the development of HE and of mortality in patients with L-FFMI and L-TSA.

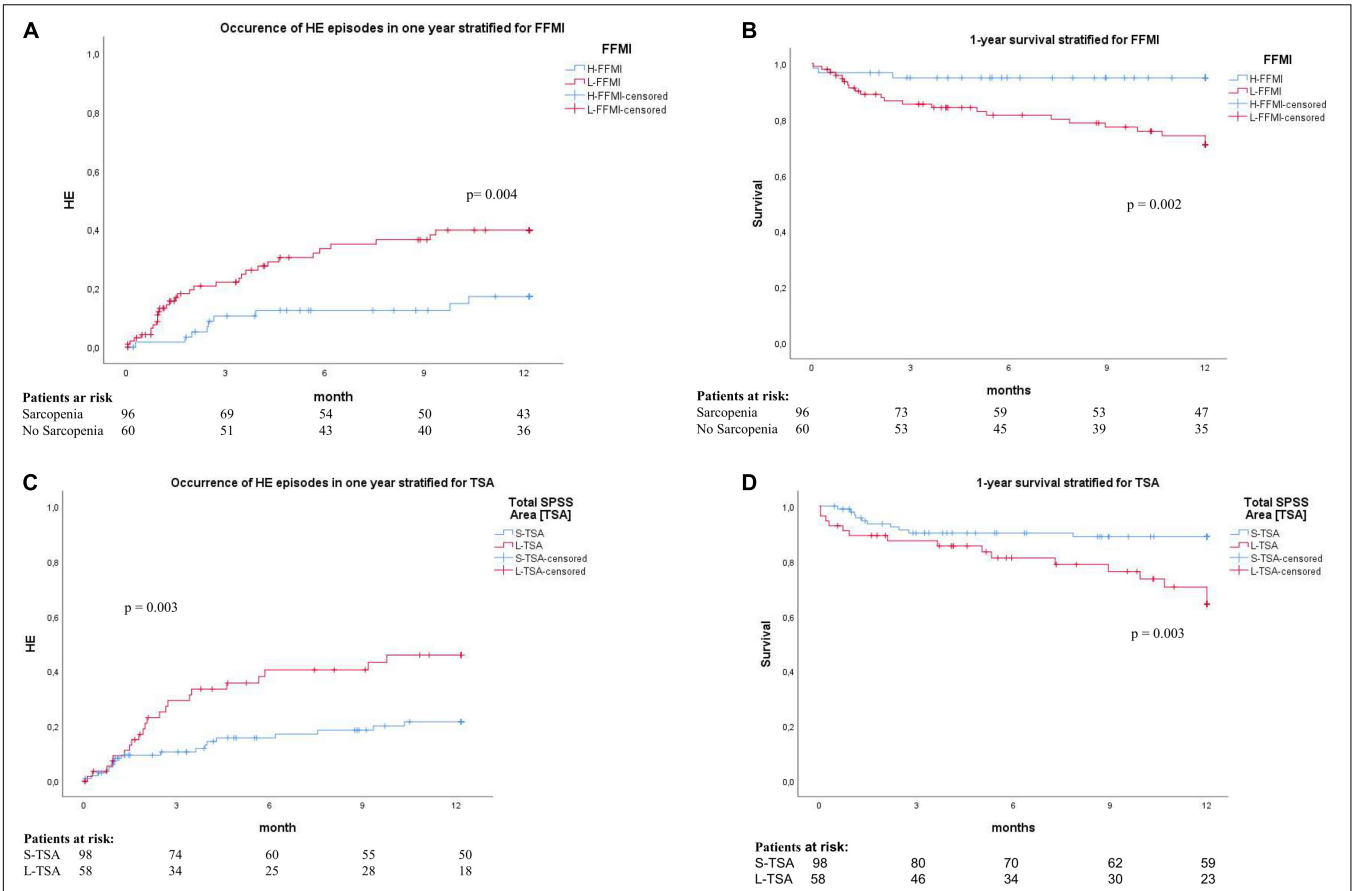

FIGURE 2 | (A) Cumulative incidence of development HE in the 1-year follow-up, stratified by FFMI. H-FFMI (high FFMI, blue line); L-FFMI (low FFMI, red line). P by log-rank. **(B)** Kaplan–Meier survival plot for 1-year survival, stratified by FFMI. H-FFMI (high FFMI, blue line); L-FFMI (low FFMI, red line). P by log-rank. **(C)** Cumulative incidence of development HE in the 1-year follow-up, stratified by TSA. S-TSA (small TSA, blue line); L-TSA (large TSA, red line). P by log-rank. **(D)** Kaplan-Meier survival plot for 1-year survival, stratified by TSA. S-TSA (small TSA, blue line); L-TSA (large TSA, red line). P by log-rank.

TABLE 2 | Univariate/multivariate Cox regression analysis for 1-year HE-development.

Parameters	univariate Cox-Regression				multivariate Cox-Regression			
	p	HR	CI Lower	CI Upper	p	HR	CI Lower	CI Upper
L-FFMI	0.006	2.809	1.336	3.908	0.015	2.685	1.215	5.932
L-TSA	0.005	2.460	1.318	4.591	0.005	2.500	1.324	4.718
Previous HE	<0.001	1.848	1.344	2.542	<0.001	2.001	1.425	2.809
CLIF-C-AD	0.003	1.050	1.017	1.085	0.023	1.039	1.005	1.073
MELD	0.042	1.049	1.002	1.098				
Child-Pugh	0.007	1.221	1.056	1.413				
Age	0.007	1.048	1.013	1.084	0.090			
Bilirubin	0.034	1.132	1.009	1.269	0.402			
Platelets	0.690	0.999	0.996	1.003				
CRP	0.444	1.004	0.993	1.016				

L-FFMI, low fat-free muscle index; L-TSA, large total shunt area; CLIF-C-AD, chronic-liver-failure Consortium Acute Decompensation Score; MELD, model for end-stage liver disease; CRP, C-reactive protein.

DISCUSSION

The present study describes the interplay of sarcopenia and TSA/SPSS in patients with liver cirrhosis. The L-FFMI and L-TSA as measures of sarcopenia and large portosystemic shunting, respectively, were identified as independent predictors for deleterious outcomes in patients with liver cirrhosis (3, 8, 10). Notably, these factors were observed not to be

TABLE 3 | Univariate/multivariate Cox regression analysis for 1-year mortality.

Parameters	univariate Cox-Regression				multivariate Cox-Regression			
	P	HR	CI Lower	CI Upper	p	HR	CI Lower	CI Upper
L-FFMI	0.006	5.460	1.639	18.191	0.007	7.683	1.749	33.743
L-TSA	0.005	3.069	1.391	6.772	0.009	3.050	1.323	7.035
CLIF-C-AD	<0.001	1.083	1.045	1.122	0.004	1.061	1.019	1.105
MELD	<0.001	1.149	1.087	1.215				
Child-Pugh	<0.001	1.724	1.414	2.102				
Age	0.062	1.040	0.998	1.083				
Bilirubin	<0.001	1.168	1.084	1.259	0.023	1.144	1.018	1.285
Platelets	0.211	0.997	0.992	1.002				
CRP	0.004	1.014	1.004	1.023	0.096			

L-FFMI, low fat-free muscle Index; L-TSA, large total shunt area; CLIF-C-AD, chronic-liver-failure Consortium Acute Decompensation Score; MELD, model for end-stage liver disease; CRP, C-reactive protein.

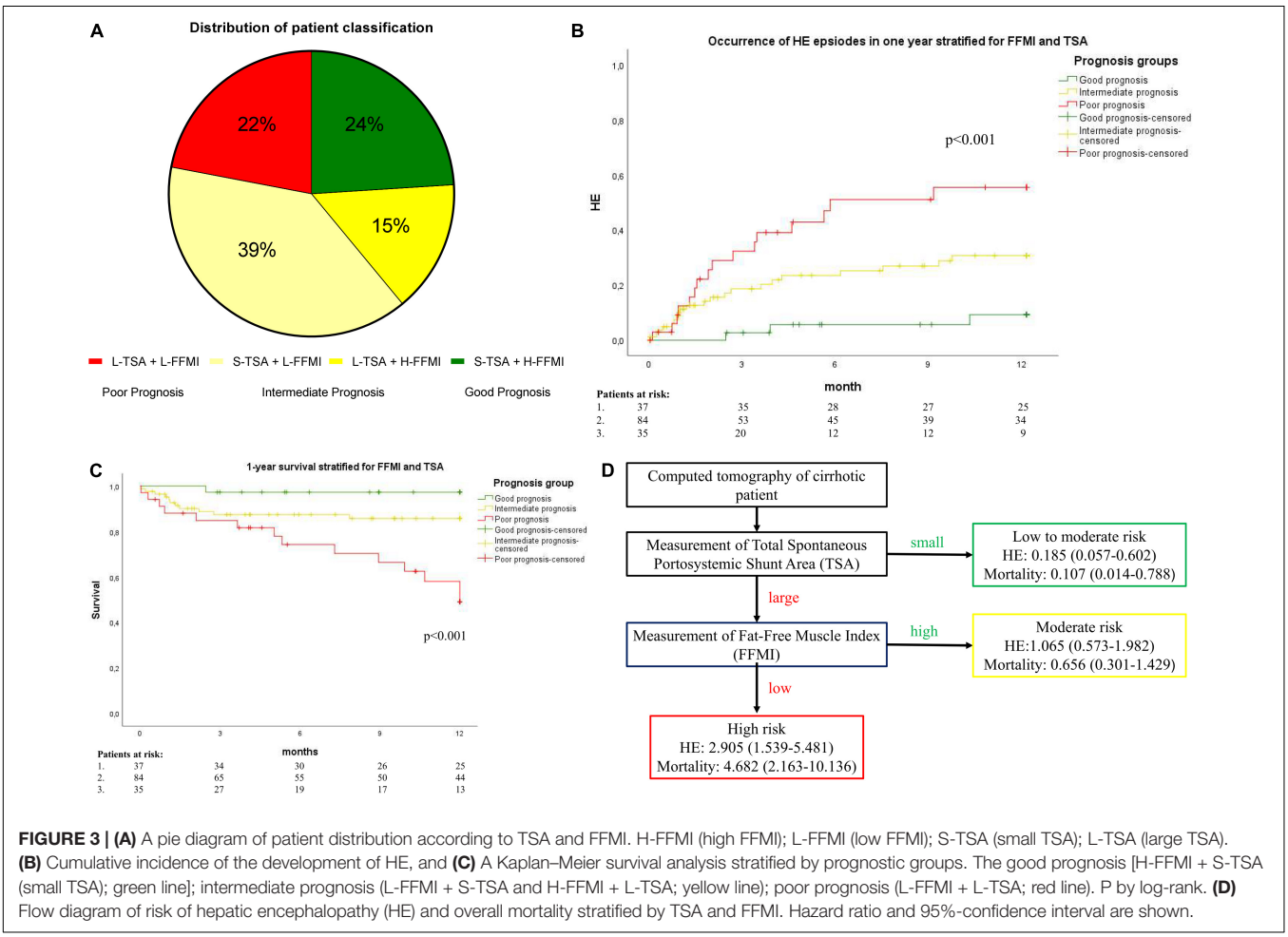

FIGURE 3 | (A) A pie diagram of patient distribution according to TSA and FFMI. H-FFMI (high FFMI); L-FFMI (low FFMI); S-TSA (small TSA); L-TSA (large TSA). **(B)** Cumulative incidence of the development of HE, and **(C)** A Kaplan–Meier survival analysis stratified by prognostic groups. The good prognosis [H-FFMI + S-TSA (small TSA); green line]; intermediate prognosis (L-FFMI + S-TSA and H-FFMI + L-TSA; yellow line); poor prognosis (L-FFMI + L-TSA; red line). P by log-rank. **(D)** Flow diagram of risk of hepatic encephalopathy (HE) and overall mortality stratified by TSA and FFMI. Hazard ratio and 95%-confidence interval are shown.

interrelated with one another in our cohort. If, however, both L-FFMI and L-TSA were present simultaneously, the risk for the development of HE and mortality within 1-year follow-up increases exponentially, independent of liver function. As both factors can be easily quantified from routine diagnostic CT-imaging, they may represent promising new imaging biomarkers for outcome stratification. In this context, an easy-to-apply prognostic algorithm is proposed by this study to complement the established methods of risk stratification.

Sarcopenia is generally accepted as a major risk factor for worsened outcomes in chronic diseases (16–18). As it was also

TABLE 4 | Univariate/multivariate Cox regression analysis for 1-year HE development comparing lower fat-free muscle index (L-FFMI) and large-total portosystemic shunt area(L-TSA) classification with high fat-free muscle index (H-FFMI) and small-TSA (S-TSA) classification.

1-year HE development	univariate Cox-Regression				multivariate Cox-Regression			
	p	HR	CI Lower	CI Upper	*p*	HR	CI Lower	CI Upper
L-FFMI & L-TSA	<0.001	9.013	2.615	31.064	0.001	12.790	2.928	55.864
Previous HE	<0.001	1.848	1.344	2.542	0.006	1.842	1.194	2.841
CLIF-C-AD	0.003	1.050	1.017	1.085	0.086			
Age	0.007	1.048	1.013	1.084	0.180			
Bilirubin	0.034	1.132	1.009	1.269	0.184			
CRP	0.444	1.004	0.993	1.016				

L-FFMI, low fat-free muscle Index; H-FFMI, high fat free muscle index; S-TSA, small total shunt area; L-TSA, large total shunt area; CLIF-C-AD, chronic-liver-failure Consortium Acute Decompensation Score; CRP, C-reactive protein.

TABLE 5 | Univariate/multivariate Cox-Regression for 1-year mortality comparing L-FFMI and L-TSA classification with H-FFMI and S-TSA classification.

1-year mortality	univariate Cox-Regression				multivariate Cox-Regression			
	p	HR	CI Lower	CI Upper	*p*	HR	CI Lower	CI Upper
L-FFMI and L-TSA	0.004	20.312	2.666	154.774	0.013	13.660	1.752	106.495
CLIF-C-AD	<0.001	1.083	1.045	1.122	0.011	1.085	1.019	1.155
Age	0.062	1.040	0.998	1.083				
Bilirubin	<0.001	1.168	1.084	1.259	0.110	1.172	0.965	1.424
CRP	0.004	1.014	1.004	1.023	0.350			

L-FFMI, low fat-free muscle Index; H-FFMI, high fat free muscle index; S-TSA, small total shunt area; L-TSA, large total shunt area; CLIF-C-AD, chronic-liver-failure Consortium Acute Decompensation Score; CRP, C-reactive protein.

shown to be related to adverse outcomes in patients with liver cirrhosis, it was recently included in international guidelines such as the Clinical Practice Guidelines of the European Association for the Study of the Liver (EASL) (12). Several studies dealt with the association of sarcopenia with clinical outcomes and mortality in patients with cirrhosis. One study showed that patients with sarcopenia were more likely to have a minimal HE and a higher risk of developing an episode of overt HE (OHE) (8). In other studies, sarcopenia was shown to predict complication rates and waiting-list mortality in patients prior to liver transplantation (9, 19). Studies on the outcomes following liver transplantation evaluated pre-transplant sarcopenia and showed poorer 1-year survival rates for sarcopenic recipients of liver transplants (11).

A CT-derived assessment of muscle areas is based on densitometric thresholds. Therefore, particularly in patients with liver disease, a distortion of anatomical composition and, thereby, precision of body compartment measurements due to fluid overload is a real concern. To mitigate the impact of ascites on the accuracy of muscle measurements, we decided to quantify skeletal muscles from the paraspinal compartment, as this site is distant from the abdominal cavity and was recently shown to allow for the estimation of skeletal muscle mass (15). Moreover, previous studies indicated that myosteatosis is an independent risk factor for HE development (20) and that, beyond mere muscle mass, a fat-free muscle fraction as another indicator of muscle quality seems to be of prognostic value, particularly in patients with liver disease (5, 10, 21, 22). Hence, the lean muscle fraction normalized for body height as an objective and comparable indicator of muscle quality, which can be opportunistically derived from diagnostic imaging, was investigated in this study.

Spontaneous portosystemic shunting is not only a risk factor for poor clinical outcomes on its own (2, 3). Moreover, patients with SPSS in combination with transjugular intrahepatic portosystemic shunt (TIPS) have been shown to have a higher risk of developing episodes of HE (23). However, the interplay of SPSS with other risk factors has not been studied, so far. In the presented study, the prognostic value of SPSS/TSA was complemented by adding FFMI, opportunistically measured from the same CT scans.

Particularly in patients with decompensated liver cirrhosis, adequate risk stratification and identification of high-risk patients (24, 25) are crucial due to a plethora of severe resulting complications.

Among the available risk factors, sarcopenia and portosystemic shunting particularly appeared suitable for evaluation as these factors were not only shown to predict severe complications and increased mortality but also represent potential therapeutic targets (26–28). Shunt embolization was shown to significantly decrease the risk of developing OHE (23, 29), while amelioration of sarcopenia prior to TIPS was demonstrated to enhance the clinical outcome, and a muscular activity has been shown to improve portal hypertension (10, 30, 31). Importantly, previous episodes of HE could be confirmed as a strong independent predictor of HE development and, thus, underlines the robustness of our data.

Although some previous studies indicated a potential interrelation between portosystemic shunts and sarcopenia (2, 13), these factors were observed to predict fatal outcomes

independent from one another in liver cirrhosis, as well as independent from liver function in our study. According to the survival analysis conducted in our study, both factors seemed to contribute to an increased risk for HE development at a similar rate; the risk exponentially increased when both factors were present simultaneously. This observation may indicate a potentially synergetic impact on the outcome for these factors and, therefore, may imply an additional predictive value by simultaneous measurement. It should be pointed out that this study does not aim to replace existing algorithms for sarcopenia screening and treatment, like the one presented in the current EASL Clinical Practice Guidelines (12), but rather complements them by adding SPSS/TSA as another aspect that can be easily measured opportunistically from the same scans.

The present study, therefore, warrants active screening for both sarcopenia and portosystemic shunting in patients with liver cirrhosis for several reasons. First, screening for these factors may help to facilitate the identification of high-risk patients, who may require intensified monitoring. Beyond that, early detection of sarcopenia and relevant portosystemic shunting ensure punctual therapeutic interventions and may contribute to a more precise and individualized treatment approach in these patients. A diagnostic CT is performed for several indications in patients with liver cirrhosis, such as evaluation for liver transplantation, TIPS, or hepatocellular carcinoma. Here, both sarcopenia and portosystemic shunts may be opportunistically quantified from available imaging and do not require additional efforts for assessment.

We acknowledge several limitations of this study. As with other monocentric retrospective investigations, the generality of the observation cannot be warranted, and validation studies are needed. Additionally, the impact of etiology could not be explored due to the small sample size and the diverse cohort, even though risk factors for sarcopenia and shunting vary depending on the cause of cirrhosis. Our results should be validated to define the relevance in specific etiologies.

Moreover, the retrospective character precluded functional assessment of muscle function. However, our results indicate that both sarcopenia and portosystemic shunting, which are frequent among patients with liver cirrhosis in various stages of the disease, may have a substantial impact on outcomes in these patients, independent from liver function. As these factors can be easily quantified from routine diagnostic CT imaging, our findings, therefore, are legitimately larger and have especially prospective investigations, which ultimately may reinforce the utility of our findings for clinical routine.

Due to the retrospective design, a selection bias cannot be ruled out. The reasons for patient exclusion were mainly missing follow-up data or poor image quality. Also, even though the main characteristics did not differ significantly between the included and the excluded patients, those who were eliminated from the study had significantly higher MELD- and Child-Pugh-Scores, as well as slightly less alcoholic liver disease. Therefore, our findings need further validation, especially in patients with advanced liver disease, but this was beyond the scope of our study.

It should also be noted that despite the presented prognostic value of our algorithm, liver function is still the main predictor of clinical outcomes, including HE development and mortality. Due to the lack of a validation cohort, we were not able to establish and properly calibrate a combined score like the MELD-sarcopenia score (32). This could be researched in future.

In conclusion, this study may indicate a synergistic impact of sarcopenia and portosystemic shunting on the outcome with an exponential risk increase for HE development and mortality, when both factors are present. Underlining the strength of our data, the role of sarcopenia and portosystemic shunting as biomarkers for deleterious outcomes in patients with liver cirrhosis was confirmed. This may suggest a great value of opportunistic screening for both sarcopenia and portosystemic shunts, from a routine diagnostic CT in patients with liver cirrhosis, in identifying high-risk patients with an easy-to-apply prognostic algorithm.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the dataset is restricted by GDPR. Requests to access the datasets should be directed to MP, michael.praktiknjo@ukbonn.de.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethikkommission der Medizinischen Fakultät der Universität Bonn. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AF and JA-O: acquisition of data, analysis and interpretation of data, drafting of the manuscript, and statistical analysis. JC, NB, AS, and CJ: acquisition of data, analysis and interpretation of data. UA, AL, JR, and JT: administrative support, interpretation of data, and critical revision of the manuscript regarding important intellectual content. JL and MP: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript regarding important intellectual content, funding recipient, administrative, technical and material support, and study supervision. All authors contributed to the article and approved the submitted version.

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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.2022.831005/full#supplementary-material

Supplementary Figure 1 | (A) Cumulative incidence of development HE in the 1-year follow-up, stratified by L3-SMI. No sarcopenia (blue line); sarcopenia (red line). P by log-rank. **(B)** Kaplan–Meier survival plot for 1-year survival, stratified by L3-SMI. No sarcopenia (blue line); sarcopenia (red line). P by log-rank.

Supplementary Figure 2 | Correlation plot for FFMI and TSA.

Supplementary Figure 3 | Competing risk analysis for HE and death/liver transplantation (LT).

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Predictors of Morbidity and Mortality After Colorectal Surgery in Patients With Cirrhotic Liver Disease–A Retrospective Analysis of 54 Cases at a Tertiary Care Center

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Background: Despite various existing scores that predict morbidity and mortality of patients with cirrhotic liver disease (CLD), data on specific risk stratification of patients with CLD undergoing colorectal surgery (CRS) are rare. The aim of this study was to assess in-hospital morbidity and mortality of patients with liver cirrhosis scheduled for CRS, with specific focus on possible pitfalls of surgery in this special cohort.

Methods: Between 1996 and 2018, 54 patients with CLD undergoing CRS were identified and included in this study cohort. Postoperative morbidity and mortality were assessed using the Clavien/Dindo (C/D) classification as well as by type of complication. Univariate and multivariate analyses were performed to analyze the predictive factors for increased postoperative morbidity.

Results: Of the patients, 37% patients died during the procedure or postoperatively. Major complications were seen in 23.1% of patients (>C/D IIIb). Patients with Child B or C cirrhosis as well as patients undergoing emergency surgery experienced significantly more major complications ($p = 0.04$ and $p = 0.023$, respectively). The most common complications were bleeding requiring blood transfusion (51.1%) and cardiocirculatory instability due to bleeding or sepsis (44.4%). In 53.7% of patients, an anastomosis was created without a protective ostomy. Anastomotic leakage occurred in 20.7% of these patients. Multivariate analysis showed that a primary anastomosis without a protective ostomy was the strongest risk factor for major complications ($p = 0.042$).

Discussion: Morbidity and mortality after CRS in patients with CLD remains high and is not only influenced by liver function but also by surgical variables. Considering the high rate of anastomotic leakage, creating a protective or definitive ostomy must be considered with regard to the underlying pathology, the extent of CLD, and the patient's condition. Moreover, our data suggest that surgery in these most fragile patients should be performed only in experienced centers with immediate contact to hepatologists and experts in hemostasis.

Keywords: cirrhotic liver disease, colorectal surgery, morbidity, mortality, colon, rectum

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INTRODUCTION

Cirrhotic liver disease (CLD) is the common end stage of a plethora of chronic injuries to the liver such as viral hepatitis, non-alcoholic steatohepatitis, and alcoholic hepatitis. These pathogenic stimuli first result in structural changes with excessive accumulation of extracellular matrix proteins such as collagen (fibrosis). This results in the destruction of the cellular architecture of the liver (cirrhosis). Consequently, functional liver tissue is reduced with a consecutive insufficiency of normal function. With decreasing liver function, the reduction of renal function and pulmonary function can also be observed. Increasing stiffness of liver tissue causes portal hypertension with the development of collaterals to the mesentericoportal circulation and spontaneous portosystemic shunts (1). Data from the Global Burden of Disease study showed that the age-standardized incidence rate of CLD was 20.7 per 100,000 in 2015, a 13% increase from 2000, with men being affected significantly more often than women (2). Because of pathophysiologic changes in homeostasis, patients with CLD have a higher risk of morbidity and mortality following surgery.

Surgery of the colon and rectum is among the most common surgical procedures in daily practice. The evaluation of clinical fitness to undergo colorectal surgery (CRS) in pre-existing CLD remains a clinical dilemma. Complications of cirrhosis such as coagulopathy, portal hypertension with varices, and ascites increase the immediate surgical risk. Surgery strongly increases the risk of acute or chronic liver failure (ACLF) postoperatively (3–5). It has been shown that morbidity and mortality among patients with CLD undergoing surgery for CRS are higher during hospitalization and up to 30 days, 90 days, and 1 year postoperatively (6, 7). On the other hand, minimally invasive technique, a more elaborate, patient-oriented surgical approach, and improved medical management of liver cirrhosis, has improved the surgical outcomes in the past. Nevertheless, deciding whether patients with CLD are clinically ready to undergo major abdominal surgery necessitates a specific, surgical risk assessment, especially since surgical decision making in these cases becomes more frequent, with an increase in the proportion of patients with CLD undergoing CRS to 1 in 100 in the past decades (7). If surgery is inevitable, for instance because of colonic perforation, a better understanding of risk factors associated with postoperative morbidity and mortality depending on the severity of cirrhosis and severity of the abdominal catastrophe is desirable. Despite existing methods for staging general morbidity and mortality of patients with CLD (i.e., Child–Turcotte–Pugh [CTP] stage and Model of End-Stage Liver Disease [MELD] score), data on specific surgical risk assessment of patients with CLD undergoing CRS (CRS) remain scarce.

In this study, we analyzed intraoperative and postoperative variables in patients after CRS and macroscopically or histologically confirmed liver cirrhosis. The aim of the study was to identify potentially modifiable risk factors to optimize the patient's condition prior to surgery and identify pitfalls of CRS in this special patient group to reduce postoperative morbidity and mortality.

PATIENTS AND METHODS

Patient Selection

We retrospectively identified all patients who subsequently underwent CRS between 1 January 1996 and 31 December 2018 at the University Hospital of Bonn, Germany. Only patients with a CLD diagnosis based on histological examination or intraoperative findings were included in this study (**Figure 1**). Data were obtained from the patients' medical charts, discharge letters, surgical reports, and anesthesiologic protocols. Demographics and laboratory data, medical or interventional therapy, and histological reports were analyzed.

Surgery

All patients underwent surgery of the colon and rectum. Procedures included right hemicolectomy, left hemicolectomy, resection of the transverse colon, sigmoid resection, subtotal, or total colectomy, proctocolectomy, colostomy, and reversal of Hartmann's procedure. Few patients received minor additional surgery (hernia repair, biopsy of the liver).

Severeness of Liver Cirrhosis

To determine severeness of CLD, CTP stage, and MELD scores were calculated.

Morbidity and Mortality

The Dindo/Clavien (D/C) score was used to classify the postoperative complications. Severe complications were defined as D/C grade $\geq$ IIIB (8). Morbidity was further categorized as follows:

- Cardiocirculatory instability requiring administration of vasopressors (caused by various reasons, i.e., bleeding, sepsis, and cardiac shock)
- Bleeding requiring transfusion of two or more units of red blood cells
- Surgical site infection
- Peritonitis
- Respiratory complications (necessity of thoracocentesis and mechanical ventilation)
- Renal complications (renal replacement therapy)
- Hydropic decompensation (necessity of abdominocentesis)
- Anastomotic leakage
- Infectious complications (other than surgical site infection).

Statistical Analysis

The patient data were evaluated using the IBM SPSS Statistics computer program (Version 25.0, IBM Corporation, Armonk, USA). The level of significance was chosen as $\alpha = 0.05$.

For the statistical description of the data, the frequency, the arithmetic mean, the median, the range with minimum and maximum, the standard deviation, and p-values were determined and are presented below. Various statistical tests were used to determine the influencing factors. For the analysis of variance, the Fisher test was used. The test for exact significance was carried out on both sides. The Cochran–Armitage trend test was used to check whether several variables can be viewed as varying linearly. The ranks formed were tested

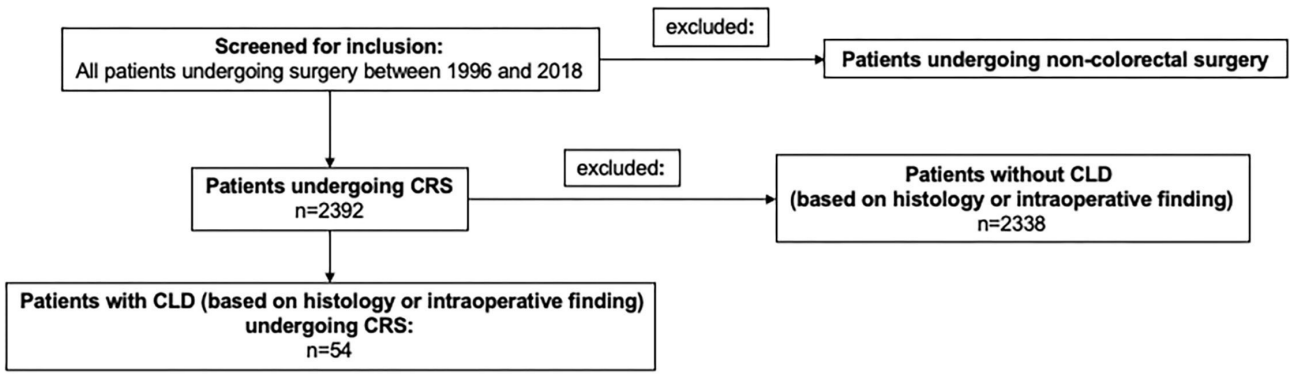

FIGURE 1 | Flow chart on patient selection and exclusion.

for significance by the Mann–Whitney *U* test. Since there were more than 30 patients, the asymptotic significance was calculated. Spearman's rank correlation and chi-square tests were used. In addition, a univariate regression analysis was carried out to test relationships, the significant values of which were finally analyzed in a multivariate model.

RESULTS

Patient Characteristics

Of all patients who underwent CRS between 1 January 1996 and 31 December 2018 at the University Hospital of Bonn, Germany; 54 patients with CLD diagnosis based on histological examination or intraoperative findings were included in this study and further analyzed.

Of the patients, 42 (77.8%) patients were male and the median age was 61.5 years (30–90 years). The median body mass index (BMI) was 25.4 (17.7–35.4). The morbid obesity was found in 14 patients (25.9%). Most of patients had alcoholic CLD (42.6%), followed by 29.6% of patients in whom no underlying cause of cirrhosis was found. Considering the high prevalence of diabetes and obesity in our cohort, a relevant number of these patients defined as cryptogenic conceivably "suffered" from nonalcoholic steatohepatitis (NASH). Among the 29.6% patients with cryptogenic cirrhosis, five showed BMI >30 (9.3%) and most likely suffered from NASH cirrhosis. Median MELD was 9 while over half (53.7%) of the patients exhibited CTP A cirrhosis. Median albumin was 3.1 mg/dl (interquartile range [*IQR*] 1.0–4.0) (**Table 1**). Regarding preoperative physical status classification (American Society of Anesthesiologists [ASA] score), 48.1% ($n = 26$) of patients were considered ASA III, 27.8% ($n = 15$) of patients were designated ASA II, and 7.4% ($n = 4$) of patients considered ASA IV, and 16.7% ($n = 9$) of patients were classified as ASA V. Of note, only one of the ASA V patients survived the procedure (**Table 2**).

Of the patients, 25 (50%) patients suffered from prior cardiac disorders (atrial fibrillation, coronary heart disease, chronic heart failure, and prior myocardial infarction), 22 (40.7%) patients suffered from acute or chronic renal failure, 11 (20.4%) patients suffered from prior stroke or epilepsy, and 10 (18.5%) patients suffered from diabetes mellitus or obesity (BMI > 30 kg/m^2). Pulmonary comorbidities (e.g., chronic obstructive pulmonary disease) were seen in 19 patients (35.2%) and peripheral arterial disease and other atherosclerotic diseases were seen in 25 patients (46.3%) (**Table 3**). The most common comorbidity was arterial hypertension ($n = 32$, 59.3%). No a single patient showed signs of acute or chronic liver failure prior to surgery.

Surgical Therapy

Surgical variables are presented in **Table 2**. Right-sided hemicolectomy (29.6%) and resection of the sigmoid colon (27.8%) were the most common procedures. In 53.7% ($n = 29$) of the procedures, a primary anastomosis without a protective ostomy was performed, while 14.8% of patients received a diverting loop ileostomy. Most cases were performed with an open surgical approach (88.9%). In two patients, a primary laparoscopic approach was converted to an open laparotomy (3.7%). Of the procedures, 61.1% ($n = 33$) were elective and 38.9% ($n = 21$) were in an emergency setting. In addition to CRS, 13% ($n = 7$) patients received a cholecystectomy, and hernia repair was needed in 7.4% ($n = 4$) of all surgeries. Liver biopsy was performed in 59.3% ($n = 32$) of patients.

Indications for Surgery

Cancers of the colon or rectum ($n = 24$, 44.4%), colon perforation ($n = 10$, 18.5%), and diverticulitis without perforation ($n = 9$, 16.7%) were the most common indications for surgery. Surgery was also performed because of ischemia ($n = 4$, 7.4%), bleeding ($n = 2$, 3.7%), stenosis of a pre-existing ostomy ($n = 2$, 3.7%), and reversal of Hartmann's procedure ($n = 2$, 3.7%).

Intraoperative Transfusion of Blood Products

Transfusion of red blood cells was needed in 22 patients during the procedure, with a maximum of 16 units of red blood cells. There were 32 patients who received fresh frozen plasma, with a maximum of 12 units. Using Fisher's test, no significant correlation was found between necessity of transfusion and emergency surgery. The Cochran–Armitage trend test showed no significant trend concerning transfusion of red blood cells

TABLE 1 | Patient characteristics.

Characteristics	Mean (SD)/f (f %)	Median	Min.-Max.
Age (years)	61.0 ± 13.5	61.5	30–90
Sex			
Male	42 (77.8 %)		
Female	12 (22.2 %)		
Height (cm)	174.1 ± 8.9	173	156–193
Weight (kg)	81.3 ± 19.4	80	48–130
BMI	26.4 kg/m² ± 4.9	25.4	17.7–35.4
Alcoholic	23 (42.6 %)		
Cryptogenic (inter alia, NASH)	16 (29.6 %)		
Viral	6 (11.1 %)		
PBC	6 (11.1 %)		
Autoimmune hepatitis	1 (1.9 %)		
Other	2 (3.7 %)		
Child-Turcotte-Pugh stage			
A	29 (53.7 %)		
B	14 (25.9 %)		
C	6 (11.1 %)		
MELD score	12.3 ± 6.9	9	6–31
Laboratory values			
INR	1.2 ± 0.4	1.10	0.9–3.8
Bilirubin (mg/dl)	1.7 ± 2.4	0.92	0.2–10.8
Creatinine (mg/dl)	1.7 ± 1.7	1.0	0.4–9.0
Albumin (g/dl)	3.0 ± 0.7	3.1	1.0–4.0
Thrombocytes (G/l)	220.6 ± 111.0	223	24–439
Ascites			
No/little	30 (55.6 %)		
Moderate/treatable	9 (16.7 %)		
Massive/refractory	5 (9.3 %)		
Portal hypertension	19 (35.2 %)		
Esophageal varices	15 (27.8 %)		
Splenomegaly	14 (25.9 %)		
Icterus	6 (11.1 %)		
Hepatocellular carcinoma	4 (7.4 %)		
Encephalopathy	3 (5.6 %)		

PBC, Primary biliary cholangitis; MELD, Model-of-end-stage-liver-disease; INR, International Normalized Ratio; SD, standard deviation; f, frequency; f %, frequency in percent; N = 54.

TABLE 2 | Surgical variables.

Characteristics	Mean (SD) / f (f %)	Median	Min.-Max.
Procedure			
Right hemicolectomy	16 (29.6 %)		
Left hemicolectomy	8 (14.8 %)		
Resection of the transverse colon	5 (9.3 %)		
Colectomy	4 (7.4 %)		
Sigmoid resection	15 (27.8 %)		
Reversal of Hartmann's procedure	2 (3.7 %)		
Colostomy	4 (7.4 %)		
Postoperative intestinal continuity			
Primary anastomosis	29 (53.7 %)		
Hartmann's procedure	17 (31.5 %)		
Diverting ostomy	8 (14.8 %)		
Surgical approach			
Open	48 (88.9 %)		
Laparoscopic	4 (7.4 %)		
Conversion	2 (3.7 %)		
Urgency			
Emergency	21 (38.9 %)		
Elective	33 (61.1 %)		
ASA classification			
1	0 (0 %)		
2	15 (27.8 %)		
3	26 (48.1 %)		
4	4 (7.4 %)		
5	9 (16.7 %)		

ASA, American Society of Anesthesiologists; SD, standard deviation; f, frequency; f %, frequency in percent; N = 54.

TABLE 3 | Comorbidities.

	f (f %)
Arterial hypertension	32 (59.3 %)
Cardiac CM	27 (50.0 %)
Vascular CM	25 (46.3 %)
Renal CM	22 (40.7 %)
Pulmonal CM	19 (35.2 %)
Neurological CM	11 (20.4 %)
Diabetes mellitus	10 (18.5 %)

CM, Comorbidity; f, frequency; f %, frequency in percent; N = 54.

and postoperative major complications (>D/C IIIb) in contrast to transfusion of plasma, where a significant trend toward major complications was found ($p = 0.031^*$). Interestingly, using Spearman's rank correlation, a statistically significant interdependence between the number of red blood cell-units and units of plasma and severity of postoperative complications was found ($p = 0.04^*$ and $p = 0.036^*$, respectively).

Postoperative Complications

General Post-operative Complications

More than half of patients experienced major complications (> D/C IIIb) (59.3%, $n = 22$). Of the 54 included patients 20 (37%) died intraoperatively or in the postoperative course (D/C V, **Table 4**). In detail, seven patients died on table, five during Hartmann's procedure, and 13 patients died in the postoperative course. In 15 patients, redo procedures were necessary (27.8%). A significantly higher risk of redo procedures was observed after emergency CRS ($p = 0.013^*$). In 44.4% of cases ($n = 24$) cardiocirculatory instability requiring vasopressor therapy occurred, with bleeding ($n = 21$, 38.9%), surgical site infection ($n = 20$, 37%), and peritonitis ($n = 17$, 31.5%) being the most common accompanying complications (**Table 5**).

TABLE 4 | Complications according to Dindo/Clavien.

D/C classification	f (f %)
I	5 (9.3 %)
II	15 (27.8 %)
IIIa	2 (3.7 %)
IIIb	11 (20.4 %)
IV	1 (1.9 %)
V	20 (37 %)

D/C, Dindo/Clavien; f, frequency; f %, frequency in percent; N = 54.

TABLE 5 | Post-operative complications.

	f (f %)
Cardiocirculatory instability	24 (44.4 %)
Bleeding	21 (38.9 %)
Surgical site infections	20 (37.0 %)
Peritonitis	17 (31.5 %)
Respiratory complications	16 (29.6 %)
Renal complications	16 (29.6 %)
Hydropic decompensation	13 (24.1 %)
Anastomotic leakage	6 (11.1 %)
Infection	
Urinary tract	5 (9.3 %)
Pneumonia	5 (9.3 %)
Other	15 (27.8 %)

f, frequency; f %, frequency in percent; N = 54.

TABLE 6 | Mortality and rate of anastomotic leakage.

	Total	Mortality	Insufficiency
Primary anastomosis	29	9	6
Hartmann's procedure	17	8	2
Diverting ostomy	8	3	0

Severity of preoperative presence of ascites correlated with morbidity according to Spearman's rank correlation ($p = 0.024^*$). Decompensated ascites was seen in 13 patients (24.1%) postoperatively, although no correlation with a higher-grade morbidity was found. Postoperative peritonitis was also significantly correlated with severity of postoperative complications using the chi-square test ($p = 0.038^*$).

Anastomotic Leakage and Mortality

Anastomotic leakage occurred in six of the 29 cases in which a primary anastomosis was created (20.7%), and leakage of the colonic or rectal stump after Hartmann's procedure was observed in two of 17 cases (11.8%). Of the 29 patients receiving a primary anastomosis, nine (31%) died (**Table 6**).

TABLE 7 | Univariate analysis of risk factors indicating postoperative morbidity.

	p	Odds ratio	95% confidence interval
CTP value	0.038*	1.633	1.028–2.594
ASA classification	0.022*	2.237	1.125–4.452
MELD Score	0.034*	1.112	1.008–1.228
Emergency procedure	0.003*	8.143	2.001–33.144
Albumin value	0.027*	0.269	0.084–0.861
Malignant disease	0.021*	0.260	0.083 –0.817
Primary anastomosis	0.006*	5.667	0.052–0.602
Hartmann's procedure	0.026*	4.926	1.210–20.050

Significance at 5% level/ = $p < 0.05$. N = 54.*

Univariate Analysis of Post-operative Morbidity and Mortality

Univariate regression analysis showed significant correlation of ASA status and major complications as well as death ($p = 0.022^*$ and $p = 0.000^*$, respectively). In contrast to procedures performed in an elective setting, emergency surgery was accompanied by an increased risk of major complications and mortality ($p = 0.003^*$ and $p = 0.008^*$, respectively). The CTP stage showed significant correlation with the occurrence of major complications and mortality in univariate regression analysis ($p = 0.038^*$ and 0.006^*, respectively). The probability of mortality increases with CTP stage: 7% of patients in CTP stage A died, 14% in CTP B, and 67% in CTP C. This finding was also observed using the MELD score (**Table 7**). While morbidity and mortality significantly increased with higher CTP stage and MELD scores, using the Mann–Whitney *U* test, no correlation between CTP stage and MELD score and the probability of anastomotic leakage was found (CTP: $p = 0.888$; MELD: $p = 0.435$).

When investigating laboratory values, only low albumin was significantly correlated with an increased risk of morbidity ($p = 0.027^*$) but not mortality. Low platelets, low Internationally Normalized Ratio (INR), and bilirubin were not correlated with increased morbidity or mortality.

Although surgery for malignant disease increased the risk of major complications in the postoperative course ($p = 0.021^*$), surgery in presence of hepatocellular carcinoma did not ($p = 0.182$).

Only pre-existing renal dysfunction (not creatinine value alone) significantly predicted morbidity in univariate regression analysis ($p = 0.043^*$).

Interestingly, factors such as age, morbid obesity, pre-existing diabetes, and intraoperative transfusion did not significantly predict morbidity or mortality in univariate regression analysis.

Multivariate Analysis of Post-operative Morbidity and Mortality

All variables significantly predicting morbidity in univariate regression analysis were considered for multivariate regression analysis. Here, only a primary anastomosis without diverting ostomy was associated with increased morbidity ($p = 0.013^*$).

DISCUSSION

Cirrhotic liver disease is a known major risk factor for perioperative morbidity and mortality after surgery in general and much so after CRS. In patients undergoing non-transplant surgery, the usual scoring systems determining the severity of cirrhosis are correlated with increased postoperative morbidity, i.e., CTP stage in estimating 30-day morbidity and MELD for estimating mortality within the first 3 months postoperatively (9). Our data underline this finding, demonstrating increased mortality with increased CTP stage and a higher morbidity depending on both, CTP stage and MELD scores. Meunier et al. reported a 26% mortality in 41 patients with CLD undergoing CRS for various indications (10). Our data exceeded this already high mortality, showing 37% in-hospital mortality irrespective of an elective or emergency setting. The results of this study correlate well with the data of Nguyen et al. showing a 35.8% mortality in a cohort of a nationwide inpatient sample with cirrhosis and portal hypertension undergoing emergency CRS (11). Indeed, our data as well suggest a strong trend toward morbidity and mortality depending on the acuteness of surgery and CTP/MELD stage.

Several parameters related to the acuteness of surgery and CTP and MELD stages have been evaluated as possible predictors of morbidity and mortality in CLD patients undergoing CRS. The previous studies have identified hepatic coagulopathy complicating surgery in this patient group (12). Indeed, we were able to determine a 38.9% bleeding complication rate in our cohort although altered standard coagulation laboratory tests did not predict morbidity or mortality. Interestingly, in a previous study of ours, hemorrhage was the most common complication after small bowel surgery in presence of CLD, although no correlation to altered coagulation parameters or stage of liver disease (13). This agrees with clinical experience that because of the patients' altered intra-abdominal hemodynamic balance and hepatic coagulopathy but normal classical laboratory tests (INR and/or prothrombin time), patients with CLD are at a much higher risk for relevant hemorrhage. Hepatic insufficiency alters pro- and anti-hemostatic pathways, providing a fragile balance to the patient's coagulation status, which is additionally unbalanced by an operative trauma including, for example, hypothermia (14). Contrary to our findings, prothrombin time prolongation predicted morbidity in a multivariate analysis of 161 patients with CLD undergoing CRS (15), stressing that a thorough examination of the patient's hemostatic situation is particularly desirable. Planning any surgical intervention must, therefore, include close consultation of hemostasis experts and a comprehensive laboratory workup (i.e., repetitive global coagulation tests) seems advisable.

Low albumin presented as a significant predictor of postoperative morbidity in our cohort. Preoperative low albumin has been identified as an independent risk factor for postoperative morbidity and mortality after CRS irrespective of a pre-existing liver disease even in highly advanced surgery within enhanced recovery pathways (16, 17). Low albumin reflects on poorer nutritional status and immune functions in CLD patients. Different scores and ratios consisting of albumin, platelets, hemoglobin, and lymphocytes representing the inflammatory status of the patient prior to surgery have been evaluated and correlated with morbidity and mortality in one form or another (18–20). In general, an equation of preoperative immune status and nutritional status seems to correlate with postoperative outcome and potentially even long-term oncological survival. Yet final validation of these scores and introduction into clinical practice are still absent. In patients with CLD, decreased hepatic synthesis results in altered immune function and malnutrition. Malnutrition is commonly seen in patients with cirrhosis with an incidence of 60–100% in patients with advanced CLD. Malnutrition is a poor prognostic factor associated with lower survival and morbidity in patients with CLD even without a traumatic stimulus such as abdominal surgery but especially thereafter (21–26). Cirrhosis-associated immune dysfunction describes an increased inflammatory phenotype of systemic immune cells with a decreased antipathogenic functionality, resulting in infectious and inflammatory complications. This becomes especially relevant in patients with intestinal dysbiosis due to therapeutic interventions let alone antibiotic therapy. In this context, the gut–liver–immune axis has become an important target in therapy of CLD. Alterations of this axis by surgical manipulation of the intestine lead to alterations in immune homeostasis and potentially decrease residual liver function (27). In our cohort, surgical site infection and peritonitis were two of the most common complications following CRS in patients with CLD, reflecting on the patients' altered immune response and highlighting the importance of preoperative optimization of the functional and nutritional status of patients with CLD.

The most interesting result of our study is the significantly higher rate of major complications and thus morbidity when a primary anastomosis without a protective ostomy was created. The rate of anastomotic leakage, the most catastrophic complication in our cohort, was very high, 20.7%, leading to a higher overall morbidity and mortality. CLD has not yet been clearly identified as a risk factor for anastomotic leakage in the recent literature (28, 29). In detail, a study by Sabbagh et al. (27) showed no differences in anastomotic healing in 40 patients with CLD compared to 80 non-CLD patients. Of note, the acuteness of surgery and Child stage of patients must be taken into consideration when comparing the results of these studies. To our knowledge, only one study stratified the higher risk of anastomotic leakage in the presence of CLD (30). In a retrospective analysis of patients of a prospective colorectal database, Käser et al. showed a 12.5% rate of anastomotic leakage in patients with CLD compared to a 2.5% rate of anastomotic leakage in non-CLD patients. Arguably, with only 24 patients in the CLD cohort *vs*. 1,851 patients in the non-CLD cohort, the sample size was rather small and underlying reasons for defective anastomotic healing were not given. Altered intra-abdominal hemodynamics due to portosystemic collaterals, ascites, intestinal dysbiosis, and altered immune response can be responsible for this finding (13, 31). In an animal model of anastomotic healing in rats with CLD, a decreased concentration of hydroxyproline was found in anastomotic regions of cirrhotic rats compared to healthy controls. Since

hydroxyproline is important in stabilizing the collagen in the anastomotic junction, this finding should be evaluated in further studies (32). Malnutrition and low protein levels have been evaluated as an influence in animal studies as well (33). It has also been shown in a retrospective study in humans that low albumin is associated with disturbances in anastomotic healing (34). It might be hypothesized that in our cohort, a primary anastomosis was only created in patients with mild cirrhosis. Still, these patients were more likely to develop severe complications, with a mortality of 31%. In accord with the literature, low albumin and intestinal dysbiosis with an altered gut–immune–liver axis as a marker of liver dysfunction could be associated with the poor outcomes after CRS (35, 36). Additionally, in our cohort, a considerable number of patients (18.5%) suffered from diabetes, which is also a risk factor for anastomotic leakage and should be taken into consideration. A retrospective analysis of patients undergoing anterior rectal resection showed an incidence of anastomotic leakage of 9.3% in patients with diabetes (odds ratio = 2.906, 95% confidence interval 1.130–7.475; $p = 0.027$) (37). In our cohort, the incidence of anastomotic leakage was higher with 20.7%. Furthermore, it was not investigated whether patients continuously consumed alcohol or had already stopped drinking before surgery. It is hypothesized that continued alcohol abuse itself is a risk factor for anastomotic leakage. An incidence of anastomotic leakage of 21.3% was described in patients with continued alcohol abuse (38), although alcohol consumption was not associated with leakage in a large meta-analysis of 17 studies (39). Pathologic abuse of alcohol might be a surrogate for poor nutritional status and decreased liver function with consecutively decreased the protein status in these patients, even if no manifest CLD has developed yet. In this context reasons for anastomotic leakage in cirrhotic patients and patients with continued alcoholism are multifactorial (portal hypertension with impaired regulation of splanchnic blood flow, protein metabolism disorder, immune dysfunction syndrome especially in the presence of ascites). When investigating anastomotic leakage in patients with CLD specifically, protein status, presence of ascites, peritonitis, acuteness of surgery, requirement for vasopressor therapy, etc., must be considered and should be analyzed in further upcoming studies.

It should be mentioned that the performance of Hartmann's procedure was also predictive of a higher morbidity. Arguably, there might be a bias here as discontinuing resection of the colon was performed only in high-risk patients with severe cirrhosis susceptible to a higher complication rate. Interestingly, no correlation between CTP and MELD stage and the occurrence of anastomotic leakage was found in our cohort, suggesting that anastomotic leakage, the most feared complication after CRS, can occur at any stage of cirrhosis with fatal consequences.

Because of the high rate of anastomotic leakage in our cohort, the creation of a protective ostomy must be considered in patients with liver cirrhosis. Of note, various factors such as pre-existing diabetes, continued alcohol abuse, and the presence of peritonitis and ascites must be taken into consideration when deciding whether to create a primary anastomosis in patients with CLD. Interestingly, CTP stage and MELD score did not influence the probability of anastomotic leakage in our cohort, although this might be attributed to the small sample size.

Limitations of this study are its retrospective nature with a relatively small sample size and thus an obvious lack of statistical power. Interestingly, this is a problem of most studies investigating the postoperative outcomes in patients with CLD, with the most recent study investigating the colectomy in patients with CLD identifying only 248 patients with CLD in a cohort of 36,380 patients after CRS (7). However, we tried to reduce any bias by including all consecutive patients at a single tertiary referral center who underwent CRS with or without anastomosis. Despite these limitations, our study delivered valid results to be considered by colorectal surgeons, especially concerning the risk of anastomotic leakage. Potential preoperative optimization of functional and especially nutritional status should be considered and further investigated in prospective studies concerning surgery in patients with CLD.

CONCLUSIONS

Morbidity and mortality after CRS in patients with CLD remain high and are influenced not only by liver function but also by surgical variables. Considering the high rate of anastomotic leakage, construction of a protective or definitive ostomy must be considered, taking pre- and intraoperative variables into account. Preoperative optimization of patients' functional and nutritional status should be considered if possible. Moreover, our data suggest that surgery in these most fragile patients should be performed only in experienced centers with immediate contact to medical specialists and experts in hemostasis.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Conceptualization, data curation, formal analysis, investigation, and writing–original draft: CVB, TV, and CB. Methodology: CVB, LB, MW, CB, and TV. Resources: JK. Supervision: HM, SM, and JK. Writing–review and editing: CVB, AS, HM, SM, LB, MW, JK, and TV. All authors have read and agreed to the published version of the manuscript.

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A deep learning approach for detecting liver cirrhosis from volatolomic analysis of exhaled breath

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Liver disease such as cirrhosis is known to cause changes in the composition of volatile organic compounds (VOC) present in patient breath samples. Previous studies have demonstrated the diagnosis of liver cirrhosis from these breath samples, but studies are limited to a handful of discrete, well-characterized compounds. We utilized VOC profiles from breath samples from 46 individuals, 35 with cirrhosis and 11 healthy controls. A deep-neural network was optimized to discriminate between healthy controls and individuals with cirrhosis. A 1D convolutional neural network (CNN) was accurate in predicting which patients had cirrhosis with an AUC of 0.90 (95% CI: 0.75, 0.99). Shapley Additive Explanations characterized the presence of discrete, observable peaks which were implicated in prediction, and the top peaks (based on the average SHAP profiles on the test dataset) were noted. CNNs demonstrate the ability to predict the presence of cirrhosis based on a full volatolomics profile of patient breath samples. SHAP values indicate the presence of discrete, detectable peaks in the VOC signal.

KEYWORDS

deep learning, cirrhosis, volatile organic compound, prediction, breath

Introduction

Cirrhosis of the liver is an advanced stage of disease in which the liver is damaged from scarring or fibrosis as a result of chronic hepatic injury that can arise from conditions such as chronic infection with Hepatitis B or C virus, excess alcohol or other causes (1). Cirrhosis can be classified as compensated or decompensated. A diagnosis of compensated cirrhosis can be challenging as these patients can be asymptomatic and may have normal laboratory or imaging findings.

The presence of cirrhosis can be inferred from clinical, laboratory, radiologic, or elastographic findings, but a liver biopsy represents the gold-standard for diagnosis (2). Cirrhosis is a preneoplastic condition and a major risk factor for hepatocellular carcinoma (HCC) which is the sixth most prevalent cancer and third leading cause of

cancer-related death (3). Once diagnosed, close monitoring for progression as well as active surveillance for onset of HCC are essential (4). The onset of complications such as ascites, varices, and hepatic encephalopathy define decompensated cirrhosis, and are associated with a higher risk of death (5).

Liver disease has long been recognized to be associated with detectable changes in a patient's breath, e.g., fetor hepaticus (6, 7). These result from the presence of diverse range of Volatile Organic Compounds (VOCs), which may consist of byproducts of liver metabolism that are released into the bloodstream and eventually eliminated in the patient's breath. Importantly, VOCs have been associated with liver cirrhosis (8) and fibrosis (9). Thus, a reliable detection of disease-associated VOC or other metabolites altered by liver damage have the potential for use as a non-invasive test for the diagnosis and monitoring of cirrhosis.

Global volatolomic analyses can be performed on exhaled breath samples by separating and detecting individual VOCs. The approaches for detection of individual VOC are laborious and time consuming, and often require the use of sophisticated equipment. A limitation of several prior breath-based biomarker studies is that they rely on identification of a single VOC such as limonene (10), which may miss more complex signatures of disease. The large number and variability of VOC in exhaled breath have hampered the development of individual breath-based biomarkers for disease. Recognizing the inherent variability and diversity of individual VOCs with biomarker potential, we sought to evaluate approaches for an unbiased global volatolomic profiles as disease biomarkers. For our study, global volatolomic profiling was performed using thermal desorption (TD) with gas chromatography (GC) based separation coupled with field asymmetric ion mobility spectrometry (FAIMS) for biomarker discovery.

Analysis of volatolomic profiles has been greatly aided with the use of artificial intelligence (AI) algorithms such as deep CNNs which can analyze relationships between all detectable compounds represented in a breath sample.

This study builds upon existing techniques to diagnose liver cirrhosis from non-invasive breath samples using an artificial neural network based on TD-GC-FAIMS signal. We demonstrate that cirrhosis results in detectable, quantifiable changes in the volatolomic profile of a patient's breath. Furthermore, by utilizing Shapley Additive Explanations, we demonstrate a set of volatolomic features that correspond to disease prediction and reflect biomarkers that can be used for the detection of disease without the need to rely on identification of individual VOCs.

Materials and methods

Study participants

This prospective study was conducted under a Mayo Clinic institutional review board (IRB) approved protocol and conformed to the ethical guidelines of the Declaration of Helsinki. Informed consent was obtained from study participants in writing. The trial is registered at clinicaltrials.gov (NCT04341012).

All participants in this single-center prospective study were enrolled between September 2019 and March 2020. The study inclusion criteria were the ability to provide informed consent and age greater than 18 years. Healthy volunteers were employees of the hospital who were recruited to participate through word-of-mouth. Exclusion criteria for healthy controls included a history of liver disease. Patients were categorized into groups based on absence or presence of cirrhosis and/or portal hypertension, and of individual complications as determined on clinical bases which included histologic, clinical, laboratory, or imaging features. Participants with non-cirrhotic portal hypertension were excluded.

Variable definitions

A clinical diagnosis of cirrhosis served as our ground truth training label and reference standard. Cirrhosis was classified as stage I, stage II, or stage III. Stage I was defined as compensated cirrhosis with the absence of varices or other clinical complications. Stage II (compensated) cirrhosis was defined as presence of varices but no other complications. The presence of varices in patients with compensated cirrhosis is a prognostic factor and indicates a higher risk of decompensation. Stage III (decompensated) cirrhosis was defined as the presence of ascites, variceal hemorrhage, or hepatic encephalopathy. Diagnoses of cirrhosis and presence of clinical complication were determined independently by two hepatologists.

Sample collection

A flow-chart of sample collection and volatolomic analysis is shown in **Figure 1**. Each study participant provided a single breath sample collected using the ReCIVA breath sampler (Owlstone Medical, Cambridge, UK) and passed through thermal desorption tubes to capture VOCs, then separated using high temperatures and GC and passed onto FAIMS (Owlstone Medical, Cambridge, UK), a spectrometry device which separates ions based on size and charge to create a data matrix that represents a volatolomic profile of the breath sample (11). FAIMS has been used for VOC detection in many settings (12–18).

Data collection using this approach (TD-GC-FAIMS) has been described previously (19). Each study participant provided a breath sample totaling to 1-L of exhaled air onto Bio-Monitoring TD tubes (Markes International, South Wales, United Kingdom). Samples were divided into four technical replicates which were derived from the same 1 L breath sample and were collected simultaneously on four separate collection

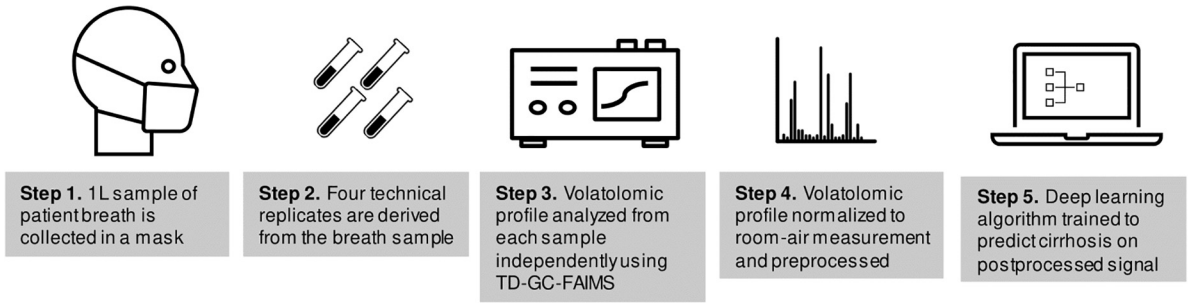

FIGURE 1
Flow-chart of sample collection and analysis.

tubes by the collection mask. Samples were collected by a trained technician (J.T.) after patients had fasted at least 4 h from food or drink besides water. A subset of 11 samples from 11 individuals (1 sample per individual) had been stored for a period of time exceeding 6 weeks; these samples were excluded from the analysis because the effects of long-term cold storage on breath VOCs are poorly understood (20, 21).

Analysis of volatile organic compounds

The TD-GC-FAIMS data output was preprocessed to separate the ion intensities from each dispersion field (DF) setting and subtract out environmental VOCs and background current fluctuations using air filter field control blanks. All technical replicates were analyzed independently. The negative and positive ion intensity mesh matrices at each respective DF were combined and outer matrix cells with intensity values below the overall maximum baseline intensity (0.0104 pA) were removed. Compensation field (CF) scan points were limited to between −3 V and + 3 V. In addition, 30 terminal time resolved values, approximately 40 s at the end of the GC run, were removed for each DF data matrix. Data preprocessing was conducted in Matlab version 2019b (MathWorks, Matick, MA).

For purposes of training deep-learning models, outputs were additionally processed by dividing by the maximum value. The signal was downsampled from a 2D to a 1D signal by taking the maximum value for each row. The final output of the workflow was a signal with 3,400 rows. Note that although the signal is sampled into 3,400 discrete values, there are not 3,400 features present in the signal; a single TD-GC-FAIMS peak spans several rows, and the dataset is sparse with many rows having a value of zero. This is analogous to DL prediction based off electrocardiography signal, where a sample of 10,000 points will capture 10–12 discrete peak features (22). During model training, data were randomly augmented with 5% Gaussian random noise.

Training of the deep learning model

For model evaluation purposes, all samples from 22 individuals (totaling 75 samples), including 17 positive patients (59 samples) and 5 healthy controls (16 samples) were randomly selected and set aside as a test dataset; these samples were excluded from the model development process. The dataset was split at the patient level such that no patients had samples in both the training and test dataset. The 24 individuals (82 samples), which included 18 positive patients (64 samples) and 6 healthy controls (18 samples) were used for training and validating the neural network model. The ground truth label was taken to be the presence of cirrhosis as determined by clinical experts. All results are reported on the test dataset.

The 24 individuals were randomly divided into four splits using stratified group fourfold cross-validation; each split consisted of three analysis folds and one assessment fold where the analysis folds were used for training and the assessment fold was used for validating the model. The same cross-validation (CV) split was used for all iterations of hyperparameter tuning. Partitioning was done at the patient level such that no individual had samples in more than a single CV fold. Although partitioning patients into each fold was random, we attempted to preserve the distribution of our outcome with stratification, where at least one healthy patient was represented in each CV split; this was necessary to ensure proper training of the model. **Figure 2** displays the data partition; **Supplementary Table 2** provides additional details.

Model development

Several potential deep learning model architectures were evaluated to predict presence of cirrhosis. We selected a custom convolutional neural network (CNN) model for architecture and hyperparameter-tuning process, which outperformed several other models in an initial phase of experimentation

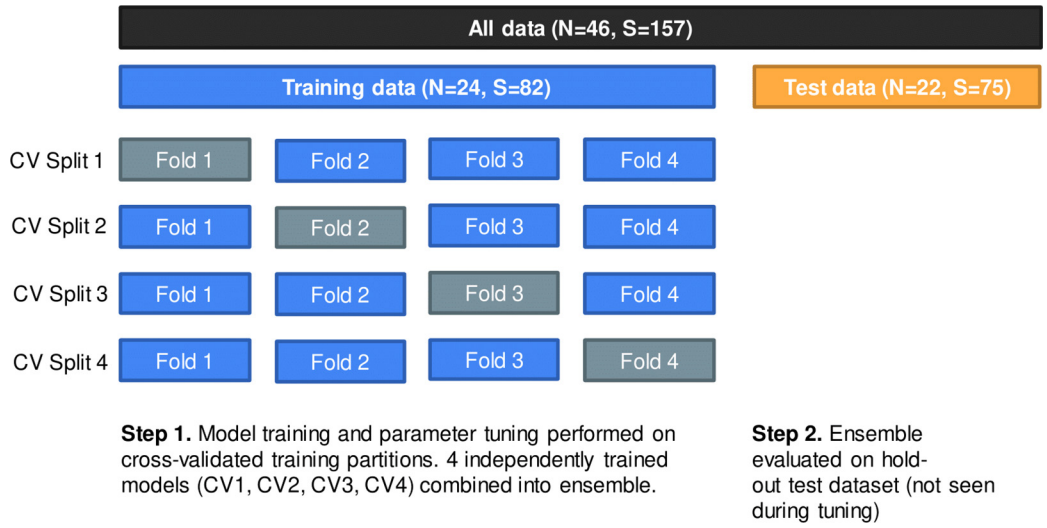

FIGURE 2
Data partition using group fourfold cross-validation (CV) method. Within training data, each split represents one independently trained model. Models were evaluated on a hold-out test dataset of 22 patients (75 samples).

(including pretrained ResNet and a custom fully connected deep-neural network).

The CNN model architecture and hyperparameters were optimized through grid search. Parameters which were considered included the number of convolutional layers, number of kernels per layer, number of fully connected ("dense") layers at the output end of the model, learning rate, batch size as well as alternate methods of augmenting the data to account for data imbalance. **Figure 3** displays the architecture of the best-performing CNN model discovered through hyperparameter tuning. **Supplementary Table 3** lists all parameters considered (23). Additionally, model architecture and hyperparameter optimization grid search results were summarized with analysis (training) and assessment (validation) accuracy and loss curves in an interactive R markdown document (R version 4.0.3 with Shiny 1.6.0).

The optimal model architecture and hyperparameter configuration was selected by assessing the average highest performing validation accuracy and lowest validation loss across all four CV splits. The best-performing four individual CNN models, one for each corresponding CV split, were combined into an ensemble model by taking the average of model outputs.

Model development with hyperparameter tuning was performed on the Google Cloud Platform (GCP) and was accelerated using 1 Nvidia T4 GPU (16GB RAM).

Model evaluation

The primary endpoint of this study was diagnosis of liver cirrhosis at the patient level, which was achieved by combining the four individual constituent CNN models from four cross-validation splits (CV1, CV2, CV3, CV4) (i.e., at the dataset level) into an "ensemble" prediction by taking the mean probability across all four models, and then by taking the median value of this ensemble prediction across the 3–4 technical replicates per patient. Additionally, to evaluate the reproducibility of model prediction across multiple technical replicates, the AUC curve and summary metrics are reported at the sample level.

To investigate the algorithm's ability to discriminate cirrhosis patients from healthy controls, the ensemble model's predicted probabilities were tabulated by cirrhosis stage and visualized with boxplots.

Model explainability

Interpretation of AI algorithms is an increasingly important approach to validate their performance and lend insight to the modeling process. To aid in the interpretation of the results of the CNN, we utilized SHapley Additive ExPlanations (SHAP) (RRID:SCR_021362) to determine which features contribute to the detection of liver disease (24). SHAP identifies features which are important in determining the model output by allocating contributions of the model output across input parameters. SHAP was implemented in the SHAP package version 0.39.0 for Python 3.7.8. SHAP values were computed individually for the four CNN models.

SHAP feature importance plots were summarized on the training and test datasets for each CV split with "beeswarm" scatter plots (24). To identify individual compounds from TD-GC-FAIMS which are most important for detecting the

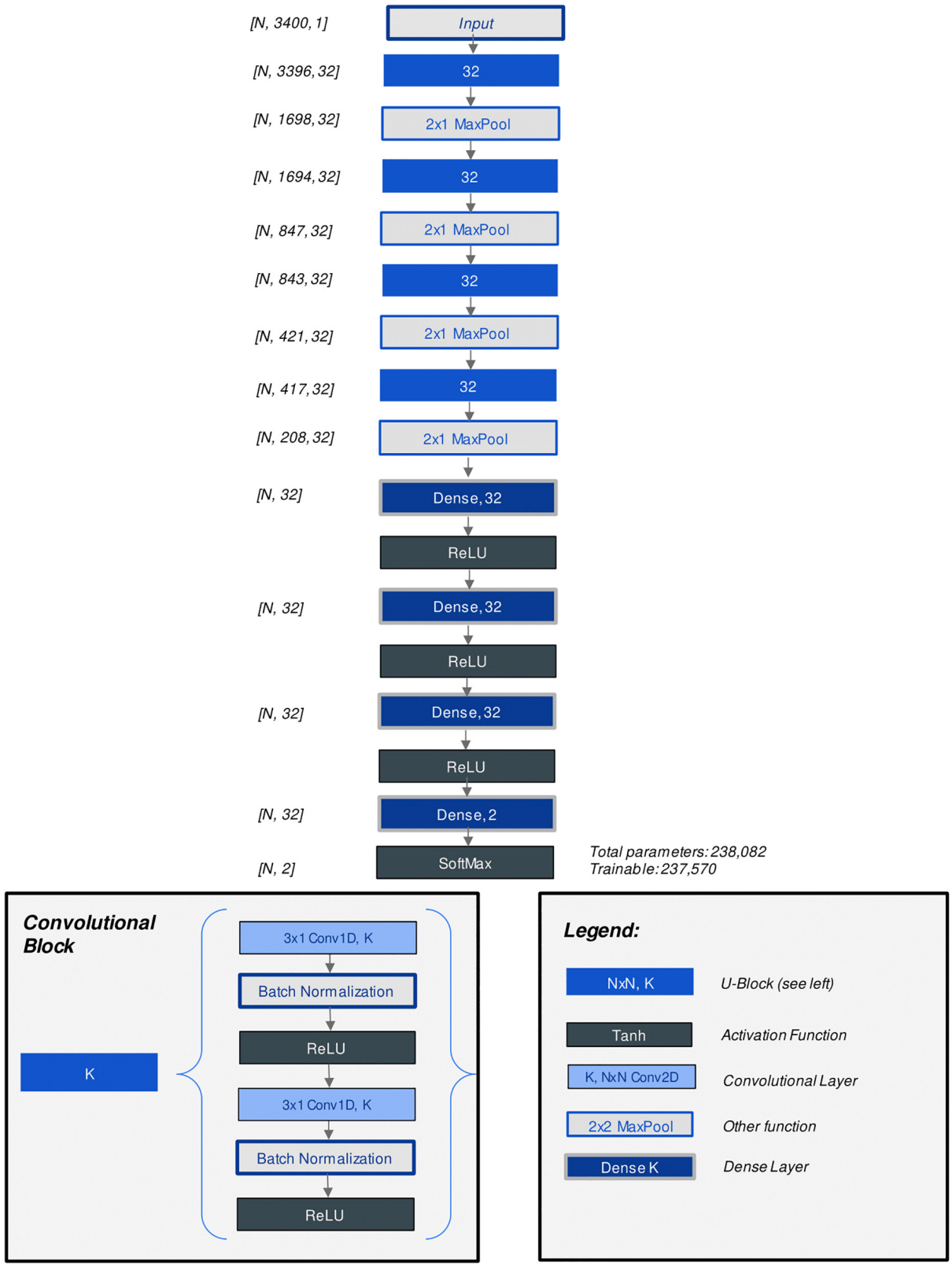

FIGURE 3
Diagram of custom CNN model architecture.

presence of liver disease, the five features with the largest magnitude (largest absolute SHAP value) were selected per each instance in the test dataset (75 samples) and were overlayed on the sample's VOC signal, creating "heatmaps" which identify peaks important for predictions. The heatmaps were visualized with darker red representing the higher number of times the same peak was detected across four constituent models. For each patient, the final ensemble predicted probability was annotated.

Statistical analysis

Clinical demographics and laboratory test results data were summarized with the median and range for the continuous variables and with the number and percentage of patients for the categorical variables. The Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables were used to compare demographics between healthy controls and cirrhosis patients; Kruskal-Wallis rank sum test was used to compare laboratory test results between stage I, stage II, and stage III cirrhosis.

Model performance was assessed for the four individual cross-validated models as well as the ensemble model at the sample and patient levels using the area under the receiver operating characteristic curve (AUC), accuracy, sensitivity, specificity, positive predictive value, negative predictive value, and F1 score. A threshold cutoff value of > 0.50 was used to classify a sample or patient as positive (presence of any stage of cirrhosis). The exact 95% confidence intervals were computed for AUC, accuracy, sensitivity, and specificity metrics at the patient level (Pearson-Klopper method).

To explore patterns in patient subgroups, subgroup analysis was performed on the final ensemble model with respect to age, BMI, and sex at the patient level.

Model development and hyperparameter tuning were performed using Tensorflow version 2.3.0 for Python version 3.7.8. Data summaries, statistical analysis, visualizations, and model evaluation were performed using R Statistical Software (version 4.0.3); R Foundation for Statistical Computing, Vienna, Austria.

Results

Patient demographics

A total of 46 individuals (157 samples) were included in this study (123 samples from 35 patients with decompensated or compensated cirrhosis and 34 control samples from 11 healthy individuals). Among the 46 patients included, median age was 57 (Range: 24–76), 35/46 (76%) had history of liver cirrhosis, 23/46 (50%) were men. A comparison of demographics between healthy and cirrhosis patients is depicted in Table 1. In comparison to healthy controls, cirrhosis patients had an older age at diagnosis (median: 61 vs. 45 years, $P = 0.001$) and were more likely to be obese (51.3% vs. 27.3%).

TABLE 1 Comparison of demographics between healthy and cirrhosis patients.

	Median (minimum, maximum) or No. (%) of patients		
	Disease ($N = 35$)	**Healthy ($N = 11$)**	***P*-value**
Sex (Male)			1.00
Female	17 (48.6%)	6 (54.5%)	
Male	18 (51.4%)	5 (45.5%)	
Age (years)	61.0 (33.0, 76.0)	45.0 (24.0, 60.0)	**<0.001**
Age group (years)			**0.002**
(20, 50)	7 (20.0%)	8 (72.7%)	
(50, 80)	28 (80.0%)	3 (27.3%)	
Body mass index (kg/m^2)	30.2 (20.2, 41.3)	27.6 (21.0, 41.8)	0.42
Body mass index (categorical)			0.20
Healthy weight (18.5–24.9)	5 (14.3%)	2 (18.2%)	
Overweight (25.0–29.9)	10 (28.6%)	6 (54.5%)	
Obesity (>30.0)	20 (57.1%)	3 (27.3%)	

P-values result from a Wilcoxon rank sum test (continuous variables) or Fisher's exact test (categorical variables). Bold values denote statistical significance at the $p < 0.05$ level.

Within the disease cohort, 14 patients (35.9%) had stage I cirrhosis, 15 patients (38.5%) had stage II cirrhosis, and 10 patients (25.6%) had stage III cirrhosis. Two persons with stage III cirrhosis had a history of hepatic encephalopathy that was well controlled and not clinically manifest at time of collection. A comparison of laboratory test results across cirrhosis stages is shown in Table 2. As expected by the cirrhosis classifications, stage III cirrhosis had highest model for end-stage liver disease (MELD), aspartate aminotransferase to platelet ratio index (APRI), and Fibrosis-4 index for liver fibrosis (FIB-4) scores with medians 13, 0.9, 6, respectively. Supplementary Table 1 expands upon Table 2, including additional laboratory test results.

Model performance at the sample and patient levels

The CNN model was successful in differentiating breath samples taken from patients with cirrhosis vs. healthy controls; four models trained on separate CV splits classified the presence of cirrhosis with an average AUC of 0.79 at the sample level (clustering between technical replicates precludes accurate

TABLE 2 Comparison of characteristics across disease stage for the cirrhosis study population.

	Median (minimum, maximum) or No. (%) of patients			
	Cirrhosis stage I, compensated ($N = 13$)	**Cirrhosis stage II, compensated ($N = 12$)**	**Cirrhosis stage III, decompensated ($N = 10$)**	***P*-value**
Ascites	0 (0.0%)	0 (0.0%)	10 (100.0%)	**<0.001**
Varices	0 (0.0%)	12 (100.0%)	8 (80.0%)	**<0.001**
Platelets	185.0 (123.0, 272.0)	92.5 (44.0, 279.0)	83.0 (36.0, 238.0)	**0.014**
MELD	8.0 (6.0, 20.0)	10.0 (7.0, 19.0)	13.0 (7.0, 28.0)	**0.041**
APRI	0.4 (0.2, 1.1)	0.8 (0.2, 3.5)	0.9 (0.3, 3.5)	0.16
FIB4	2.4 (0.6, 4.2)	3.7 (1.2, 10.7)	6.0 (1.3, 14.8)	**0.013**
Etiology				0.13
Non-alcoholic steatohepatitis (NASH)	10 (76.9%)	8 (66.7%)	3 (30.0%)	
Alcoholic liver cirrhosis (ALC)	0 (0.0%)	2 (16.7%)	0 (0.0%)	
Hepatitis C Virus (HCV)	1 (7.7%)	1 (8.3%)	1 (10.0%)	
HCV + ALC	0 (0.0%)	0 (0.0%)	2 (20.0%)	
Primary sclerosing cholangitis 2 (PSC 2)	2 (15.4%)	1 (8.3%)	3 (30.0%)	
Hemochromatosis	0 (0.0%)	0 (0.0%)	1 (10.0%)	

P-values result from a Kruskal-Wallis rank sum test (continuous variables) or Fisher's exact test (categorical variables). MELD, model for end-stage liver disease; APRI, aspartate aminotransferase to platelet ratio index; FIB4, Fibrosis-4 index for liver fibrosis. Bold values denote statistical significance at the $p < 0.05$ level.

estimation of the exact 95% CI at the sample level; these values are reported for the primary endpoint of patient diagnosis only). When these models were combined into an ensemble by averaging prediction probabilities, the AUC was 0.90 as depicted by Figure 4.

At the patient level, the ensemble model prediction outperformed the four constituent CV models in detecting the presence of cirrhosis in patients. Individual models discriminated between cirrhosis individuals and healthy controls with an average AUC of 0.80 (range: 0.54, 1.00), their ensemble achieved an AUC of 0.90 (95% CI: 0.75, 1.00). At a 50% classification threshold, the ensemble model yielded the following performance metrics: sensitivity of 1.00 (perfect), specificity of 0.40, positive predicted value of 0.85, negative predicted value of 1.00, and F1 score of 91.9.

All diagnostic performance measures for the ensemble and its constituent CV models are reported in Table 3 at both sample and patient levels.

The subgroup analysis did not reveal any significant differences in model performance between subgroups (age, BMI, or sex) indicating that age is not a confounding factor in classification of breath samples.

Performance based on the cirrhosis stage

At the 50% threshold, the model correctly classified 100% of patients with stage I, stage II, or stage III cirrhosis (17/17 patients; 59/59 samples), i.e., the model achieved perfect sensitivity. The model correctly identified 2/5 healthy individuals (6/16 healthy samples) but incorrectly classified 3/6 healthy individuals (10/16 healthy samples) as having cirrhosis (Figure 4). Evaluation of the ensemble model at classifying the presence or absence of cirrhosis at several stages of cirrhosis is shown (Figure 5). The model displayed higher confidence when the patient had stage II or II cirrhosis (median probabilities $>$ 0.99) than when they had stage I disease (median probability $>$ 0.76).

Identification of volatile-organic compound features

The SHAP values which identify peaks in the signal that contributed most to the prediction are depicted by the beeswarm summary plots in Figure 6. For each CV model, we identified the top 10 peaks which selected a total of 22 unique compounds in the TD-GC-FAIMS signal; 14 compounds (64%) were identified by at least two independently trained CV models, two compounds were identified by three CV models, and one compound was identified by all four CV models. Figure 7 displays an example of four patients (one from each stage of cirrhosis and one healthy control) whose VOC profiles' signal is visualized with overlaying heatmaps, which depict the five most important compounds in the classification of liver cirrhosis identified by each model.

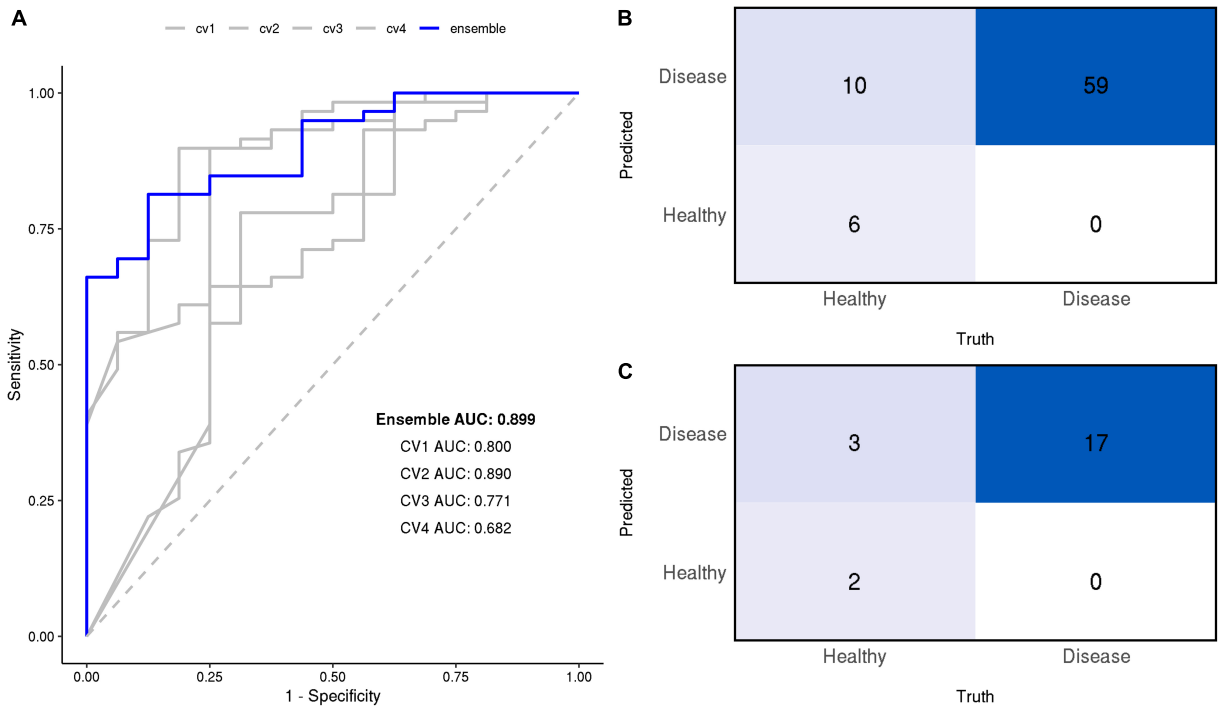

FIGURE 4
(A) Receiver operating characteristic (ROC) curves of the final models at the sample level. Area under the ROC curve (AUC) is annotated for each model. The ensemble's confusion matrix heatmaps at the sample **(B)** and patient **(C)** levels summarize the frequency of True Positives (TP), False Negatives (FN), True Negatives (TN), False Positives (FP).

TABLE 3 Model performance metrics at sample and patient levels at the 0.5 threshold.

	AUC (95% CI)	Accuracy (95% CI), fraction	Sensitivity (95% CI), fraction	Specificity (95% CI), fraction	PPV (95% CI), fraction	NPV (95% CI), fraction	F1 score
Sample level							
Ensemble	0.899	86.7% 65/75	100.0% 59/59	37.5% 6/16	85.5% 59/69	100.0% 6/6	92.2
CV1	0.800	86.7% 65/75	100.0% 59/59	37.5% 6/16	88.7% 55/62	69.2% 9/13	92.2
CV2	0.890	85.3%64/75	93.2% 55/59	56.2% 9/16	88.7% 55/62	69.2% 9/13	90.9
CV3	0.771	85.3% 64/75	98.3% 58/59	37.5% 6/16	85.3% 58/68	85.7% 6/7	91.3
CV4	0.682	81.3%61/75	93.2% 55/59	37.5% 6/16	84.6% 55/65	60.0% 6/10	88.7
Patient level							
Ensemble	0.894 (0.751, 1.000)	86.4% (65.1%, 97.1%) 19/22	100.0% (80.5%, 100.0%) 17/17	40.0% (5.3%, 85.3%) 2/5	85.0% (62.1%, 96.8%) 17/20	100.0% (15.8%, 100.0%) 2/2	91.9
CV1	0.824 (0.627, 1.000)	86.4% (65.1%, 97.1%) 19/22	100.0% (80.5%, 100.0%) 17/17	40.0% (5.3%, 85.3%) 2/5	85.0% (62.1%, 96.8%) 17/20	100.0% (15.8%, 100.0%) 2/2	91.9
CV2	0.882 (0.691, 1.000)	81.8% (59.7%, 94.8%) 18/22	88.2% (63.6%, 98.5%) 15/17	60.0% (14.7%, 94.7%) 3/5	88.2% (63.6%, 98.5%) 15/17	60.0% (14.7%, 94.7%) 3/5	88.2
CV3	0.800 (0.486, 1.000)	86.4% (65.1%, 97.1%) 19/22	100.0% (80.5%, 100.0%) 17/17	40.0% (5.3%, 85.3%) 2/5	85.0% (62.1%, 96.8%) 17/20	100.0% (15.8%, 100.0%) 2/2	91.9
CV4	0.682 (0.371, 0.994)	81.8% (59.7%, 94.8%) 18/22	94.1% (71.3%, 99.9%) 16/17	40.0% (5.3%, 85.3%) 2/5	84.2% (60.4%, 96.6%) 16/19	66.7% (9.4%, 99.2%) 2/3	88.9

95% Confidence Intervals are reported at the patient level only, clustering of technical replicates precluded calculation of the exact confidence interval at the sample level. PPV, positive predictive value; NPV, negative predictive value.

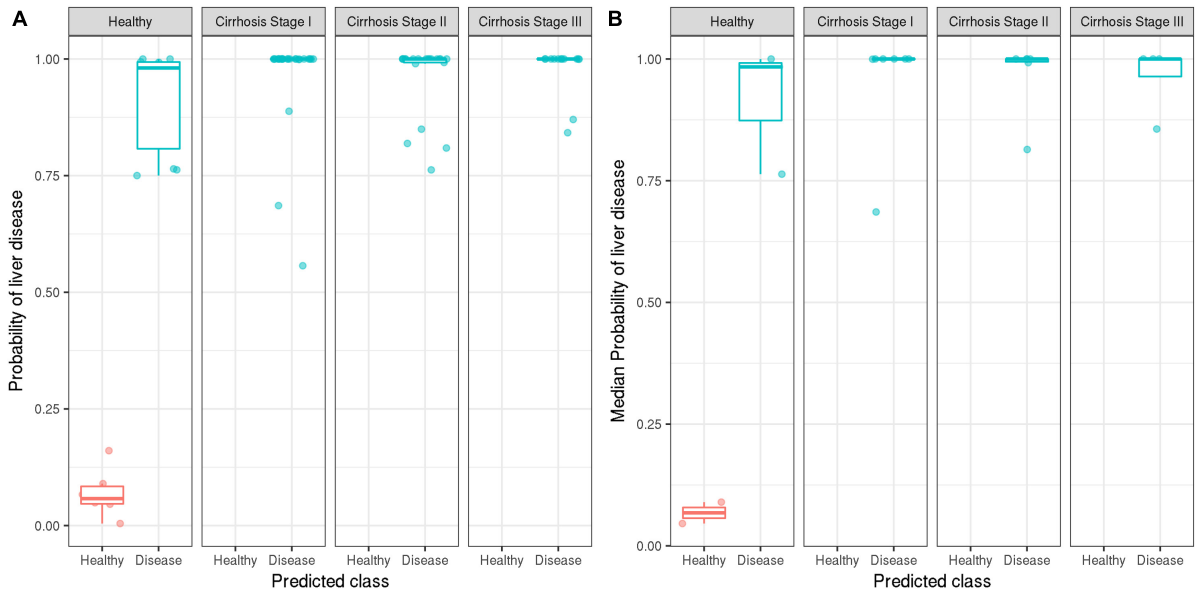

FIGURE 5
Distribution of the ensemble model's predicted probabilities for healthy vs. disease classifications stratified by the true stage of cirrhosis. Ground truth labels of healthy (red) and disease (blue) are displayed. On the *y*-axis, probability values of model output are displayed. Model performance is reported at the sample level **(A)**, as well as patient level **(B)** by aggregating based on median probabilities.

Discussion

This work presents a deep-learning based approach for detecting liver cirrhosis based on non-invasive breath samples analyzed with TD-GC-FAIMS. To our knowledge, this is the first application of deep-neural networks for the prediction of liver cirrhosis from volatolomic profiles from patient breath samples (25–28). We observed that CNNs were an effective technique for analyzing the volatolomic profiles obtained using TD-GC-FAIMS, and our optimal model displayed an AUC of 0.90 and an accuracy of 86% at the patient level. This supports the application of volatolomic analyses using TD-GC-FAIMS for non-invasive diagnosis of cirrhosis from breath samples.

Deep learning approach

Several deep-learning approaches were attempted including transfer-learning of a pretrained ResNet, and a fully connected deep neural network. Previous experiments done by this group have demonstrated machine-learning based approaches for the detection of cirrhosis (19). We observed optimal performance with a CNN model. This is consistent with extensive literature that indicates CNNs are an efficient and accurate method of analyzing sparse signals data; in the medical field, CNNs are popular model for both image analysis and signals processing (22, 29–32).

Optimal performance was observed with an ensemble of four CNNs combined by taking the mean prediction probability; the ensemble performance was slightly better than the best performing constituent models, and substantially better than the average of its four constituents. Combining several models into an ensemble is an effective technique for generating consistent predictions and reducing the impact of overfitting.

Model performance in stage I and stage II cirrhosis

Our model was effective in predicting the presence of cirrhosis with an accuracy of 86% at the patient level. The model displayed a tendency to overdiagnose the presence of cirrhosis; the ensemble model had a sensitivity of 100% but a specificity of 40% at the patient level.

At the sample level, all mistakes came from differentiating healthy controls from patients with stage I cirrhosis (e.g., the lowest stage of disease, when individuals are often asymptomatic). This suggests that the model is correctly identifying hallmarks of advanced cirrhosis with a very high level of accuracy.

Imbalance in the training dataset likely played a role in model specificity (only 11/46 individuals included in this study were healthy controls). Specificity may be modified by adjusting the prediction cutoff from 0.5 to a higher value, with the understanding that this may increase the rate of

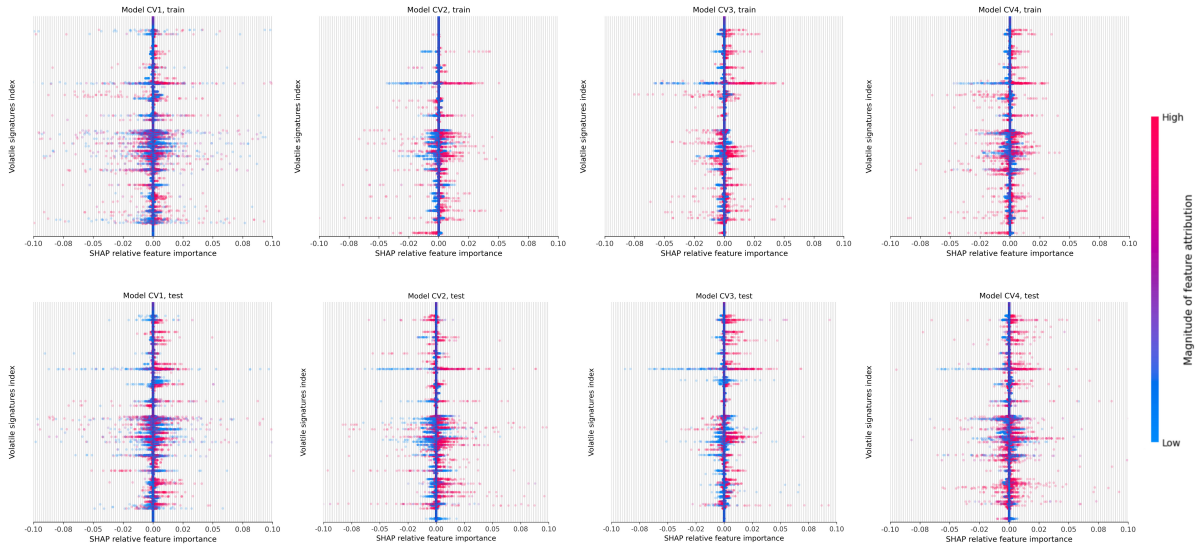

FIGURE 6
Beeswarm summary plots on train and test data. This plot combines feature importance and feature effects. Every feature (VOC) is represented as a row on the *y*-axis (3,400 total) and SHAP values are on the *x*-axis (multiple VOC may overlap at a single index). Each dot represents a Shapley value for a given sample prediction. The color intensity shows the magnitude of importance of each feature.

false negatives. A diagnostic tool with high sensitivity could be appropriate as an inexpensive, non-invasive screening tool for cirrhosis detection in an at-risk population, with the understanding that additional diagnostic tests such as imaging exams would be required to rule out false positive results in an initial screen.

Explainable artificial intelligence

The use of SHAP for explaining the predictions of the CNN model identified several discrete peaks which were consistently associated with either a positive or negative prediction. We observed that 14/22 (63%) of the top peaks detected by the ensemble model were identified by multiple independent CV models, which indicates that these features are reproducible between independently trained models. This supports the reliability of the CNN approach.

Several specific VOCs are known to be overexpressed or underexpressed in cirrhotic patients, including limonene, methanol, and 2-pentanone (8). Data-driven approaches such as deep neural networks rely on the entire volatolomic profile measurements, not only a few discrete peaks, and therefore may be incorporating VOCs which have not yet been identified, or VOC constituents which are partially metabolized from known compounds. Future work has the potential to characterize previously unknown VOCs which the model indicates are implicated in cirrhosis.

Limitations

We acknowledge several important limitations to this study. Although this is a preliminary study in a relatively modest dataset of 46 patients (157 samples) with unbalanced groups, several observations strongly support the conclusion that the model is capturing a true volatolomic signature which can diagnose disease. Firstly, all four crossvalidated models demonstrated strong predictive performance on an independent test dataset of 22 patients that were not seen at any point in the model training and validation process, and therefore is likely not the result of overfitting (AUC 0.682–0.882). Secondly, model confidence correlated to cirrhosis stage (median probabilities $>$ 0.99 for Stage II, Stage III cirrhosis, median probability $>$ 0.76 for Stage I, healthy) which is consistent with the clinical observation that it is more difficult to detect lower grade cirrhosis; furthermore, subgroup analysis did not indicate any confounding with age or sex. Thirdly, SHAP analysis identified 64% of features were identified by at least two independently trained CV models; the model is consistently identifying several discrete features in multiple patient samples. Further experimental work is needed to identify which specific compounds are identified by these peaks.

Ongoing subject recruitment focuses on the collection of additional samples, but reporting of findings on the initial dataset is required to demonstrate proof-of-concept, and to support the expensive and labor-intensive collection of additional samples, as well as to justify the recruitment of additional patient participants.

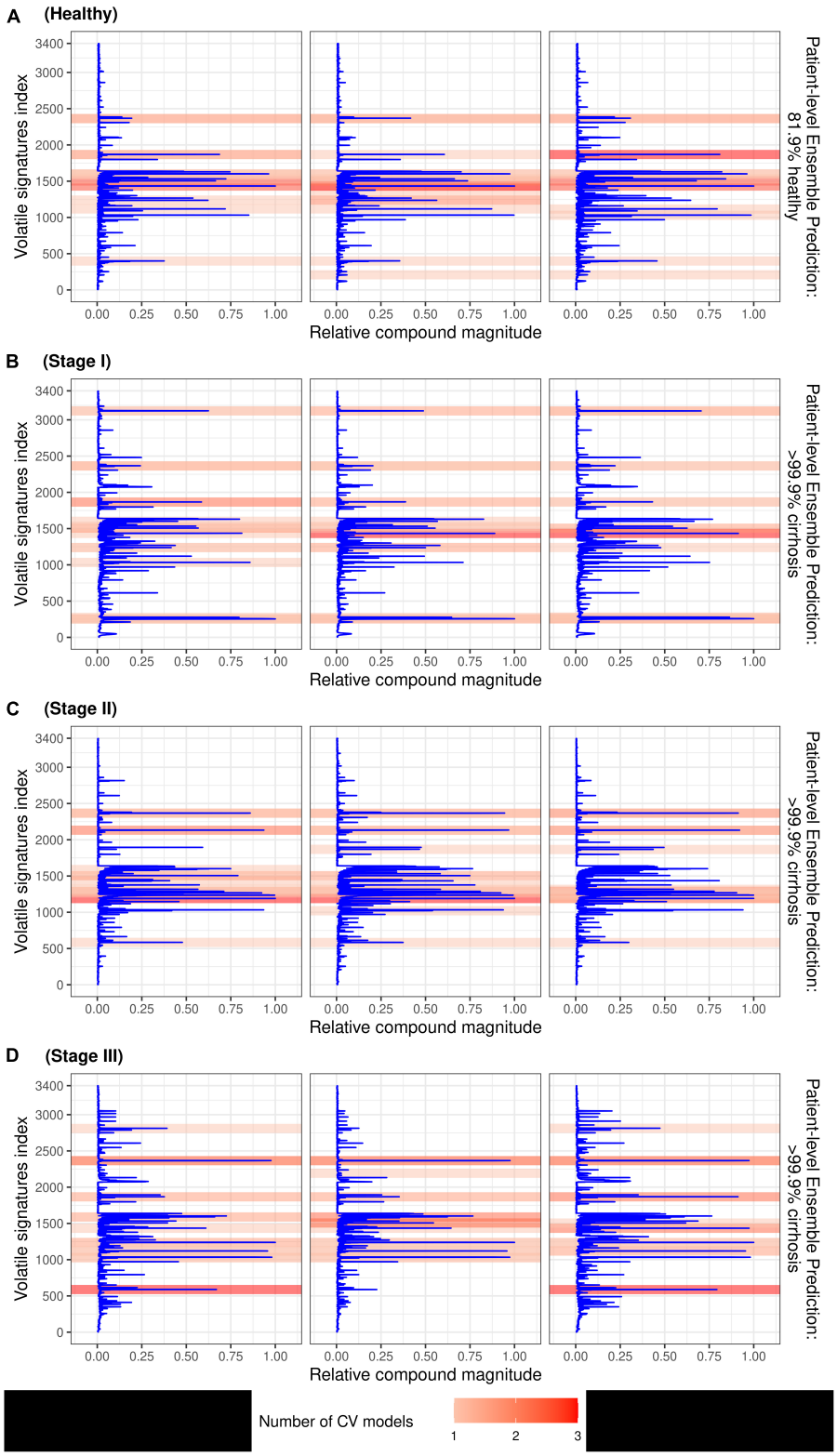

FIGURE 7
Patient breath samples with overlayed heatmaps which identify the 5 most important peaks from each CV model (up to 20 peaks total) in the classification of liver cirrhosis for a healthy control **(A)**, and 3 individuals with stage I **(B)**, stage II **(C)**, and stage III **(D)** cirrhosis, respectively. Compounds are represented by indices on the *y*-axis and VOC signal value is on the *x*-axis; darker shading indicates the feature was selected by multiple CV models.

Conclusion

A deep learning model is capable of detecting the presence of cirrhosis in volatolomic profiles obtained from analyses of exhaled breath samples from patients using TD-GC-FAIMS. Model performance had an AUC of 0.90 and a sensitivity in detecting disease of 100% at the patient level. Use of SHAP as a technique for explainable AI detected a set of unique peaks associated with both positive and negative prediction; 64% of the top 10 peaks were reproducible across multiple independently trained models. This technique demonstrates feasibility of a non-invasive clinical screening exam for diagnosing and monitoring liver cirrhosis from non-invasive breath samples without the need for detection and characterization of individual metabolites.

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 Mayo Clinic Institutional Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

ML and JT performed the experimental work and reviewed the manuscript. MW and AW performed the data analysis and modeling and drafted the manuscript. RC and TP supervised the work and reviewed 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.

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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.2022.992703/full#supplementary-material

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Hepatic perfusion as a new predictor of prognosis and mortality in critical care patients with acute-on-chronic liver failure

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Background and aims: Liver diseases are frequent causes of morbidity and mortality worldwide. Liver diseases can lead to cirrhosis, with the risk of acute-on-chronic liver failure (ACLF). For the detection of changes in hepatic hemodynamics, Doppler ultrasonography is a well-established method. We investigated hepatic hemodynamics *via* serial Doppler ultrasonography to determine the predictive value of changes in hepatic perfusion for the outcome in patients with severe liver diseases compared to established prognostic models such as the MELD (Model for End-Stage Liver Disease) or CLIF-C (Chronic Liver Failure-Consortium) ACLF score.

Methods: In this prospective cohort study, hepatic perfusion was quantified at baseline before the initiation of treatment and every third day by means of serial measurements of the hepatic artery resistance index (HARI) and the maximum portal vein velocity (PVv) using Doppler ultrasonography in 50 consecutive patients with severe liver diseases admitted to a medical intensive care unit (MICU). The recorded hemodynamic parameters were compared to the MELD score, and the CLIF-C ACLF score to analyze their utility for the prediction of the outcome of patients with severe liver diseases, liver cirrhosis, and ACLF.

Results: The changes (delta) obtained by serial measurements of the MELD score, HARI, and PVv were analyzed through scatter plots. Bivariate correlation analysis yielded a new positive linear correlation between the delta-HARI and the delta-MELD score ($r = 0.469$; $p < 0.001$). In addition, our data revealed a new negative linear correlation between delta-PVv and the delta-MELD score ($r = -0.279$, $p = 0.001$). The leading cause of MICU mortality was acute-on-chronic liver failure (ACLF). A subgroup analysis of patients with liver cirrhosis revealed a positive linear correlation between the delta-HARI and the delta-CLIF-C-ACLF score ($r = 0.252$, $p = 0.005$). Of clinical relevance, non-survivors of ACLF exhibited a significantly higher mean value for the

delta-HARI (0.010 vs. −0.005; $p = 0.015$) and a lower mean value for the delta-PVv (−0.7 vs. 1.9 cm/s; $p = 0.037$) in comparison to survivors of ACLF.

Conclusion: This study shows the prognostic value of the assessment of hepatic perfusion in critical care patients with severe liver diseases by bedside Doppler ultrasound examination and its utility as an accurate predictor of the outcome in patients with ACLF. Increasing HARI and a decreasing PVv are predictors of an adverse outcome. Delta-HARI and delta-PVv are new biomarkers of prognosis and ACLF-related mortality in patients with liver diseases. Delta-HARI and delta-PVv may be helpful in guiding clinical decision-making, especially in catecholamine and fluid management.

KEYWORDS

liver disease, liver cirrhosis, acute-on-chronic liver failure, liver perfusion, doppler ultrasound, critical care medicine

Introduction

Liver diseases are significant causes of morbidity and mortality worldwide (1). The progression of liver diseases to cirrhosis and decompensation associated with critical illness is a significant cause of mortality in these patients (2, 3). Acute-on-chronic liver failure (ACLF) can occur in patients with liver cirrhosis and is a recently described entity diagnosed in patients with chronic liver diseases and a combination of hepatic and extrahepatic organ failures (kidney, respiratory, coagulation, circulatory, brain). Early diagnosis and treatment of ACLF are essential for the outcome of these critically ill patients (4, 5).

Organ perfusion plays an essential role in liver diseases, while the mechanisms regulating hepatic perfusion in patients with liver diseases, fibrosis, cirrhosis, and ACLF are only partially known (6). Portal venous flow depends mainly on the influx of splanchnic perfusion (7).

In contrast to portal venous flow, arterial flow is subject to pressure-dependent autoregulation. A second mechanism termed hepatic artery buffer response (HABR) is a central mechanism in the intrinsic regulation of hepatic blood flow (8). HABR describes the ability of the hepatic artery to compensate for changes in the flow of the portal vein by counter-directed hemodynamic adjustment of its perfusion. HABR causes arterial vasodilation through reduced portal flow and, vice versa, arterial vasoconstriction through increased portal flow (9). HABR is also preserved in patients with inflammatory liver diseases, even in advanced liver fibrosis and cirrhosis (10, 11).

Abdominal and Doppler ultrasonography are well-established methods for evaluating liver diseases and detecting changes in hepatic hemodynamics. International guidelines recommend using ultrasound scans to evaluate liver diseases (2, 3). Ultrasound scans are an easily accessible, inexpensive, and non-invasive procedure, which can be repeated as often as needed, even at the bedside of critical care patients. Such scans have high specificity in diagnosing liver fibrosis, liver cirrhosis, and portal hypertension. The diagnosis of liver cirrhosis by conventional ultrasound is based on changes in liver morphology and signs of portal hypertension. In addition, Doppler ultrasonography is a valuable tool for evaluating hemodynamic changes in cirrhotic liver tissue (12).

Little is known about the predictive value of serial measurements of hepatic hemodynamics in patients with severe liver diseases, particularly in patients with acutely decompensated cirrhosis at risk of developing acute-on-chronic liver failure.

The aim of this study was 1. to determine the predictive value of changes in hepatic perfusion for the outcome in patients with severe liver diseases compared to well-established prognostic models such as the MELD or CLIF-C ACLF score in the context of critical care treatment (13, 14), 2. to analyze the role of liver perfusion as an early predictor of mortality due to ACLF, and 3. to identify potential new hemodynamic targets in critical care for early therapeutic intervention in ACLF.

Abbreviations: ACLF, acute-on-chronic liver failure; ALF, acute liver failure; ALI, acute liver injury; CCC, cholangiocellular carcinoma; CEUS, contrast-enhanced ultrasound; CLI, chronic liver injury; CLIF-C, chronic liver failure-consortium; CT, computed tomography; EASL, European association for the study of the liver; EDV, end-diastolic velocity; HA, hepatic artery; HARI, hepatic artery resistance index; HABR, hepatic artery buffer response; HCC, hepatocellular carcinoma; LP, liver parenchyma; MELD, model for end-stage liver disease; MICU, medical intensive care unit; PEEP, positive end-expiratory pressure; Ppeak, peak pressure; PSV, peak systolic velocity; PVv, portal vein velocity; PW, doppler, pulsed-wave-doppler; ROC, receiver operating characteristic; SD, standard deviation; TI, time interval.

Materials and methods

Study design and patient characteristics

This prospective cohort study enrolled 50 patients with severe acute and chronic liver injury (ALI and CLI) and acute-on-chronic liver failure (ACLF) (Table 1). The diagnosis of liver cirrhosis was based on non-invasive tests following the current European Association for the Study of the Liver (EASL) Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis (15). Accordingly, we diagnosed liver cirrhosis by detecting specific morphological changes of the liver by ultrasound and computed tomography (CT) in combination with examination of clinical and laboratory chemistry parameters (16, 17). In our cohort, liver cirrhosis was diagnosed in 36 of the 50 patients studied. The patients were treated in a medical intensive care unit (MICU) of a German University Hospital that specializes in the treatment of liver diseases.

The aim of the study was to assess the potential of Doppler ultrasound as a predictor of the outcome of patients with severe liver diseases and as a novel prognostic biomarker for ACLF. The study was approved by the Ethics Committee of the University of Regensburg, Regensburg, Germany (registration number 18-920-101). All patients provided written, informed consent before the study, in accordance with the principles of the Declaration of Helsinki [revision of (18)].

A flow chart of eligible patients with liver disease, data collection, and analysis is given in Figure 1.

TABLE 1 Clinical characteristics of the study cohort.

Characteristics	Total study cohort ($n = 50$)
Age [years]: mean ± SD (range)	59.7 ± 10.3 (39–90)
Sex: *n* (%)	
Female	16 (32)
Male	34 (68)
MICU stay [days]: mean ± SD (range)	15.3 ± 13.8 (2–72)
Mortality in the MICU: *n* (%)	
Deceased patients[a]	16 (32)
Survived patients	34 (68)
Liver diseases: *n* (%)	
Alcohol-related liver cirrhosis	22 (44)
Acute liver failure[b]	6 (12)
Autoimmune liver disease[c]	4 (8)
Viral hepatitis[d]	4 (8)
Liver cirrhosis of idiopathic origin	3 (6)
HCC	3 (6)
CCC	3 (6)
Other liver diseases[e]	5 (10)
Liver cirrhosis: *n* (%)	36 (72)
Child A/B/C	1 (2.8)/12 (33.3)/23 (63.9)
Precipitating events for ACLF: *n* (%)	15 (30)
Infections	9 (60)
Gastrointestinal bleedings	6 (40)
Life support in the MICU: *n* (%)	
Renal replacement using dialysis: required/not required	23/27 (46/54)
Mechanical ventilation: required/not required	13/37 (26/74)
Delta-PEEP [cmH2O]: mean ± SD (range)	−0.7 ± 2 (−4.7 to 4)
Delta-Ppeak [cmH2O]: mean ± SD (range)	−1.2 ± 4.2 (−14 to 10)
Vasoactive drugs: required/not required	29/21 (58/42)
MELD score [points]: mean ± SD (range)	
Values at admission ($n = 50$)	25.2 ± 8.6 (8–40)
Absolute values ($n = 187$)	25.8 ± 9 (7–40)
Delta-values ($n = 137$)	−0.3 ± 4 (−18 to 12)
HARI: mean ± SD (range)	
Values at admission (n = 50)	0.74 ± 0.07 (0.57–0.9)
Absolute values (n = 187)	0.74 ± 0.08 (0.55–0.95)
Delta-values (n = 137)	−0.003 ± 0.057 (−0.17 to 0.16)
Maximum PVv [cm/s]: mean ± SD (range)	
Values at admission ($n = 50$)	19.1 ± 14.1 (−39.8 to 45.7)
Absolute values ($n = 187$)	19.2 ± 15.7 (−43.8 to 49.2)
Delta-values ($n = 137$)	0.4 ± 7 (−39.5 to 20.3)

Presentation of the baseline demographic (age, sex) and clinical (MICU stay, mortality in the MICU, liver diseases, liver cirrhosis, precipitating events for ACLF, life support in the ICU, MELD score, HARI, maximum PVv) characteristics of the total study cohort ($n = 50$). [a]main cause of death was ACLF ($n = 15$) which resulted in septic multiorgan failure or coagulation failure with bleeding, [b]drug-induced liver injury, [c]autoimmune liver disease: primary sclerosing cholangitis ($n = 2$), primary biliary cholangitis ($n = 2$), [d]viral hepatitis: hepatitis A ($n = 1$), B ($n = 1$), C ($n = 1$), B + C + E ($n = 1$), [e]includes liver transplantation ($n = 1$), cavernous transformation of the portal vein ($n = 1$), cholangiosepsis ($n = 1$), secondary sclerosing cholangitis ($n = 1$), and hemochromatosis ($n = 1$).

Ultrasound and doppler analyses and prognosis

To quantify hepatic perfusion, the hepatic artery resistance index (HARI) and the maximum portal vein velocity (PVv) were determined at admission to the MICU and then every third day using Doppler ultrasonography. A total of 187 ultrasound and Doppler examinations were performed in 50 patients (mean 3.74; range 1–12). Seventeen patients were examined once, 8 patients twice, 4 patients three times, and 21 patients four or more times. A standardized protocol was used for the positioning and breathing/ventilation of the patients during the ultrasound examination and Doppler sonography according to the literature (19, 20). We determined the hepatic artery resistance index (HARI) and maximum portal vein velocity (PVv).

Ultrasound scans were performed by experienced examiners. Imaging and processing of the recordings were carried out with the mobile ultrasound system Noblus® (Hitachi Aloka Medical, Ltd., Japan). The hemodynamic parameters were recorded using a convex transducer with a

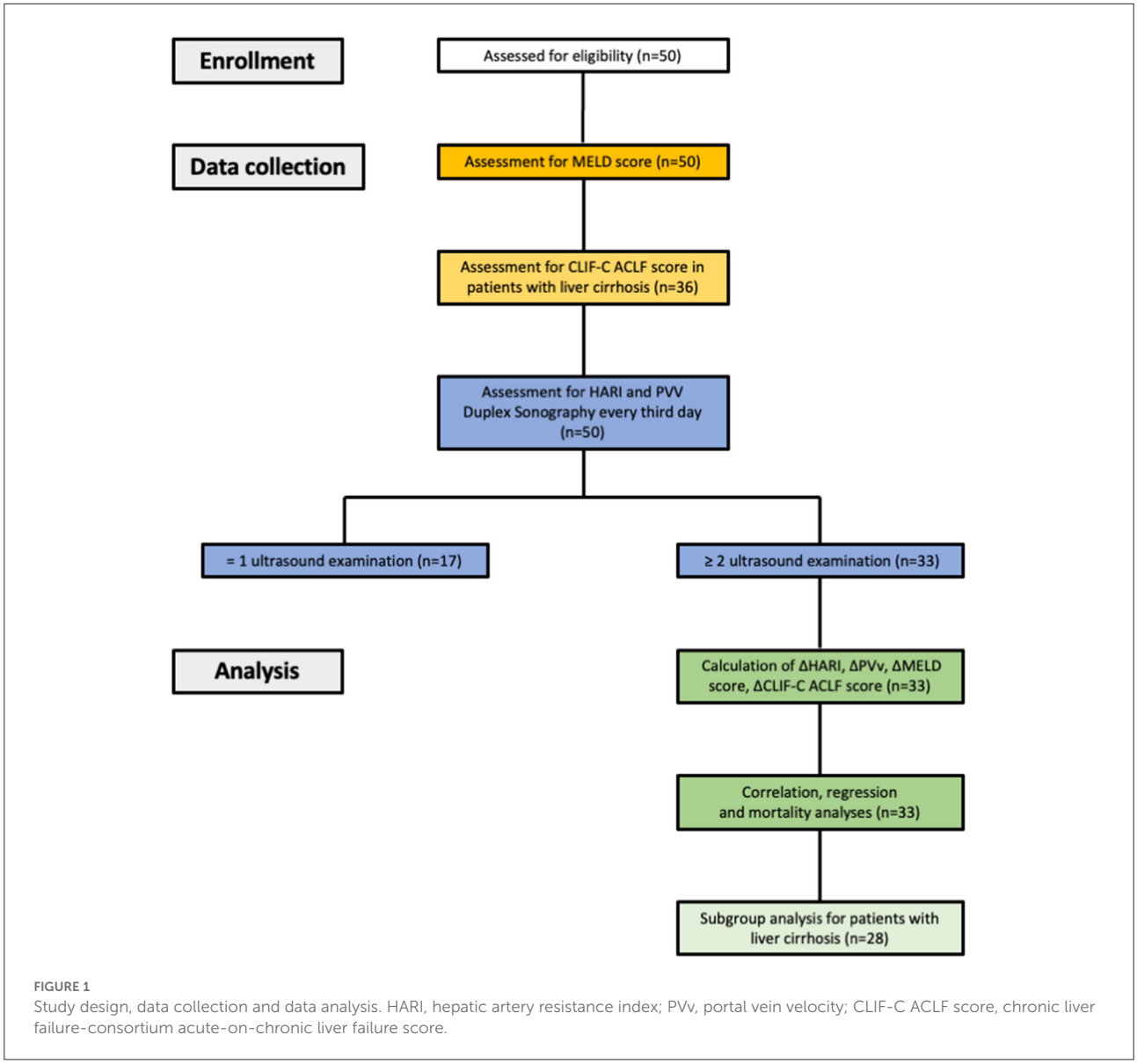

FIGURE 1
Study design, data collection and data analysis. HARI, hepatic artery resistance index; PVv, portal vein velocity; CLIF-C ACLF score, chronic liver failure-consortium acute-on-chronic liver failure score.

1–5 MHz frequency range. Each examination consisted of three independent measurements of PVv and the HARI. The mean values were calculated and recorded. An example of a Doppler ultrasound examination in a patient with ACLF is shown in Figure 2.

The PVv was measured using Pulsed-Wave-(PW)-Doppler in the hepatoduodenal ligament at the crossing level of the proper hepatic artery and the portal vein. The HARI was recorded at the crossing level of the hepatic artery and the portal vein using PW-Doppler (Figure 2). The resistance index was automatically calculated using the following equation (21):

$$\text{HARI} = (\text{Peak systolic velocity} - \text{End} - \text{diastolic velocity})/\text{Peak systolic velocity}.$$

The MELD score, a well-established indicator of the mortality of patients with end-stage liver disease, was calculated for each patient—simultaneously with the ultrasound examination—using the following equation (22, 23):

$$\begin{aligned}\text{MELD score} = {} & 9.57 \times \ln(\text{serum creatinine}) \\ & + 3.78 \ln(\text{total bilirubin}) + 11.2 \\ & \times \ln(\text{international normalized ratio}) + 6.43\end{aligned}$$

In patients with decompensated liver cirrhosis—in addition to the MELD score—the Chronic Liver Failure Consortium (CLIF)-C ACLF score, a score derived and validated by the CLIF consortium, was determined to predict the mortality of patients with ACLF (24). The following formula was used for

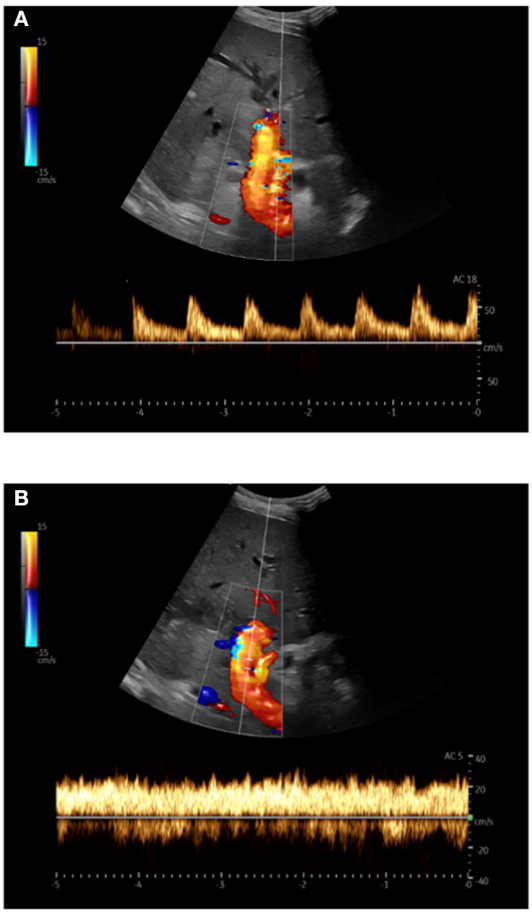

FIGURE 2
Doppler sonography in triplex technique (B-image + color Doppler + spectral Doppler) in a patient with acute-on-chronic liver failure; **(A)** Hepatic artery: Derivation of the arterial flow signal; **(B)** Portal vein: The maximum flow velocity was measured at the level of the hepatic artery after angle correction.

the calculation, wherein CLIF-C OF score was raised according to (24):

$$\text{CLIF} - \text{C ACLF score} = 10\times\ (0,33\times\text{CLIF} - \text{C} - \text{OFs} + 0,04 \times\text{Age} + 0,63\times\ \ln(\text{WBC count in } 10^3/\mu\text{l}) - 2)$$

To analyze the impact of life support in the MICU on liver perfusion, we recorded whether invasive ventilation (Servo-i®, Getinge, Sweden), renal replacement therapy (multiFiltrate Ci-Ca®, Fresenius, United States of America) or catecholamine therapy was required during intensive care treatment. Ventilation was pressure-controlled or pressure-supported; positive end-expiratory pressure (PEEP) and peak pressure (Ppeak) at the time of the ultrasound examination were collected. None of the patients received non-invasive ventilation during the ultrasound examination. The continuously administered catecholamines during the ultrasound examination were recorded in their respective dosage (Norepinephrine in mg/h, epinephrine in mg/h, dobutamine in mg/h, terlipressin in mcg/h, and vasopressin in IU/h).

Statistical analyses

Data were analyzed using SPSS Statistics, version 25 (IBM, USA). Correlation analyses of perfusion parameters, the MELD score, and the CLIF-C ACLF score were performed according to Pearson. The strength and direction of the correlations were described by the determined correlation coefficient (r). In addition, linear regression analyses of the perfusion parameters and the MELD score were carried out and described using the R^2-value. As part of the study, differences in parameters were determined for specific groups. Mann-Whitney-U-tests were used for non-normally distributed variables and t-tests for normally distributed variables with equal variance. A p-value of 0.05 was set as the level of significance. Multiple regression analyses were performed to examine the extent to which liver perfusion was affected by life support at the MICU.

Results

Baseline characteristics of the patients

A total of 50 patients were enrolled in the study. The demographic and clinical characteristics of these 50 study patients are summarized in Table 1. Thirty-four patients were male, and 16 were female. The age of the cohort ranged from 39 to 90 years (mean 59.7; SD ± 10.3 years). The study included patients with different stages of acute and chronic liver diseases. Thirty-six patients were diagnosed with liver cirrhosis. The leading etiology of liver cirrhosis was alcohol-related ($n = 25$), which was diagnosed in 3 patients with hepatocellular carcinoma (HCC). Other causes of liver cirrhosis were autoimmune liver diseases ($n = 4$), viral hepatitis ($n = 4$), and cirrhosis of idiopathic origin ($n = 3$). The patients with liver cirrhosis were categorized according to the Child-Pugh classification, 1 patient was classified with liver cirrhosis Child-Pugh A, 12 patients with liver cirrhosis Child-Pugh B, and 23 patients with liver cirrhosis Child-Pugh C. In summary, the majority of our patient cohort had advanced stages of liver cirrhosis and were at high risk of developing acute-on-chronic liver failure (Table 1).

The patients without underlying liver cirrhosis ($n = 14$) had been admitted to the MICU due to drug-induced acute liver failure (ALF) ($n = 6$), cholangiosepsis ($n = 5$), and liver diseases of different etiologies ($n = 3$).

On average, the patients were treated at the MICU for 15.3 (SD ± 13.8) days. The length of the MICU stay ranged

TABLE 2 Correlation and regression analyses between perfusion parameters and delta-MELD score.

Analyses	Statistical parameter	Delta-HARI ($n = 137$)	Delta-PVv ($n = 137$)
Correlation[a] with the delta-MELD score	Correlation coeff. r	0.469	−0.279
	P-value	0.007×10^{-6}*	0.001*
Regression[b] with the delta-MELD score	R-value	0.469	0.279
	R^2-value	0.22	0.078
	P-value of regression model	0.007×10^{-6}	0.001
	Coeff. of constant	−0.25	−0.27
	Regression coeff.	32.76	−0.16
	P-value of regression coeff.	0.007×10^{-6}	0.001
	95% confidence interval	22.27–43.25	−0.25 to −0.07

Results of the statistical analyses on the association between the delta-MELD score and the perfusion parameters delta-HARI and delta-PVv. [a]Correlation analyses according to Pearson for the delta-HARI/delta-PVv and the delta-MELD score. For each correlation, the coefficient (r) and the p-value are listed. [b]Regression analyses for the delta-HARI/delta-PVv and the delta-MELD score. For each regression, the R-/R^2-value, the p-value of the regression model, the constant coefficient, the regression coefficient with its p-value, and the 95% confidence interval are listed. *The correlations are statistically significant at the level of 0.05.

from a minimum of 2 days to a maximum of 72 days. Twenty-three of the 50 patients studied (=46%) underwent renal replacement therapy, and 13 patients (=26%) required mechanical ventilation therapy during intensive care treatment. Twenty-nine patients (=58%) required vasoactive medication for circulatory support during the MICU stay. Sixteen of the 50 examined patients (32%) died during the MICU stay. The leading cause of death was acute-on-chronic liver failure (93% of deceased patients, $n = 15$). In the non-survivors, ACLF resulted, despite maximum intensive care therapy, in multiorgan failure, coagulation failure, and circulation failure (15/16 patients). One patient (1/16 patients) newly diagnosed with congestive hepatopathy died of septic shock due to severe pneumonia.

Precipitating events for ACLF were infections and gastrointestinal bleedings. In 9 patients, infections were the precipitating events for ACLF (6/9 patients with pneumonia and 3/9 patients with urosepsis). In 6 patients, gastrointestinal bleeding was the precipitating events for ACLF (4/6 patients with varicose bleeding and 2/6 patients with non-varicose upper gastrointestinal bleeding).

HARI, PVv, and MELD score were collected and analyzed at the time of admission to the ICU. Overall $n = 50$ patients were studied. On average, the HARI was 0.74, the maximum PVv was 19.1 cm/s, and the MELD score was 25.2 at admission (Table 1). The mean values of HARI, PVv, and MELD score were compared for deceased and survived patients by t-test and Mann-Whitney U-test, respectively (Table 4A). On admission to the ICU, non-survivors had, on average, a higher MELD score (29.7 vs. 23.2, $p = 0.010$). Liver perfusion at admission did not differ significantly between non-survivors and survivors [HARI (0.75 vs. 0.73, $p = 0.342$), PVv (14.4 cm/s vs. 21.3 cm/s, $p = 0.129$)].

Dynamic changes of the liver perfusion parameters HARI and PVv

The primary goal of our study was to analyze the changes over time of the perfusion parameters HARI and PVv and to investigate their utility as prognostic biomarkers. In patients ($n = 33$) who were examined more than once during their MICU stay, the course over time parameters ($n = 137$) was calculated from the varying absolute values of each examination and are further referred to as delta-values (Table 1). The mean of the MELD score of the patients during their MICU stay was 25.8 points and decreased by 0.3 points with each examination. The mean HARI was 0.74 and decreased by 0.003 during the MICU stay. In contrast, the mean maximum PVv was 19.2 cm/s and increased by 0.4 cm/s during the MICU stay.

Liver perfusion parameters and the MELD score

The prognostic value of routine Doppler evaluation of hepatic perfusion on the MICU was determined by means of a correlation analysis of the delta-HARI and delta-PVv with the delta-MELD score. Correlation analysis was performed by bivariate correlation analyses according to Pearson (Table 2). There was a significant positive linear correlation between the delta-MELD score and the delta-HARI ($r = 0.469$; $p < 0.001$) and a negative linear correlation between the delta-MELD score and delta-PVv ($r = -0.279$, $p = 0.001$). The correlations between delta-MELD score, delta-HARI, and delta-PVv are shown in scatter plots in Figure 3. In summary, patients with increasing HARI or decreasing PVv showed an increase in their MELD Score, which reflects the worsening of their liver disease.

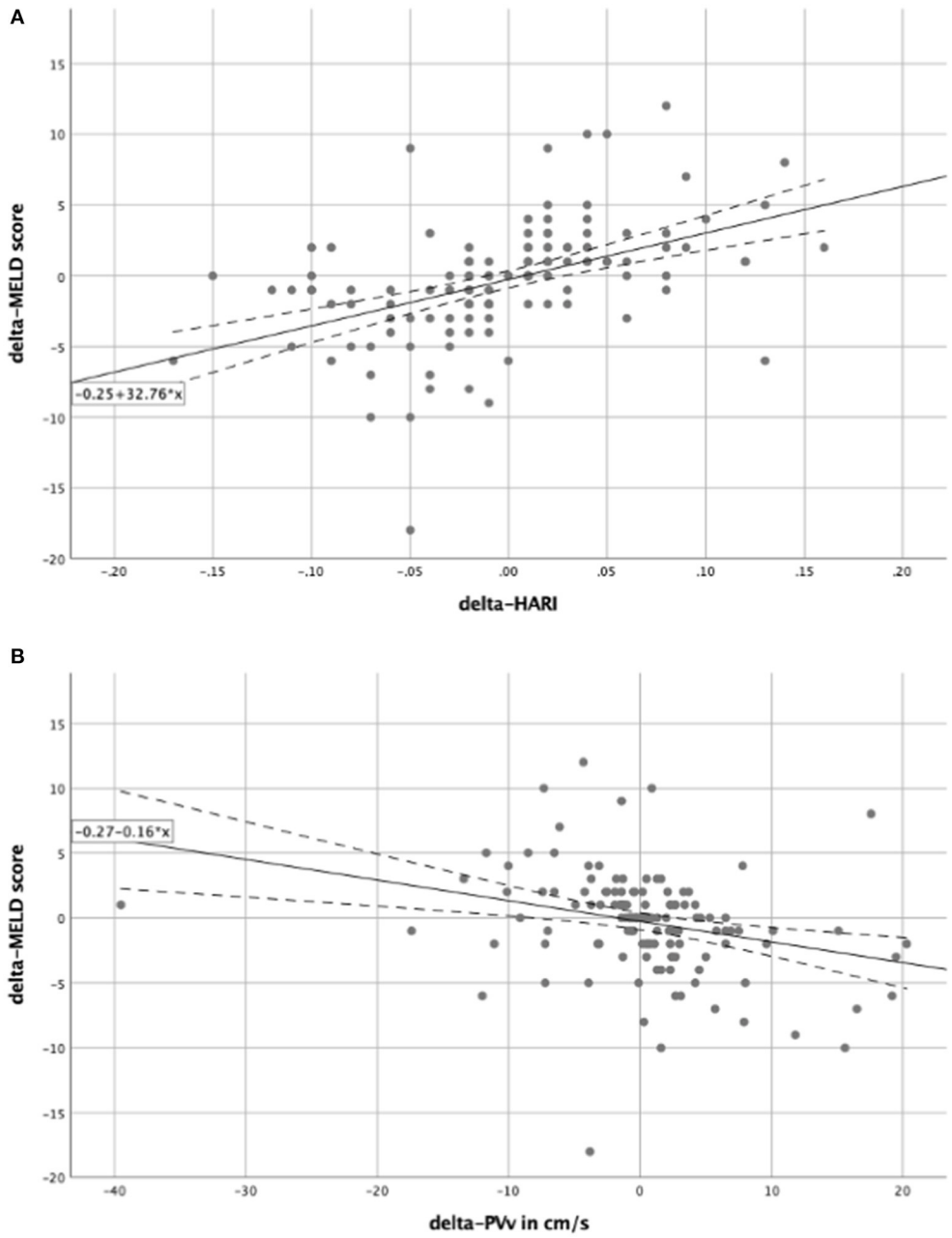

FIGURE 3
Scatter plots for the correlation between delta-MELD score and the perfusion parameters. In both scatter plots, the solid line represents the regression equation, which is also shown in the box. The dashed lines equate to the 95% confidence interval. **(A)** Showing a positive linear correlation between the delta-MELD score and the delta-HARI; **(B)** showing a negative linear correlation between the delta-MELD score and delta-PVv. HARI, hepatic artery resistance index; PVv, portal vein velocity.

Relation of liver perfusion parameters and the MELD score

To further investigate the influence of the delta-HARI and delta-PVv on the delta-MELD score, regression analyses (Table 2) showed an R^2-value of 0.220 and 0.078. Both regression models presented p-values of <0.05. The regression of the delta-HARI and the delta-MELD score resulted in a regression coefficient of 32.76 with a p-value of <0.001 and a 95% confidence interval ranging from 22.27 to 43.25. The regression coefficient for delta-PVv and the delta-MELD score was −0.16 with a p-value of 0.001 and a 95% confidence interval ranging

TABLE 3A Liver perfusion parameters as predictors of mortality.

Parameters	Deceased patients ($n = 16$)	Survived patients ($n = 34$)	*P*-value	Cohens's d[a]
(A) Liver perfusion parameters at admission as predictors of mortality				
HARI at admission	0.75 ± 0.06 (0.65–0.84)	0.73 ± 0.08 (0.57–0.9)	0.342[†]	0.291 (weak)
PVv at admission	14.4 ± 15 (−20.9 to 29.9)	21.3 ± 13.2 (−39.8 to 45.7)	0.129*	0.44 (weak)
MELD score at admission	29.7 ± 6.2 (21–40)	23.2 ± 8.8 (8–40)	0.01[†]	0.809 (strong)

Mean comparison of HARI, PVv and MELD score at admission for deceased and survived patients. The results are expressed as mean ± SD (range). Only the MELD score at admission shows statistically significant differences in its means values. [a]Cohen's d indicates effect size, calculated using https://www.psychometrica.de/effektstaerke.html, *Mann-Whitney-*U*-test used for non-normally distributed PVv at admission, [†] *t*-test used for normally distributed HARI and MELD score at admission.

TABLE 3B Differences in perfusion parameters over time predicting mortality.

Parameters	Deceased patients ($n = 13$)	Survived patients ($n = 20$)	*P*-value	Cohens's d[a]
Delta-HARI	0.01 ± 0.06 (−0.1 to 0.16)	−0.005 ± 0.043 (−0.07 to 0.13)	0.015*	0.934 (strong)
Delta-PVv	−0.7 ± 2.1 (−4.7 to 3)	1.9 ± 5.3 (−11.1 to 15.6)	0.037*	0.778 (middle)
Delta-MELD score	1.3 ± 2.3 (−2 to 6)	−1.9 ± 2.9 (−10 to 3)	0.002[†]	1.203 (strong)

Mean comparison of delta-HARI, delta-PVv and delta-MELD score for deceased and survived patients. The results are expressed as mean ± SD (range). The *p*-value of each of the three parameters shows statistically significant differences in their means. [a]Cohen's d indicates effect size, calculated *via* https://www.psychometrica.de/effektstaerke.html, *Mann-Whitney-U-test used for non-normally distributed delta-HARI and delta-PVv, [†] *t*-test used for normally distributed delta-MELD score.

from −0.25 to −0.07. The determined coefficients were used to set up the following regression equations to be able to predict the course over time of the MELD score as a function of the delta-HARI and delta-PVv:

$$\text{delta-MELD score} = -0.25 + 32.76 \times \text{delta-HARI}$$ (Figure 3A).

$$\text{delta-MELD score} = -0.27 - 0.16 \times \text{delta-PVv}$$ (Figure 3B).

Furthermore, the relation between delta-HARI and delta-PVv was analyzed by bivariate correlation analyses according to Pearson, $r = -0.159$, $p = 0.063$. This suggests that the hepatic artery buffer response (HABR) is impaired in our patient cohort. If the HABR were functional, we would expect a positive correlation (10).

Hepatic perfusion as a predictor of ACLF and mortality at the MICU

The mean values of delta-HARI, delta-PVv, and delta-MELD score were calculated for the 33 patients who had been examined more than once during their MICU stay. The mean values of these parameters were then compared to evaluate their utility as prognostic markers in patients with severe liver disease (Table 3). Patients who did not survive ACLF were characterized by an increase of their MELD score on average by 1.3 points and of the hepatic artery resistance index by 0.01, whereas maximum portal vein velocity decreased on average by 0.7 cm/s per examination. In survivors, the MELD score decreased on average by 1.9 points and the hepatic artery resistance index by 0.005, whereas maximum portal vein velocity increased on average by 1.9 cm/s per examination. The distribution of delta-HARI, delta-PVv, and delta-MELD score for non-survivors and patients who recovered is shown in boxplots in Figure 4. The comparison of non-survivors and survivors showed statistically significant differences in the mean values of delta-HARI ($p = 0.015$), delta-PVv ($p = 0.037$), and delta-MELD score ($p = 0.002$). Of clinical relevance, each of the three parameters was useful as a prognostic biomarker for patients with ACLF. Cohen's d was calculated and interpreted to quantify the size of each effect. Figure 4 shows the newly described statistically significant differences in the mean values of delta-HARI (Figure 4A) and delta-PVv (Figure 4B) between deceased and surviving patients.

Thus, delta-HARI and delta-PVv can predict the mortality of critical care patients with severe liver diseases to a similar extent as the delta-MELD score. Differences in the mean values of the delta-MELD score indicate a slightly stronger effect on mortality and higher prognostic predictive value of the delta-MELD in comparison to the delta-HARI. In our dataset, the area under the curve (AUC) for the prediction of ICU mortality for delta-HARI was 0.76 (95% Confidence Interval: 0.58–0.94, $p = 0.012$) and thus only slightly lower than that of the delta-MELD with an AUC of 0.84 (95% Confidence Interval: 0.70–0.97, $p = 0.01$). Increasing HARI and decreasing PVv are early predictors of an adverse outcome of patients with severe liver diseases. A summary of the data is given in Figure 5.

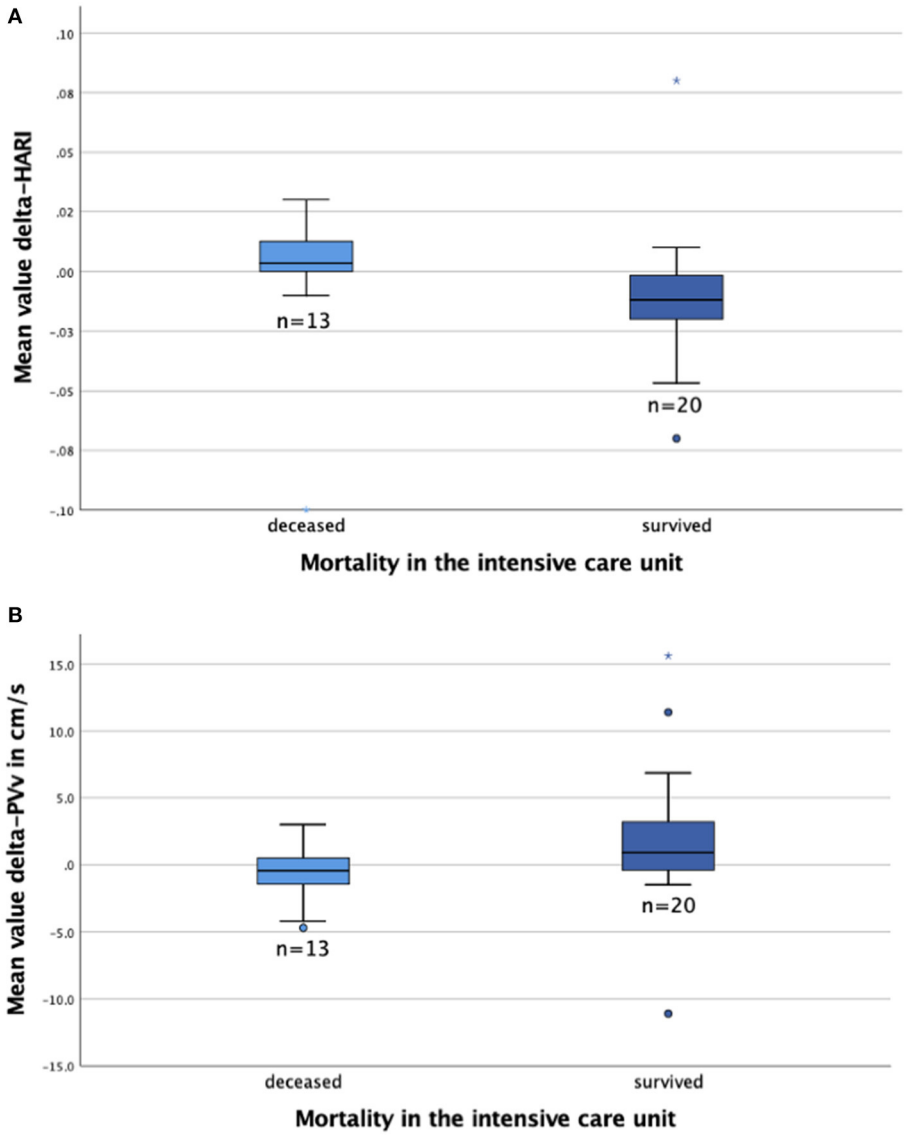

FIGURE 4
Boxplots to compare the MELD score and perfusion parameters for deceased ($n = 13$) and survived ($n = 20$) patients. The figures show the median (line in the middle of the box), 1st/3rd quartile (lower/upper edge of the box), minimum (lower whisker), maximum (upper whisker) and outliers*°. **(A)** Delta-HARI is higher in deceased patients than in survived patients; **(B)** Delta-PVv is lower in deceased patients than survived patients. HARI, hepatic artery resistance index; PVv, portal vein velocity.

Effect of life support in the MICU on liver perfusion

Multiple regression analyses were performed to examine the extent to which delta-HARI and delta-PVv were affected by factors other than the delta-MELD score. Here, we primarily focused on the effects of intensive care therapeutic procedures.

Forty-six percentage of the patients underwent renal replacement therapy (Table 1). In our study, no ultrasound examinations were performed while the patients were dialyzed. Liver perfusion did not significantly differ in patients with dialysis or without dialysis (delta-HARI 0.006 vs. −0.008, $p = 0.074$, delta-PVv 0.8 cm/s vs. 0.9 cm/s, $p = 0.868$, Mann-Whitney U-test). Twenty-six percentage of the patients required mechanical ventilation during intensive care treatment. There was no correlation between ventilation pressures (PEEP and Ppeak) and liver perfusion over time. Fifty-eight percentage of the patients required vasoactive medication for circulatory support during the ICU stay, with norepinephrine being the most frequently used catecholamine. A combination therapy of vasoactive agents was required in 11 of the 29 patients receiving vasoactive medication. Delta-norepinephrine ($r =$

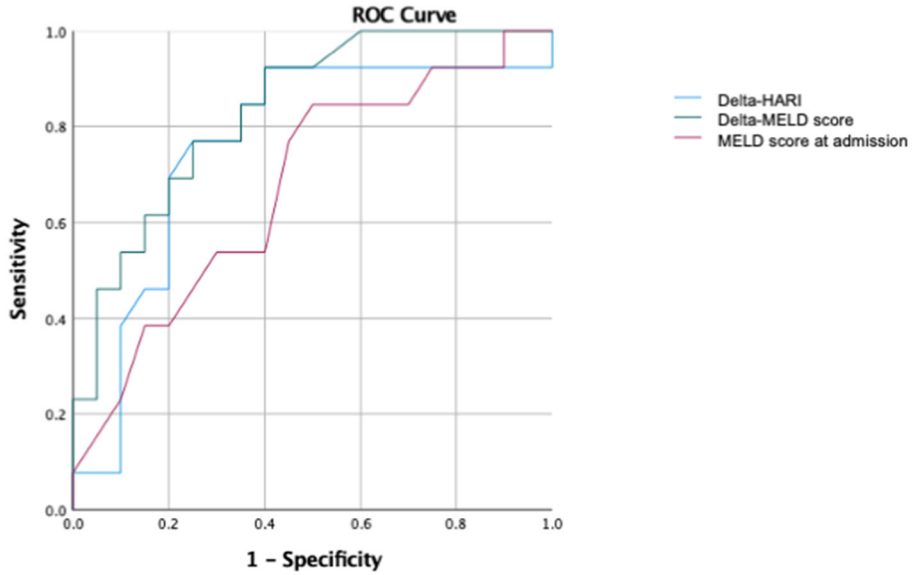

FIGURE 5
ROC analysis for the prediction of ICU mortality. AUC of delta-HARI was 0.76 (95% Confidence Interval: 0.58–0.94, $p = 0.012$), AUC of delta-MELD was 0.84 (95% Confidence Interval: 0.70–0.97, $p = 0.01$), and AUC of MELD-Score at admission was 0.71 (95% Confidence Interval: 0.53–0.89, $p = 0,049$).

TABLE 4A Life support and liver perfusion.

Life support in the MICU		Delta-HARI		Delta-PVv	
(A) Effect of life support in the MICU on liver perfusion					
Renal replacement therapy	Required ($n = 23$)	0.006 ± 0.06 (−0.1 to 0.16)		0.8 ± 4.1 (−4.7 to 15.6)	
	Not required ($n = 27$)	−0.008 ± 0.03 (−0.04 to 0.08)		0.9 ± 5.2 (−11.1 to 11.4)	
	P-value	0.074*		0.868*	
Mechanical ventilation		Delta-PEEP	Delta-Ppeak	Delta-PEEP	Delta-Ppeak
	Correlation[a] coeff. r	0.086	0.08	0.156	0.016
	P-value	0.522	0.551	0.243	0.906
	Required ($n = 13$)	−0.01 ± 0.01 (−0.04 to 0.01)		−0.2 ± 4.1 (−11.1 to 6.9)	
	Not required ($n = 37$)	0.01 ± 0.06 (−0.01 to 0.16)		1.5 ± 4.8 (−4.7 to 15.6)	
	P-value	0.321*		0.75*	
Vasopressor therapy		Delta-Norepinephrine	Delta-Epinephrin	Delta-Dobutamine	
	Correlation[b] coeff. r	0.247	0.244	−0.18	
	P-value	0.004†	0.004†	0.036†	

Effect of renal replacement therapy, mechanical ventilation, and vasopressor therapy on delta-HARI and delta-PVv. Comparison of the mean value of delta-HARI and delta-PVv for patients who require renal replacement therapy or mechanical ventilation and for those who do not. No statistically significant differences could be found. *Mann-Whitney-U test was used for non-normally distributed delta-HARI and delta-PVv. [a]Pearson's correlation analyses for delta-HARI/delta-PVv and delta-PEEP and delta-Ppeak, coefficient (r) and *p*-value given for each correlation. [b]Pearson's correlation analyses for delta-HARI and delta-Norepinephrine and delta-Epinephrine as well as delta-PVv and delta-Dobutamine. Statistically significant correlations between delta-HARI and delta-PVv with vasopressors are shown. †Correlations are statistically significant ($p < 0.05$).

0.247, $p = 0.004$) and delta-epinephrine ($r = 0.244$, $p = 0.004$) showed a positive correlation with the delta-HARI. Delta-dobutamine ($r = -0.18$, $p = 0.036$) correlated statistically significantly with delta-PVv. Details concerning the effect of life support in the MICU on liver perfusion are shown in Table 4A.

In multiple regression analyses, factors that potentially influence liver perfusion such as delta-norepinephrine, delta-epinephrine, delta-dobutamine, delta-terlipressin, delta-vasopressin, delta-PEEP, and delta-Ppeak were compared with the delta-MELD score concerning their effect on the respective liver perfusion parameters. The regression for delta-HARI

TABLE 4B Multiple regression analyses between life support in the MICU and liver perfusion.

Life support	Delta-HARI			Delta-PVv		
	Stand. coeff.	*P*-value	95% confidence interval	Stand. coeff.	*P*-value	95% confidence interval
Delta-PEEP	0.112	0.565	−0.021 to 0.043	0.04	0.063	−0.044 to 1.697
Delta-Ppeak	−0.285	0.155	−0.004 to 0.001	−0.024	0.28	−0.538 to 0.157
Delta-Norepinephrine	0.189	0.033*	0.002–0.037	0.067	0.495	−1.6 to 3.293
Delta-Epinephrine	0.153	0.211	−0.098 to 0.440	−0.06	0.659	−45.248 to 28.718
Delta-Terlipressin	0.063	0.422	−0.0001 to 0.0002	−0.085	0.338	−0.032 to 0.011
Delta-Dobutamine	0.168	0.027*	0.001–0.014	−0.219	0.01*	−2.116 to 0.29
Delta-Vasopressin	0.087	0.497	−0.021 to 0.043	−0.056	0.695	−5.334 to 3.567
Delta-MELD score	0.439	0.007×10^{-6}*	0.004–0.008	−0.281	0.001*	−0.797 to 0.196

Results of the multiple regression analyses between perfusion parameters delta-HARI and delta-PVv and potentially influencing factors of the life support in the MICU. For each regression, the standardized coefficient beta, the *p*-value, and the 95% confidence interval are given. Delta-MELD score shows the most significant and highest effect on delta-HARI and delta-PVv.
*The regressions are statistically significant ($p < 0.05$).

yielded a corrected R^2 of 0.290 with a model significance of $p < 0.001$. Delta-MELD score ($p < 0.001$), delta-norepinephrine ($p = 0.033$), and delta-dobutamine ($p = 0.027$) showed a significant effect on the course of HARI, with delta-MELD score clearly exerting the most significant influence (standardized coefficient beta: 0.439 vs. 0.189 and 0.168, respectively) (Table 4B). The regression for delta-PVv yielded a corrected R^2 of 0.116 with a model significance of $p = 0.003$. For delta-MELD score ($p = 0.001$) and delta-dobutamine ($p = 0.010$) a significant effect on delta-PVv could be determined. Again, delta-MELD score affected the course of PVv more pronounced than delta-dobutamine (standardized coefficient beta: −0.281 vs. −0.219) (Table 4B).

In summary, delta-HARI and delta-PVv are significantly influenced by the delta-MELD score and not by dialysis or mechanical ventilation. Norepinephrine and dobutamine have a mild to moderate impact on liver perfusion, while the delta-MELD score exerts the most significant effect on the course of the perfusion parameters HARI and PVv. Our analyses also reveal that optimized catecholamine therapy and fluid management are potential therapeutic targets to improve liver perfusion.

Analysis of liver perfusion in patients with liver cirrhosis—Comparison with CLIF-C ACLF and MELD score and early prediction of mortality in the MICU

Of clinical relevance, the leading cause of death in our patient cohort was an acute-on-chronic liver failure (93% of deceased patients, $n = 15$). Therefore, we performed a correlation analysis between delta-HARI and delta-PVv with the delta-MELD and delta-CLIF-C ACLF score in a subgroup analysis of the 36 patients with liver cirrhosis.

The CLIF-C ACLF score is a score that was derived and validated by the chronic liver failure (CLIF) consortium to predict the mortality of patients with ACLF (24). The CLIF-C ACLF score combines the age of the patient and the white blood cell count with the chronic liver failure (CLIF) organ failure score (CLIF OF score), which is a modified version of the Sequential Organ Failure Assessment (SOFA) score (24–26). The CLIF OF score system comprises the organs/systems liver, kidney, brain, coagulation, circulatory, and respiratory with the respective subscores 1–3 (24).

For the subgroup analysis of patients with liver cirrhosis ($n = 33$), HARI, PVv, and MELD score were collected at admission to the MICU and compared for deceased and survived patients by *t*-test and Mann-Whitney *U*-test, respectively (Table 6A). Analogous to the complete patient collective, the liver perfusion parameters at admission showed no significant differences regarding the mortality of the patients. MELD score was a predictor of mortality on admission.

Dynamic changes over time of the liver perfusion parameters in patients with liver cirrhosis

Changes over time in the liver perfusion parameters were analyzed in the subgroup of patients with liver cirrhosis. Our analyses showed a significant positive linear correlation between the delta-HARI and the delta-MELD score ($r = 0.517$; $p < 0.001$) and the delta-CLIF-C ACLF score ($r = 0.252$; $p = 0.005$). In addition, we could demonstrate a concomitant negative linear correlation between delta-PVv and the delta-MELD score ($r = -0.316$; $p < 0.001$).

TABLE 5 Subgroup analyses—Correlation and regression analyses between perfusion parameters and delta-MELD score and delta-CLIF-C ACLF score for patients with liver cirrhosis.

Analyses	Statistical parameter	Delta-HARI ($n = 122$)	Delta-PVv ($n = 122$)
Correlation[a] with the delta-MELD score	Correlation coeff. R	0.517	−0.316
	P-value	1.070×10^{-9}*	3.870×10^{-4}*
Regression[b] with the delta-MELD score	*R*-value	0.517	0.316
	R^2-value	0.267	0.1
	P-value of regression model	1.070×10^{-9}	3.870×10^{-4}
	Coeff. of constant	−0.01	−0.07
	Regression coeff.	33.68	−0.17
	P-value of regression coeff.	1.070×10^{-9}	3.870×10^{-4}
	95% confidence interval	23.60–43.75	−0.25 to −0.08
Correlation[a] with the delta-CLIF-C ACLF score	Correlation coeff. r	0.252	−0.106
	P-value	0.005*	0.245
Regression[b] with the delta-CLIF-C ACLF score	*R*-value	0.252	0.106
	R^2-value	0.063	0.011
	p-value of regression model	0.005	0.245
	Coeff. of constant	0.06	-4×10^{-3}
	Regression coeff.	29.77	−0.1
	P-value of regression coeff.	0.005	0.245
	95% confidence interval	9.1–50.45	−0.27 to −0.07

Results of the correlation and regression analyses between perfusion parameters delta-HARI and delta-PVv and delta-MELD score and delta-CLIF-C ACLF for patients with liver cirrhosis. [a]Correlation analyses according to Pearson for delta-HARI/delta-PVv and delta-MELD score and delta-CLIF-C ACLF score. For each correlation, the coefficient (*r*) and the *p*-value are listed. [b]Regression analyses for the delta-HARI/delta-PVv and the delta-MELD score and the delta-CLIF-C ACLF score, respectively. For each regression, the *R*-/R^2-value, the *p*-value of the regression model, the constant coefficient, the regression coefficient with its *p*-value, and the 95% confidence interval are listed. *The correlations are statistically significant at the level of 0.05.

There was no significant correlation between delta-PVv and delta-CLIF-C ACLF score ($r = -0.106$, $p = 0.246$).

For further investigation of the correlation of delta-HARI or delta-PVv with the delta-MELD score and the delta-CLIF-C ACLF score, regression analyses were performed. These showed an R^2-value of 0.261 for the influence of delta-HARI on the delta-MELD score and an R^2-value of 0.063 for the influence of delta-HARI on delta-CLIF-C ACLF score, respectively. Both regression models showed *p*-values of <0.05. For the effect of the delta-PVv on the delta-MELD score an R^2-value of 0.1 was calculated ($p < 0.05$).

Regression analyses for the effect of delta-PVv on the delta-CLIF-C ACLF score showed R^2-values of 0.011 and were not significant, $p = 0.245$.

Data are shown in Tables 5, 6 and Figure 6.

Using the determined coefficients, the following regression equitation can predict the course over time of the delta-MELD score and the delta-CLIF-C-ACLF score as a function of delta-delta-HARI and delta-PVv.

Delta-MELD score = −0.01 + 33.68 x delta-HARI (Figure 6A).

Delta-CLIF-C ACLF score = 0.06 + 29.77 x delta-HARI (Figure 6B).

Delta-MELD score = −0.07 to 0.17 × delta-PVv

Delta-CLIF-C ACLF score was not calculated as a function of delta-PVv as the regression analyses were not significant.

In summary, in our subgroup analysis for patients with liver cirrhosis and ACLF, we could confirm the correlation of the delta-HARI and delta-PVv with the delta-MELD score, which were evident in the entire cohort. The correlations in the subgroup of patients with liver cirrhosis and ACLF were even higher than in the entire cohort, highlighting the relevance of our findings for patients with cirrhosis and ACLF.

Furthermore, we identified a new significant correlation of the delta-HARI with the delta-CLIF-C ACLF score in this cohort, which is of high clinical relevance because the CLIF-C ACLF score is -up to date- the prognostic score, which is the best predictor of mortality in ACLF (26). The correlations between delta-HARI and delta-CLIF-C ACLF are lower than the correlations between the delta-HARI and the delta-MELD score. We attribute this to the fact that all organ systems are included in the prediction of mortality of the CLIF-C ACLF score, whereas in our study, the ultrasound examinations were focused on the liver. In this context, the MELD score is more specific for the system "liver" in terms of the parameters included, which

TABLE 6A Subgroup analyses—Liver perfusion parameters as predictors of mortality in patients with liver cirrhosis.

Parameters	Deceased patients ($n = 15$)	Survived patients ($n = 21$)	*P*-value	Cohens's d[a]
(A) Subgroup analyses—Perfusion parameters at admission as predictors of mortality in patients with liver cirrhosis				
HARI at admission	0.75 ± 0.06 (0.65–0.84)	0.75 ± 0.06 (0.61–0.85)	0.952[†]	0.021 (no effect)
PVv at admission	14.3 ± 15.6 (−20.9 to 29.9)	22.4 ± 8.2 (8.5–45.7)	0.446*	0.259 (weak)
MELD score at admission	30.0 ± 6.3 (21–40)	24.0 ± 8.3 (9–40)	0.023[†]	0.803 (strong)

Mean comparison of HARI, PVv and MELD score at admission for deceased and survived patients with liver cirrhosis. The results are given as mean ± SD (range). Only the MELD score at admission shows statistically significant differences in its means. [a]Cohen's d indicates effect size, calculated using https://www.psychometrica.de/effektstaerke.html, [†] *t*-test used for normally distributed HARI and MELD score at admission, [*]Mann-Whitney-*U*-test used for non-normally distributed PVv at admission.

TABLE 6B Subgroup analyses—Differences in perfusion parameters over time predicting mortality in patients with liver cirrhosis.

Parameters	Deceased patients ($n = 13$)	Survived patients ($n = 15$)	*P*-value	Cohens's d[a]
Delta-HARI	0.01 ± 0.05 (−0.10 to 0.16)	−0.01 ± 0.03 (−0.07 to 0.08)	0.011*	1.065 (strong)
Delta-PVv	−0.7 ± 2.1 (−4.7 to 3)	1.9 ± 6.1 (−11.1 to 15.6)	0.13*	0.6 (middle)
Delta-MELD score	1.3 ± 2.3 (−2 to 6.0)	−1.5 ± 2.8 (−10.0 to 3)	0.004*	1.239 (strong)

Mean comparison of delta-HARI, delta-PVv and delta-MELD score for deceased and survived patients with liver cirrhosis. The results are given as mean ± SD (range). The *p*-values of delta-HARI and delta-MELD score show statistically significant differences in their means. Only delta-PVv shows no significant differences in its means. [a]Cohen's d indicates effect size, calculated *via* https://www.psychometrica.de/effektstaerke.html, [*]Mann-Whitney-*U*-test used for non-normally distributed delta-HARI, delta-PVV and delta-MELD score.

explains the better correlation of the delta-HARI with the delta-MELD score.

In addition, in the subgroup of patients with liver cirrhosis, ROC (Receiver operating characteristic) analyses were performed to predict ICU mortality (Figure 7). In this subgroup of patients with liver cirrhosis, the AUC for the prediction of ICU mortality for delta-HARI was 0.78 (95% Confidence Interval: 0.60–0.97, $p = 0.011$) and thus only slightly lower than that of the delta-MELD 0.81 (95% Confidence Interval: 0.65–0.97, $p = 0.005$). The AUC of delta-CLIF-C ACLF in this subgroup was highest with 0.815 (95% Confidence Interval: 0.66–0.97, $p = 0.005$), which is in accordance with the literature regarding the CLIF-C ACLF score as the best prognostic score of ACLF.

In summary, we show a positive correlation of the delta-HARI with the delta-MELD score ($r = 0.469$, $p < 0.001$) and a negative correlation of the delta-PVv with the delta-MELD score ($r = -0.279$, $p = 0.001$). Compared with the mean values of the delta-HARI (−0.003) and the delta-PVv (0.4 cm/s), the MELD score decreased throughout the MICU stay with simultaneously decreasing resistance indices of the hepatic artery and increasing maximum portal flow velocity. As a decreasing MELD score is a positive prognostic factor, both decreasing HARI and increasing PVv can be considered as novel positive prognostic factors for patients with severe liver diseases. In contrast, increasing HARI and decreasing PVv constitute negative prognostic biomarkers for patients with severe liver diseases. These findings were confirmed in a subgroup analysis of patients with liver cirrhosis. Here we established a new correlation between the delta-HARI and the delta-CLIF-C-ACLF score ($r = 0.252$; $p = 0.005$) and confirmed the delta-HARI as an accurate predictor of the outcome of patients with ACLF.

Discussion

This prospective cohort study aims 1. to determine the predictive value of changes in hepatic perfusion for the outcome in patients with severe liver diseases, 2. to analyze the role of liver perfusion as a new predictor for mortality due to ACLF, and 3. to establish perfusion-based biomarkers as early readouts for therapy guidance in patients with severe liver diseases and ACLF.

We have analyzed changes in hepatic perfusion of critical care patients with acute and chronic liver diseases over the course of their MICU stay to establish new biomarkers of prognosis and therapy guidance. This is the first report of a prospective time series measurement using Doppler sonography in patients with liver disease in the MICU. An increase in the hepatic artery resistance index (HARI) and a decrease in portal vein velocity (PVv) during the MICU stay predicted an adverse outcome and increased mortality.

Previous studies have focused on hepatic hemodynamics in general and on the importance of ultrasound examination in particular (27–30), and data were collected retrospectively or as part of a cross-sectional study (31–34). Only a few studies

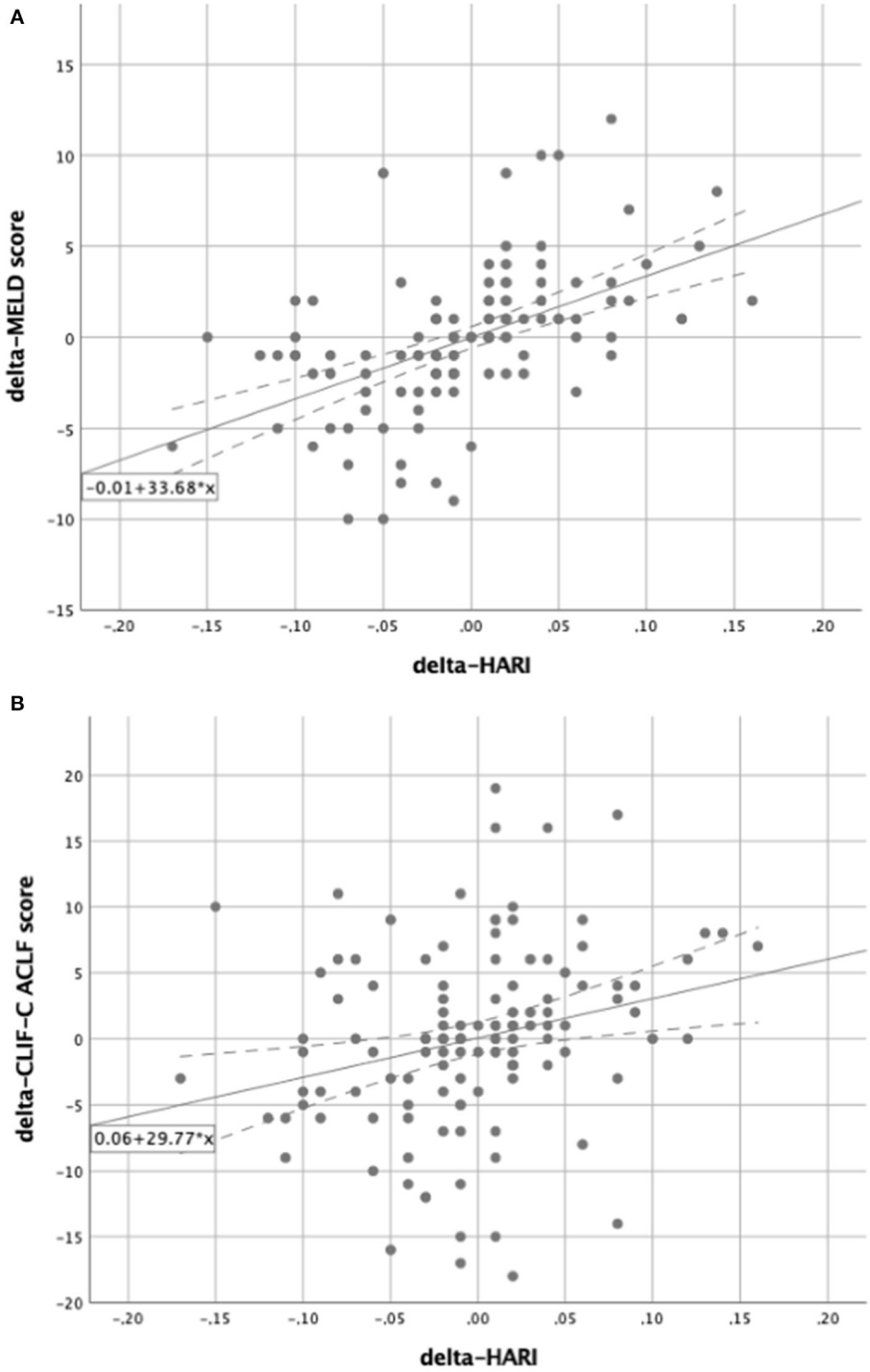

FIGURE 6
Subgroup analysis for patients with liver cirrhosis. Scatter plots for the correlation between delta-HARI and delta-MELD or delta-CLIF-C ACLF score for patients with liver cirrhosis. **(A)** Showing a positive linear correlation between delta-HARI and delta-MELD score. **(B)** showing a positive linear correlation between delta-HARI and delta-CLIF-C ACLF score.

have examined the correlation between Doppler sonographic measurements of hepatic perfusion and the MELD score so far (31–33, 35, 36).

The patients included in our study showed a mean hepatic artery resistance index of 0.74 ± 0.08 (range 0.55–0.95) and a maximum portal vein velocity of 19.2 ± 15.7 cm/s (range

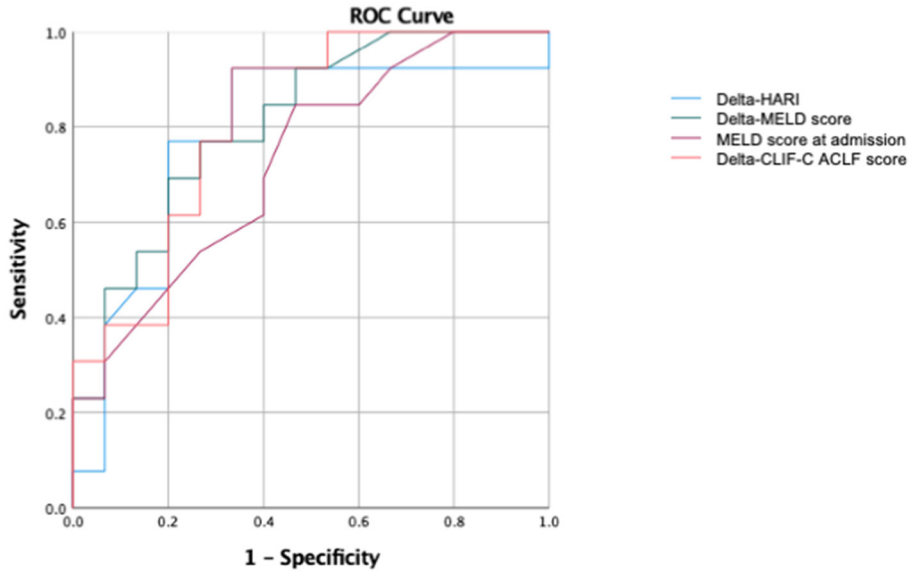

FIGURE 7
Subgroup ROC analysis for patients with liver cirrhosis of the prediction of ICU mortality. AUC of delta-HARI was 0.78 (95% Confidence Interval: 0.60–0.97, $p = 0.011$), AUC of delta-MELD was 0.81 (95% Confidence Interval: 0.65–0.97, $p = 0.005$), AUC of MELD-Score at admission was 0.73 (95% Confidence Interval: 0.54–0.914, $p = 0.040$), and AUC of delta-CLIF-C-ACLF was 0.815 (95% Confidence Interval: 0.66–0.97, $p = 0.005$).

−43.8 to 49.2 cm/s). These results are comparable to those of other studies in patients with liver cirrhosis of alcoholic vs. viral etiology (14, 21, 37, 38). In our study, the absolute mean values of the HARI and PVv reflect the momentary/current status of hepatic perfusion, whereas the time series measurement of hepatic perfusion—reflected by the parameter delta-HARI and delta-PVv—accurately describe the development of hepatic perfusion over time during hospitalization at the MICU. Our study recorded a mean of −0.003 ± 0.057 (range −0.170 to 0.160) for delta-HARI and a mean of 0.4 ± 7.0 cm/s (range −39.5 to 20.3) for delta-PVv. In patients with a good prognosis arterial resistance in the liver decreased, and the maximum portal flow velocity increased over time and with recovery. On the contrary, increasing HARI and decreasing PVv were predictors of an adverse outcome in critically ill patients with different stages of acute and chronic liver diseases. Non-survivors showed a higher delta-HARI (0.010 vs. −0.005; $p = 0.015$) and lower delta-PVv (−0.7 vs. 1.9 cm/s; $p = 0.037$) in comparison to patients who survived. Of note, it is the change over time of these perfusion parameters, which most accurately predicts outcome and mortality.Thus, we identified delta-HARI and delta-PVv as early predictors of mortality in acute and chronic liver diseases.

Mortality in our patient cohort was predominantly due to acute-on-chronic liver failure (ACLF). Worldwide, ACLF is emerging as a major cause of mortality in patients with cirrhosis and chronic liver diseases (5). A systematic review of the global burden of ACLF recently reported a prevalence among patients admitted with decompensated cirrhosis of 35% and 90-day mortality of 55% (1). Of note, the exact definition of ACLF varies worldwide (3, 39–42). In Europe, ACLF is generally defined according to the European Association for the Study of the Liver-Chronic Liver Failure (EASL-CLIF) Consortium as an acute deterioration of pre-existing chronic liver disease associated with organ failure and high short-term mortality (i.e., death <28 days after hospital admission) (25). In collaboration with the ESAL-CLIF Consortium, Jalan et al. (24) established a prognostic score to predict mortality in patients with acute-on-chronic liver failure, the CLIF-Consortium ACLF (CLIF-C ACLF) score. This score combines the CLIF-C Organ Failure Score [a modification of the Sequential Organ Failure Assessment (SOFA) score] with two other independent predictors of mortality (age and white cell count). Compared to other prognostic scores, such as the MELD score and Child-Pugh score, the CLIF-C ACLF score is the best available score for the prediction of 28-day mortality among patients with ACLF (43, 44). Of note, none of these scores includes liver perfusion parameters.

By demonstrating an association between delta-HARI and delta-PVv with ACLF-related mortality, our study shows for the first time that the course over time of hepatic perfusion plays a crucial role in the prognosis of patients with ACLF. We were able to show a clear utility for liver hemodynamic parameters as prognostic biomarkers by comparing serial measurements of HARI with the established prognostic scores delta-CLIF-C-ACLF in the early prediction of ACLF-related mortality.

Our results are in accordance with Mehta et al. who showed that the development of ACLF and its associated inflammatory response markedly changes intrahepatic hemodynamics with a subsequent decrease in hepatic blood flow and an increase in intrahepatic resistance, which predicted mortality (45). Solís-Muñoz et al. reported that the portal vein velocity was significantly lower in acutely decompensated patients with cirrhosis who developed ACLF than in those who did not develop ACLF (46). Furthermore, our data are in line with the results of Kuroda et al. (34) who analyzed hepatic perfusion using contrast-enhanced ultrasound (CEUS) in patients with acute liver failure (ALF) and investigated its utility as a prognostic tool (47). The authors recorded the time interval (TI) between the time to peak of the hepatic artery (HA) and liver parenchyma (LP) by performing CEUS at baseline and after 7 days. TI (HA, LP) was significantly shorter in non-survivors than in survivors and emerged as the only independent factor for predicting adverse prognosis in patients with ALF, with a 94.4% sensitivity and 90.6% specificity. This underlines the importance of preventing increasing HARI and decreasing PVv and implementing serial Doppler sonographic or CEUS-bases liver perfusion measurements in managing patients with acute and chronic liver disease. The transferability to daily clinical practice and the cost-effectiveness in the guidance of treatment is undoubtedly easier using routine Doppler sonography in comparison with CEUS.

Thus, the newly established correlation between hepatic perfusion and mortality, delta-HARI and delta-PVv, may present new valuable targets in the guidance of critical care therapy for patients with severe liver diseases by optimizing hepatic perfusion, for example, through calculated volume therapy or modulation of vasoactive medication. Thus, initial fluid resuscitation in ACLF following the recommendations of the International Guidelines for the Management of Sepsis (48) could be guided by repeated Doppler sonographic measurements to restore hemodynamics and to optimize liver perfusion. The choice of resuscitation fluid in patients with cirrhosis and ACLF is unclear. This issue was addressed by Maiwall et al. who compared the efficacy and safety of 20% Albumin to plasmalyte in the treatment of sepsis-induced hypotension (49). In critically ill patients with cirrhosis and sepsis-induced hypotension 20% albumin restores arterial pressure more quickly but causes more pulmonary complications than plasmalyte. Plasmalyte is safer and can be considered for volume resuscitation in these patients. The optimal management of the critically ill patient with sepsis and cirrhosis has not been well-defined and follows guidelines made for management of patients without cirrhosis with sepsis. Despite the lack of strong evidence, we usually follow an analogous (to patients without cirrhosis) approach to sepsis management in patients with cirrhosis, including the choice of fluids, vasopressors, and antibiotics. According to our data, we suggest monitoring fluids and vasopressors using routine Doppler sonography of the liver in patients with liver cirrhosis and ACLF. Monitoring of vasopressors is central because vasoactive medication can affect liver perfusion. We performed multiple regression analyses to identify potential effectors on liver perfusion. Renal replacement therapy and mechanical ventilation did not affect HARI and PVv. The latter has been described by Saner et al. who reported no significant differences in liver transplanted patients for maximal PVv and HARI when ventilated with different PEEP settings (50). The identified correlations between liver perfusion parameters and vasoactive medication are in accordance with previous publications (51–53). Consequently, monitoring vasopressors by Doppler sonography may help prevent adverse effects on (delta) HARI and PVv.

Limitations

This study has several limitations. First, the sample size is small. Larger-scale prospective clinical studies are needed to confirm these findings. Second, Doppler sonography is an operator-dependent method. Third, this study is a single-center observational study which yielded clinically relevant results with respect to the use of liver perfusion parameters to guide volume and catecholamine therapy in patients with severe liver disease. The limitation lies in the observational nature of the study. A follow up interventional study should be designed including multiple participating sites to validate the efficacy of Doppler sonographic measurements of liver perfusion to guide volume resuscitation and vasopressor therapy of patients with ACLF.

Conclusions

Here, we show that delta-HARI and delta-PVv are new predictors of outcome in patients with ACLF. Furthermore, we could show that the course over time of the HARI correlates with the CLIF-C ACLF score during ICU treatment, underlining that serial measurement of liver perfusion parameter is an early predictor of mortality due to ACLF.

Our study establishes a clear utility of routine Doppler sonography evaluating hepatic perfusion in critical care patients with severe liver diseases. In addition, the correlation between hepatic perfusion and mortality, described here for the first time, may be seen as an opportunity to improve and guide the treatment of critical care patients with severe liver diseases by optimizing hepatic perfusion, for example, through balanced volume therapy or additional vasoactive medication.

We recommend introducing a regular routine Doppler sonographic evaluation of liver perfusion in critical care patients with severe liver diseases and liver cirrhosis in everyday clinical practice to assess prognosis and to guide therapeutic management.

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 Ethics Committee of the University of Regensburg, Regensburg, Germany. The patients/participants provided their written informed consent to participate in this study.

Author contributions

SSchm, JV, CM-S, SM, KG, and MM: study concept, design, drafting of the manuscript, analyses, and interpretation of data. JV, CM-S, and SSchm: acquisition of data. JV, CM-S, SM, AM, SSchl, HT, KG, MM, and SSchm: writing and critical revision of the manuscript for important intellectual content. MM: supervision. 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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Survival prediction using the Freiburg index of post-TIPS survival (FIPS) in critically ill patients with acute- on chronic liver failure: A retrospective observational study

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Background and aim: Liver cirrhosis in patients treated in the intensive care unit (ICU) is associated with high mortality. Well established scores are useful to allow for assessment of prognosis and support ICU treatment guidance. However, currently used scoring systems often do not reflect the complexity of critically ill patients. Therefore, we tested the newly developed Freiburg index-of post-TIPS survival (FIPS) score in order to assess its potential role for prognostication of cirrhotic patients in the ICU.

Methods: A total of 310 patients with liver cirrhosis treated in the ICU between 2010 and 2021 were enrolled in this retrospective observational study. Prognostic factors for mortality and 28-day mortality were assessed. Moreover, using c indices the prognostic discrimination of different prognostic scores was analyzed.

Results: The FIPS score allowed to discriminate patients with high ICU mortality and within 28-days after ICU treatment (ICU mortality: 42.2 vs. 59.9%, $p = 0.008$ and 28-day mortality: 43.3 vs. 74.1%, $p < 0.001$). However, the FIPS score in its current composition showed no superior prognostic discrimination compared to other established scores. Multivariable analyses identified the FIPS score (HR 1.25 [1.04–1.49], $p = 0.015$) and lactate at admission (HR 1.07 [1.04–1.09], $p < 0.001$) as significant predictors of ICU

mortality. Lactate at admission substantially improved patient risk stratification within each FIPS risk groups.

Conclusion: Similar to other commonly used scores, the FIPS score in its current composition does not allow a sufficiently reliable prognostication of critically ill patients treated in the ICU. However, adding lactate as additional factor to the FIPS score may improve its prognostic ability.

KEYWORDS

liver cirrhosis, acute-on-chronic liver failure, portal hypertension, intensive and critical care, prognosis

Introduction

Acute-on-chronic liver failure (ACLF) has been recognized as a distinct syndrome that may develop in approximately 30% of patients with decompensated liver cirrhosis (1). ACLF is characterized by extrahepatic organ failure and associated with a significant increase in short-term mortality (2). Depending on the extent of extrahepatic organ failure, 28-day mortality ranges from 23.3% in patients with ACLF grade I (single organ-failure) up to 75.5% in patients with ACLF grade III (three or more organ failures). Patients with ACLF may require organ support be provided on an intensive care unit (ICU). Mortality in these patients is particularly high, reaching up to 66%, according to recent trials (3, 4). Some studies even report in-hospital mortality as high as 100% for liver cirrhosis requiring ICU treatment (4). These high mortality rates question the utility and value of life-sustaining treatments. Therefore, tools for reliable prognostication are essential for selection of patients and treatment guidance. Previously introduced liver-specific scoring systems, such as the model of end-stage liver disease (MELD), MELD-sodium and Child-Pugh (CP) scores have been developed for prediction of prognosis in non-critically ill patients with liver cirrhosis. However, none of these includes factors of extrahepatic organ function, limiting their ability for prognostication of patients in the ICU. In contrast, the CLIF-C ACLF score has been specifically developed in order to overcome this limitation and incorporates several parameters for the assessment of extrahepatic organ function (5). Moreover, a modification of this score incorporating lactate as an additional factor was proposed (6).

Recently, the Freiburg index of post-TIPS survival (FIPS) has been proposed for prognostication of patients with liver cirrhosis being allocated to implantation of transjugular intrahepatic shunt (TIPS) and has also been validated in different cohorts (7–11). The use of the FIPS outside the TIPS setting, especially in patients with more advanced liver disease and particularly ACLF remains unclear. The aim of this study was to analyze the prognostic value of the FIPS compared to established scores in patients with ACLF treated on the ICU.

Abbreviations: ACLF, acute-on-chronic liver failure; FIPS, Freiburg index of post-TIPS survival; HE, hepatic encephalopathy; ICU, intensive care unit; OS, overall survival; MELD, model for end-stage liver disease; TIPS, transjugular intrahepatic portosystemic shunt.

Patients and methods

Patient selection and follow-up

In total, 310 patients with liver cirrhosis who have been treated on the Interdisciplinary Medical Intensive Care Unit (ICU) at the Freiburg University Medical Center from January 1, 2010 through December 31, 2021 were identified by database search (**Figure 1**). All patients who fulfilled diagnostic criteria for acute-on-chronic liver failure (ACLF) according to the European Foundation for the Study of chronic liver failure (EF CLIF) were selected for analysis (n = 270) (2, 12). Subsequently, 18 patients have been excluded due to missing data. Consequently, 252 patients were available for analysis. A total of 93 patients (36.9%) were alive 28 days after ICU treatment and 135 patients (53.6%) died during their stay in the ICU.

Study endpoints and definitions

Baseline parameters were recorded on admission to the ICU. Laboratory parameters were also assessed at the time of ICU admission. Serum lactate was analyzed with point-of-care testing and values were recorded at admission and after 48 h to calculate the lactate clearance within 48 h as follows:

$$\textit{Lactate clearance}\ (\%) = \frac{\textit{Lactate}\ (\textit{at ICU admission}) - \textit{Lactate}\ (48\ \textit{hours ater ICU admission})}{\textit{Lactate}\ (\textit{at ICU admission})} * 100$$

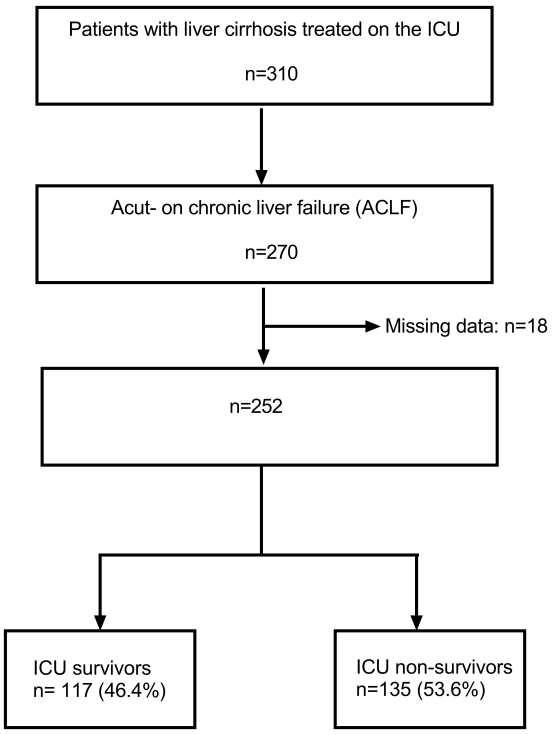

FIGURE 1
Flow chart for patient selection.

No lactate clearance was defined in values ≤0. All clinical and imaging data were extracted from the electronic patient records. Patients were followed up for 28 days after ICU admission. The primary endpoint was ICU mortality. A 28-day mortality and the course of disease during ICU treatment were assessed as secondary endpoints. The FIPS, MELD, MELD-sodium, CP scores, and CLIF-C ACLF score at the time of ICU admission were calculated for each patient, as reported (7, 13–16). For allocation to the FIPS risk groups, the proposed cut-off of 0.92 was applied (7). Liver cirrhosis was diagnosed by pathognomonic clinical findings in all patients. ACLF was graded according to the european association for the study of the liver (EASL)-CLIF criteria (2, 17). Importantly, the ACLF criteria were introduced in 2013. In all patients that have been included before 2013, data for ACLF diagnosis and staging were available in the electronic medical records.

Ethics statement

The study was approved by the Freiburg University Ethics Committee (EK 454/19) and is in accordance with the Declaration of Helsinki. Due to the retrospective design of the study informed patient consent was waived. The study was conducted following the STROBE guidelines (18).

Statistical analyses

The study is a retrospective observational analysis. Continuous variables are expressed as median with interquartile range (25th through 75th percentile). Categorical variables are shown as frequencies and percentages. Group differences were analyzed using Chi-square test or Fisher's Exact test. For continuous variables, differences were assessed with Wilcoxon rank sum, Kruskal Wallis, Wilcoxon signed rank, and Friedman tests, as appropriate.

Overall survival time (OS) was calculated using the Kaplan-Meier method with death being recorded as event. Differences in survival were assessed using log-rank tests.

Discriminatory performance of the FIPS score in comparison to the CLIF-C ACLF (lactate) score, the MELD (–sodium) and CP score was assessed using Harrell's C concordance index (c index) using STATA's somers' D package. Calibration was assessed by splitting the FIPS score in 10 similar groups and Hosmer–Lemeshow test was applied. Further, calibration was assessed by visual inspection of the Kaplan Meier curves.

In order to analyze prognostic factors, uni- and multivariable Cox regression analyses were performed. Parameters showing p-values < 0.1 in the univariable models were entered in the multivariable Cox regression models without further variable selection.

P-values < 0.05 were considered significant. Statistical analyses were performed using STATA® (Version 17.0, StataCorp Lp., College Station, TX, USA), SPSS® (Version 29.0, IBM, Armonk, NY, USA) and Prism® (Version 9.3, GraphPad Software, San Diego, CA, USA).

Results

Baseline characteristics of the included patients

Table 1 baseline characteristics of all patients included in this analysis, stratified by ICU survival [survivors (n = 117) and non-survivors (n = 135), respectively]. The leading etiology of chronic liver disease in this patient cohort was chronic alcohol abuse (50.4%). A total of 42.2% of the ICU non-survivors had alcoholic liver disease compared to 59.8% of the ICU survivors (p = 0.006). Gastrointestinal bleeding including variceal bleeding (28.2%), respiratory insufficiency (20.2%) and sepsis (18.3%) were the most common indications for ICU admission. Of note, among ICU non-survivors there were significantly more patients who were admitted to the ICU due to sepsis (24.4 vs. 11.1%, p = 0.005). In contrast, in the group of ICU survivors, there were more patients who were admitted to variceal bleeding (19.7 vs. 9.6%, p = 0.030). As expected, patients who did not survive ICU treatment needed vasopressor support

TABLE 1 Baseline characteristics of study patients stratified in intensive care unit (ICU) survivors and non-survivors.

	All patients *n* = 252	ICU survivors *n* = 117	ICU non-survivors *n* = 135	*P*-value
Age in years	61 (52–69)	63 (53–72)	60 (52–67)	0.062
Gender (male)	176 (69.8)	88 (75.2)	90 (66.7)	0.272
Etiology of chronic liver disease				
Alcohol	127 (50.4)	70 (59.8)	57 (42.2)	0.006
HCV[1]	60 (23.8)	21 (17.9)	39 (28.9)	0.054
HBV[2]	18 (7.1)	7 (6.0)	11 (8.1)	0.626
NAFLD[3]	15 (6.0)	8 (6.8)	7 (5.2)	0.604
Others	32 (12.7)	11 (9.4)	21 (15.6)	0.184
Reason for ICU admission and organ support				
Variceal bleeding	36 (14.3)	23 (19.7)	13 (9.6)	0.030
Other GI bleeding	35 (13.9)	19 (16.2)	16 (11.9)	0.365
Sepsis	46 (18.3)	13 (11.1)	33 (24.4)	0.005
Respiratory insufficiency	51 (20.2)	18 (15.4)	33 (24.4)	0.085
Renal failure including electrolyte disturbance	30 (11.9)	19 (16.2)	11 (8.1)	0.053
Loss of consciousness	33 (13.1)	18 (15.4)	15 (11.1)	0.352
Resuscitation	9 (3.6)	3 (2.6)	6 (4.4)	0.510
Others	12 (4.8)	4 (3.4)	8 (5.9)	0.391
Lowest MAP within 48 h (mmHg)	54 (45–60)	57 (49–61)	50 (37–56)	<0.001
Vasopressor support	227 (90.1)	94 (80.3)	133 (98.5)	<0.001
Norepinephrine, peak dose within 48 h (μg/min/kg)	0.40 (0.15–0.62)	0.18 (0.04–0.36)	0.58 (0.40–0.75)	<0.001
Mechanical ventilation	171 (67.9)	65 (55.6)	116 (85.9)	<0.001
CRRT	82 (32.5)	24 (20.5)	58 (43.0)	<0.001
CRRT started on ICU	68 (27.0)	18 (15.4)	50 (37.0)	<0.001
Start of CRRT after admission on ICU (days)	2 (1–5)	1 (0–3)	2 (1–6)	0.023
Decompensating events				
No ascites	52 (20.6)	25 (21.4)	27 (20.0)	0.876
Moderate ascites	148 (58.7)	73 (62.4)	75 (55.6)	0.306
Massive ascites	52 (20.6)	19 (16.2)	33 (24.4)	0.120
Spontaneous bacterial peritonitis	50 (19.8)	16 (13.7)	34 (25.2)	0.059
Hepatocellular carcinoma	39 (15.5)	17 (14.5)	22 (16.3)	0.730
Clinical scores				
CHILD-Pugh	10 (8–12)	9 (8–11)	11 (9–12)	<0.001
A	9 (3.6)	5 (4.3)	4 (3.0)	0.737
B	88 (34.9)	53 (45.3)	35 (25.9)	0.001
C	155 (61.5)	59 (50.4)	96 (71.1)	0.001
MELD	25 (18–33)	22 (15–27)	26 (21–35)	<0.001
MELD-sodium	27 (20–34)	24 (17–31)	30 (24–36)	<0.001
CLIF-C ACLF	63 (55–73)	58 (52–63)	62 (56–68)	<0.001
FIPS score	1.29 (0.56–2.00)	1.07 (0.38–1.71)	1.49 (0.85–2.16)	0.001
Low risk	90 (35.7)	52 (44.4)	38 (28.1)	0.008
High risk	162 (64.3)	65 (55.6)	97 (71.9)	

(Continued)

TABLE 1 (Continued)

	All patients n = 252	ICU survivors n = 117	ICU non-survivors n = 135	P-value
ACLF parameters				
ACLF Ia	7 (2.8)	7 (6.0)	0	0.004
ACLF Ib	19 (7.5)	17 (14.5)	2 (1.5)	<0.001
ACLF II	90 (35.7)	53 (45.3)	37 (27.4)	0.004
ACLF III	136 (54.0)	40 (34.2)	96 (71.1)	<0.001
CLIF-OF Score	12 (11–14)	11 (10–12)	13 (12–14)	<0.001
Renal failure	153 (60.7)	59 (50.4)	94 (69.6)	0.002
Liver failure	44 (17.5)	14 (12.0)	30 (22.2)	0.045
Coagulation failure	40 (15.9)	15 (12.8)	25 (18.5)	0.231
Brain failure*	35 (13.9)	15 (12.8)	20 (14.8)	0.717
Respiratory failure	174 (69.0)	65 (55.6)	109 (80.7)	<0.001
Circulatory failure	227 (90.1)	94 (80.3)	133 (98.5)	<0.001
Laboratory parameters				
WBC (ths/μl)	11.7 (7.1–17.8)	10.7 (6.7–16.5)	12.2 (7.7–18.7)	0.200
Hemoglobin (g/dl)	8.7 (7.4–10.4)	8.7 (7.5–10.5)	8.5 (7.3–10.4)	0.516
Platelets (ths/μl)	95 (59–165)	106 (74–178)	88 (47–135)	0.001
Bilirubin (mg/dl)	3.5 (1.4–8.9)	2.4 (1.3–6.7)	4.3 (1.6–11.1)	0.013
Albumin (g/dl)	2.4 (2.0–2.9)	2.5 (2.1–3.0)	2.3 (1.9–2.9)	0.182
Sodium (mmol/l)	135 (129–139)	136 (131–140)	135 (129–139)	0.379
Creatinine (mg/dl)	1.9 (1.2–3.2)	1.8 (1.1–3.1)	2.1 (1.4–3.2)	0.150
C-reactive protein (ng/ml)	42.0 (18.0–96.0)	33.0 (14.0–76.0)	55.5 (19.3–111.8)	0.009
PCT (pg/ml)	1.5 (0.5–4.5)	0.69 (0.34–2.71)	2.0 (0.8–6.1)	<0.001
Lactate (mmol/l)	3.6 (1.9–8.5)	2.7 (1.6–5.3)	6.4 (2.5–11.0)	<0.001
Lactate clearance within 48 h (%)	21.4 (−18.3 to 54.9)	34 (0.6–62.8)	−0.5 (−50.9 to 36.8)	<0.001

Continuous variables are presented as median with the interquartile range (25. and 75. percentile). Categorial variables are presented as absolute and relative frequencies [n (%)].
*Hepatic encephalopathy grade 3–4 according to the West Haven criteria.

(98.5 vs. 80.3%, $p < 0.001$), mechanical ventilation (85.9 vs. 55.6%, $p < 0.001$), and continuous renal replacement therapy (CRRT, 43.0 vs. 20.5%; $p < 0.001$) more frequently. In line with these results, 90.1% of the included patients presented with circulatory failure, 69.0% with respiratory failure and 60.7% with renal failure according the CLIF organ failure scoring system. In the ICU non-survivor group, more patients developed renal failure (69.6 vs. 50.4%; $p = 0.002$), respiratory failure (80.7 vs. 55.6%; $p < 0.001$), and circulatory failure (98.5 vs. 80.3%; $p < 0.001$). Overall, 135 patients (53.6%) died during ICU treatment and 28-day mortality was 63.1%. None of the included patients received liver transplantation during the follow-up.

The FIPS score identifies patients with a high mortality on the ICU

A total of 90 patients (35.7%) were allocated to the FIPS low risk group and 162 patients (64.3%) were classified as FIPS high risk patients. Patients allocated to the FIPS low risk group had a median OS of 31 [10.6–51.4] days compared to 9 [6.9–11.1] days in the high-risk group ($p < 0.001$, **Figure 2A**). In line with these findings, ICU mortality and 28-day mortality were higher in FIPS high risk patients compared to low risk patients (59.9 vs. 42.2%; $p = 0.008$ and 74.1 vs. 43.3%, $p < 0.001$; **Figures 2B, C**).

As the FIPS score incorporates serum creatinine levels that may be altered due to renal replacement therapy (RRT), a subgroup analysis was performed excluding patients with RRT initiated before admission to ICU ($n = 14$). Of note, the ability of the FIPS score for prognostic stratification of the patients with regard to OS and ICU and 28-day mortality was confirmed (**Supplementary Figures 1, 3** and **Supplementary Tables 1, 2**).

In order to elaborate the reasons for the higher mortality in the FIPS high risk group in more detail, various factors were assessed and compared between the FIPS low and high-risk group (**Table 2**). In patients allocated to the FIPS high risk group less often variceal bleeding (9.3 vs. 23.3%, $p = 0.003$), but more frequently infectious complications including sepsis (22.8 vs. 10.0%, $p = 0.016$) and spontaneous bacterial peritonitis (24.1 vs. 12.2%, $p = 0.008$) were the main reason for ICU admission.

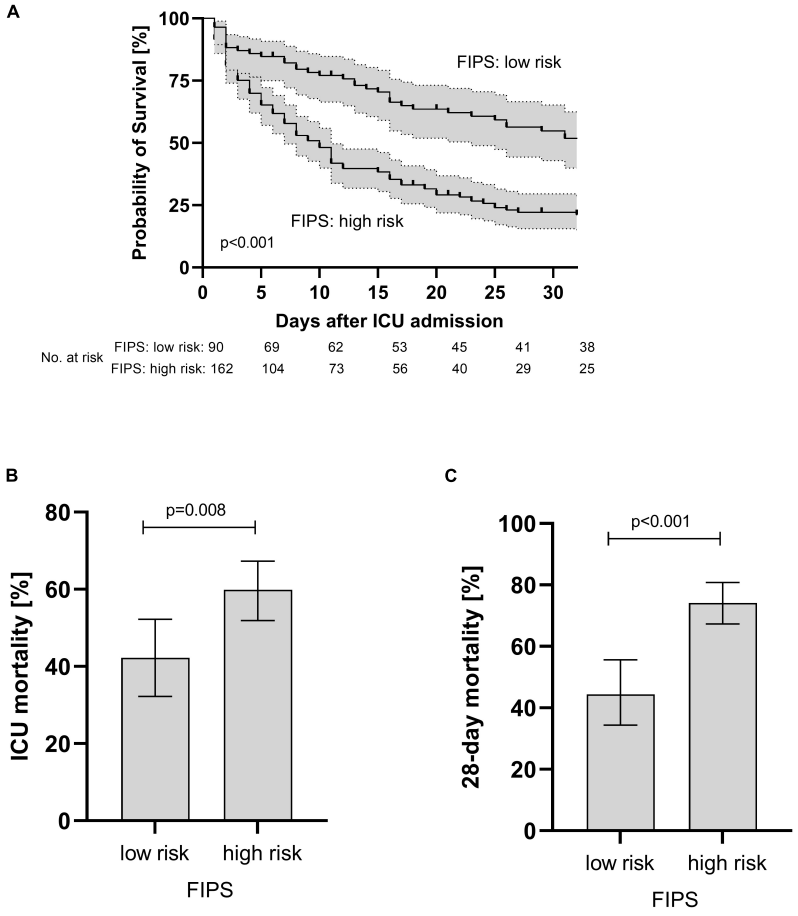

FIGURE 2
Overall survival **(A)** and ICU **(B)** and 28 day mortality **(C)** of patients with acute-on chronic liver failure (ACLF) stratified according to the FIPS score. Mortality rates are presented as relative frequencies with the corresponding 95% confidence interval. ICU, intensive care unit; FIPS, Freiburg index of post-TIPS survival.

Patients in the FIPS high risk group had significantly more often advanced ACLF (Table 2). Moreover, a significantly higher proportion of patients in the FIPS high risk patients needed CRRT on ICU compared to the FIPS low risk patients (33.3 vs. 15.6%, $p = 0.003$).

Prognostic discrimination of the FIPS score compared to the Child- Pugh-, MELD (sodium) and CLIF-C ACLF score

In order to assess prognostic discrimination capacity of the FIPS score compared to other established scores for patients with ACLF treated on ICU, c indices for ICU mortality and 28-day mortality were calculated (Table 3). The c index of the FIPS score for ICU mortality and 28-day mortality was 0.619 and 0.640 and, thus, not superior to the CLIF-C ACLF (0.584 [$p = 0.238$] and 0.626 [$p = 0.573$]), the MELD (0.590 [$p = 0.166$] and 0.629 [$p = 0.520$]), the MELD-sodium (0.585 [$p = 0.128$] and 0.626 [$p = 0.346$]), and the CP score (0.652 [$p = 0.281$] and 0.657 [$p = 0.491$]). However, the modified CLIF-C ACLF lactate score showed prognostic discrimination superior to the FIPS score (0.688 [$p = 0.018$] and 0.708 [$p = 0.004$]) indicating the prognostic relevance of lactate in these critically ill patients. In summary, the FIPS score does not show a superior prognostic discrimination capacity compared to the previously established liver-related prognostic scores in critical ill patients with ACLF.

Calibration of the FIPS score

The Hosmer-Lemeshow test confirmed similar observed and predicted ICU- and 28-day mortality across the stratified groups of the FIPS score ($\chi^2 = 7.65$, $p = 0.4680$ for ICU mortality; $\chi^2 = 7.66$, $p = 0.4675$ for 28-day mortality). In line with these results, the Kaplan Meier curves comparing observed vs. predicted survival showed acceptable calibration of the FIPS score (Supplementary Figure 2).

TABLE 2 Comparison of patients in the Freiburg index of post-TIPS survival (FIPS) low and high risk group.

	FIPS low risk group *n* = 90	FIPS high risk group *n* = 162	*P*-value
Etiology of chronic liver disease			
Alcohol	58 (64.4)	69 (42.6)	0.001
HCV[1]	21 (23.3)	39 (24.1)	0.999
HBV[2]	8 (8.9)	10 (6.2)	0.450
NAFLD[3]	3 (3.3)	12 (7.4)	0.269
Others	0	32 (19.8)	<0.001
Reason for ICU admission and organ support			
Variceal bleeding	21 (23.3)	15 (9.3)	0.003
Other GI bleeding	14 (15.6)	21 (13.0)	0.704
Sepsis	9 (10.0)	37 (22.8)	0.016
Respiratory insufficiency	20 (22.2)	31 (19.1)	0.624
Renal failure including electrolyte disturbance	6 (6.7)	24 (14.8)	0.068
Loss of consciousness	10 (11.1)	23 (14.2)	0.562
Resuscitation	6 (6.7)	3 (1.9)	0.073
Others	4 (4.4)	8 (4.9)	0.999
Lowest MAP within 48 h (mmHg)	56 (46–61)	53 (45–57)	0.034
Vasopressor support	82 (91.1)	145 (89.5)	0.827
Norepinephrine, peak dose within 48 h (μg/min/kg)	0.27 (0.08–0.59)	0.45 (0.18–0.63)	0.115
Mechanical ventilation	65 (72.2)	106 (65.4)	0.325
CRRT	19 (21.1)	63 (38.9)	0.005
CRRT started on ICU	14 (15.6)	54 (33.3)	0.003
Decompensating events			
No ascites	21 (23.3)	31 (19.1)	0.516
Moderate ascites	57 (63.3)	91 (56.2)	0.288
Massive ascites	12 (13.3)	40 (24.7)	0.035
Spontaneous bacterial peritonitis	11 (12.2)	39 (24.1)	0.008
Hepatocellular carcinoma	12 (13.3)	27 (16.7)	0.587
ACLF parameters			
ACLF Ia	4 (4.4)	3 (1.9)	0.427
ACLF Ib	11 (12.2)	8 (4.9)	0.046
ACLF II	50 (55.6)	40 (24.7)	<0.001
ACLF III	25 (27.8)	111 (68.5)	<0.001
CLIF-OF Score	11 (11–12)	13 (11–14)	<0.001
Renal failure	30 (33.3)	123 (75.9)	<0.001
Liver failure	2 (2.2)	42 (25.9)	<0.001
Coagulation failure	6 (6.7)	34 (21.0)	0.004
Brain failure*	11 (12.2)	24 (14.8)	0.704
Respiratory failure	63 (70.0)	111 (68.5)	0.887
Circulatory failure	82 (91.1)	145 (89.5)	0.827

Continuous variables are presented as median with the interquartile range (25. and 75. percentile). Categorial variables are presented as absolute and relative frequencies [*n* (%)].
*Hepatic encephalopathy grade 3–4 according to the West Haven criteria.

TABLE 3 Summary of the c index of the FIPS score compared to the CLIF C ACLF (lactate), model of end-stage liver disease (MELD) (−sodium), and CP[4] score.

	FIPS[1] c index [95% CI[4]]	CLIF C ACLF[2] c index [95% CI]	CLIF C ACLF lactate c index [95% CI]	MELD[3] c index [95% CI]	MELD sodium c index [95% CI]	CP[5] c index [95% CI]
ICU mortality	0.619 [0.559–0.679]	0.584 [0.518–0.649]	0.688 [0.612–0.741]	0.590 [0.528–0.653]	0.585 [0.523–0.647]	0.652 [0.595–0.709]
p-values vs. FIPS	–	0.238	0.018	0.166	0.128	0.281
28-day mortality	0.640 [0.591–0.689]	0.626 [0.577–0.675]	0.708 [0.665–0.751]	0.629 [0.580–0.678]	0.623 [0.573–0.673]	0.657 [0.611–0.703]
p-values vs. FIPS	–	0.573	0.004	0.520	0.346	0.491

[1]FIPS, Freiburg index of post-TIPS survival; [2]ACLF, acute-on chronic liver failure; [3]MELD, model of end-stage liver disease; [4]95% CI, 95% confidence interval; [5]CP, Child-Pugh score.

TABLE 4 Uni- and multivariable Cox regression model for prognostic factors for intensive care unit (ICU) mortality in patients with acute-on chronic liver failure (ACLF).

Parameter	Univariable model				Multivariable model			
	β[1]	HR[2]	95% CI[3]	*P*-value	β	HR	95% CI	*P*-value
Variceal bleeding	−0.25	0.78	0.44–1.38	0.387				
Sepsis	0.20	1.22	0.83–1.82	0.314				
Vasopressor support	1.31	3.71	0.91–15.10	0.067	0.94	2.56	0.63–10.45	0.192
Mechanical ventilation	0.19	1.22	0.75–1.98	0.429				
CRRT start on ICU	−0.11	0.89	0.63–1.28	0.534				
Platelets	−0.002	0.99	0.98–1.00	0.027	−0.001	0.99	0.98–1.01	0.193
C-reactive protein	−0.001	0.99	0.98–1.01	0.237				
Procalcitonin	−0.002	0.99	0.99–1.04	0.485				
Lactate	0.07	1.07	1.05–1.10	<0.001	0.07	1.07	1.04–1.09	<0.001
FIPS[4] score	0.29	1.33	1.13–1.57	<0.001	0.22	1.25	1.04–1.49	0.015

[1]β, regression coefficient; [2]HR, hazard ratio; [3]95% CI, 95% confidence interval; [4]FIPS, Freiburg index of post-TIPS survival.

TABLE 5 Uni- and multivariable Cox regression model for prognostic factors 28-day mortality in patients with acute-on chronic liver failure (ACLF) treated on the intensive care unit (ICU).

Parameter	Univariable model				Multivariable model			
	β[1]	HR[2]	95% CI[3]	*P*-value	β	HR	95% CI	*P*-value
Variceal bleeding	−0.50	0.61	0.37–1.00	0.052	−0.434	0.65	0.38–1.10	0.109
Sepsis	0.38	1.46	1.01–2.11	0.042	0.03	1.03	0.70–1.52	0.883
Vasopressor support	0.99	2.68	1.32–5.45	0.007	0.58	1.78	0.81–3.92	0.153
Mechanical ventilation	0.57	1.76	0.123–2.53	0.002	0.42	1.51	0.99–2.33	0.059
CRRT start on ICU	0.43	1.54	1.11–2.13	0.009	0.03	1.03	0.70–1.05	0.898
Platelets	−0.54	0.58	0.41–0.83	0.002	−0.001	0.99	0.98–1.00	0.335
C-reactive protein	0.001	1.00	0.99–1.01	0.393				
Procalcitonin	−0.001	0.999	0.98–1.01	0.821				
Lactate	0.09	1.09	1.07–1.11	<0.001	0.07	1.07	1.05–1.09	<0.001
FIPS[4] score	0.40	1.49	1.27–1.74	<0.001	0.36	1.44	1.19–1.73	<0.001

[1]β, regression coefficient; [2]HR, hazard ratio; [3]95% CI, 95% confidence interval; [4]FIPS, Freiburg index of post-TIPS survival.

Prognostic factors for ICU and 28-day mortality

In an attempt to determine further prognostic factors for ICU mortality a multivariable Cox regression model revealed that the FIPS score (HR 1.25 [1.04–1.49], $p = 0.015$) and lactate (HR 1.07 [1.04–1.09], $p < 0.001$) were significant and independent prognostic factors for ICU mortality in patients with ACLF treated on the ICU (Table 4). Extending the observation period to 28 days after ICU admission, the FIPS score (HR 1.44 [1.19–1.73], $p < 0.001$) and lactate (HR 1.07 [1.05–1.09], $p < 0.001$) remained statistically significant and

independent prognostic factors (**Table 5**). The combination of the FIPS score and lactate at admission substantially improved patient risk stratification. Using a lactate cut-off of 6 mmol/l (as determined by receiver operating characteristic (ROC) analysis and the Youden index), the addition of lactate allows the identification of patients with an impaired prognosis within the FIPS risk groups (**Figure 3**).

Discussion

Liver cirrhosis is associated with high mortality especially in patients admitted to the ICU (3, 4). Reliable prognostication including prediction of mortality risk is very important for the management of these vulnerable patients. Our data demonstrate that the FIPS score may help to identify patients at high risk of dying during ICU treatment. ICU mortality in patients with a high FIPS score was significantly higher than in patients with a low FIPS score (59.9 vs. 42.2%, $p = 0.008$). Extending the observation period to 28 days after admission to ICU, the differences in mortality between FIPS low and high scoring patients was even larger (74.1 vs. 43.3% $p < 0.001$) indicating that the FIPS score may also have a prognostic value even for extended observation periods after ICU discharge.

However, in direct comparison to previously established scores [MELD, MELD sodium, Child-Pugh, CLIF C ACLF (lactate) scores], the FIPS score is not superior in prognostic discrimination of critically ill patients with liver cirrhosis. Several possible reasons may explain the limited prognostic capacity of the FIPS score in these patients. First, the parameters included in the FIPS score are similar to those considered in the other scoring systems and probably they may not sufficiently represent the full complexity of ACLF. Recently, generalized inflammatory response has been recognized as an important factor in the emergence and deterioration of ACLF (19), addition of inflammatory parameters to the FIPS score may increase its prognostic value. However, our multivariable regression analyses do not support the inclusion of inflammatory parameters into FIPS score. Therefore, this aspect should be further evaluated in larger cohorts.

Another important drawback of the FIPS score limiting its use for critically ill patients may be the inclusion of serum creatinine. In patients receiving CRRT creatinine does not reliably describe renal function. Therefore, we performed a subgroup analysis excluding patients who were already on RRT before admission to the ICU. After exclusion of these patients, the prognostic discriminatory capacity of the FIPS score was confirmed (**Supplementary Figure 1**).

Finally, albumin plays an important role in the management and assessment of critically ill patients in the ICU. Data on hypoalbuminemia in cirrhotic patients suggest increased mortality in non-critically ill patients on the liver transplant waiting list (20) and a general association with a poor clinical outcome in critically ill patients (21). Therefore, inclusion of albumin in prognostic scores for critically ill patients may be important, as shown by a modification of the MELD score resulting in improved mortality prediction for patients waiting for liver transplantation (22).

But, most critically ill patients show reduced albumin levels and therefore its prognostic capacity may be reduced. Moreover, critically ill cirrhotic patients often receive albumin substitution, e.g., after large-volume paracenteses or for the management of systemic inflammation (23, 24). Therefore, serum albumin values may show significant fluctuation and may be highly influenced by the iatrogenic administration of albumin. This represents a major bias for interpretation of the prognostic relevance of albumin in these patients and may be another important reason for the FIPS score not being superior in prognostication compared to the other established scoring systems.

Our multivariable regression models suggest that that the addition of blood lactate levels might increase the prognostic accuracy of the FIPS score. Generally, lactate and lactate clearance are well established predictors of outcome in critically ill patients on the ICU (25–27). Furthermore, lactate is known to be a relevant prognostic marker for short-term mortality in patients with liver cirrhosis (6). Recent studies have attempted to improve the MELD score and the CLIF-C-ACLF score by adding lactate and have thus achieved better prognostic accuracy compared to the original scores (6, 28). Of note, since lactate levels may fluctuate over the time, the determination of lactate at a single time point may not be sufficient (6). Interestingly, there was no significant superiority of lactate determination in addition to the FIPS at time of admission versus lactate clearance in different regression models (**Supplementary Data**). Therefore, lactate at admission was used for further risk stratification. Within the FIPS risk groups, the addition of lactate levels helped identification of patients with the highest risk of mortality in the ICU in each group. In conclusion, the addition of lactate to the FIPS score may increase its prognostic capacity. Regrettably, our sample size was too small to assess a modified FIPS-lactate-score for patients with ACLF treated in the ICU. Therefore, further studies with larger sample sizes and internal and external validation cohorts are needed.

Moreover, dynamic assessment of the FIPS score during the course of ICU treatment may be of prognostic impact and may increase its prognostic accuracy. As albumin was only assessed at baseline, we were not able to analyze the FIPS dynamics during ICU treatment. Therefore, this relevant topic should be addressed in further studies.

An important limitation of our study with respect to generalization of our results, is that 69.0% of our patients presented with respiratory failure and even 90.1% with circulatory failure. Previous studies including ACLF patients only reported few patients with respiratory failure (10–16%) (29–31). In line with this observation, the median CLIF C ACLF score in our cohort was significantly higher than in previously reported cohorts (63 vs. 42.6%) indicating a very

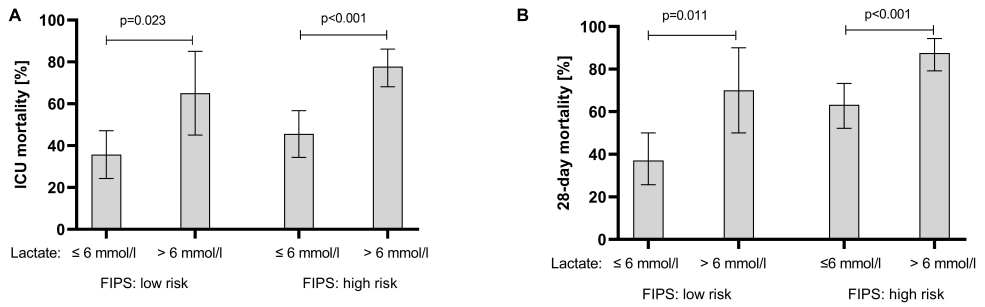

FIGURE 3
ICU mortality **(A)** and 28-day mortality **(B)** of acute-on chronic liver failure (ACLF) patients stratified according the FIPS risk groups in combination with lactate at ICU admission. Mortality rates are presented as relative frequencies with the corresponding 95% confidence interval. ICU, intensive care unit; FIPS, Freiburg index of post-TIPS survival.

selected subgroup of patients with ACLF (31). Further, another major limitation of cour study is due to the retrospective design. Patient inclusion was not consecutive and therefore an inherent selection bias cannot be completely ruled out.

Available established scores do not reflect the complexity of critically ill patients with liver cirrhosis in the ICU. However, the FIPS score in its current composition is not superior to these scoring systems and currently not recommended as an alternative. Adding lactate as an additional parameter to a revised FIPS score may improve its accuracy in critically ill patients with ACLF in the ICU. This hypothesis should be assessed in future studies.

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 Ethics Committee of the University of Freiburg, number: EK 454/19. The patients/participants provided their written informed consent to participate in this study.

Author contributions

DB, HL, and PB: design of the study. DB, HL, KS, NR, and MR: acquisition of data. DB, HL, and KS: analysis and interpretation of the data. DB: statistical analyses and consulting. DB and HL: drafting the manuscript. TW, AS, AsS, MS, CN-H, and RT: revision for important intellectual content. All authors approved the final version of the article.

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Conflict of interest

DB: Consultant Bayer Healthcare, Boston Scientific, and Shionogi. Lectures: Falk Foundation and W. L. Gore & Associates.

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.1042674/full#supplementary-material

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Beta-blockers in patients with liver cirrhosis: Pragmatism or perfection?

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With increasing decompensation, hyperdynamic circulatory disturbance occurs in liver cirrhosis despite activation of vasoconstrictors. Here, the concept of a therapy with non-selective beta-blockers was established decades ago. They lower elevated portal pressure, protect against variceal hemorrhage, and may also have pleiotropic immunomodulatory effects. Recently, the beneficial effect of carvedilol, which blocks alpha and beta receptors, has been highlighted. Carvedilol leads to "biased-signaling" *via* recruitment of beta-arrestin. This effect and its consequences have not been sufficiently investigated in patients with liver cirrhosis. Also, a number of questions remain open regarding the expression of beta-receptors and its intracellular signaling and the respective consequences in the intra- and extrahepatic tissue compartments. Despite the undisputed role of non-selective beta-blockers in the treatment of liver cirrhosis, we still can improve the knowledge as to when and how beta-blockers should be used in which patients.

KEYWORDS

beta-blockers, portal hypertension, liver cirrhosis, signaling, carvedilol, adherence

Prologue

In liver cirrhosis there is a significant change in hemodynamics in different vascular compartments, depending on the degree of decompensation (1–3). This is a major cause of organ dysfunction, concerning not only the liver but also the kidney (4), the lungs (5), the heart (6) or the intestine (7). Regardless of the etiology of cirrhosis, vasodilatation of the splanchnic vessels occurs early, followed by peripheral vasodilatation with decreased intrathoracic blood volume, resulting in hormonal counter regulation and hyperdynamic circulatory disturbance (2, 8, 9). Within the liver–in addition to the remodeling of the organ architecture and contrary to the extrahepatic situation–vasoconstriction dominates (2).

One force driving toward decompensation of liver cirrhosis is portal hypertension, often alongside a chronic inflammatory status. Such inflammation is caused and

maintained on the one hand by etiological factors such as viruses, which directly damage the liver, and on the other hand by potential stimuli derived from the bowel. Based on these pathophysiological mechanisms, the treatment of liver cirrhosis primarily aims at the interruption of etiology and in advanced stages of liver disease additionally at reduction of portal hypertension and its sequels–a main cause of complications–either by drugs or placement of a *trans*-jugular intrahepatic portosystemic shunt (TIPS).

In this context, administration of a non-selective ß-blocker (NSBB) has been firmly established for almost four decades. The therapy was introduced by Lebrec and coworkers under the hypothesis that a non-selective ß-blocker reduces tributary blood flow into the portal vein (10), thereby diminishing the risk of bleeding from varices. This hypothesis passed the test of a clinical trial followed by many others (11). In Germany, the admission rate for variceal bleeding decreased significantly between 2005 and 2018, possibly due to the widespread use of ß-blockers in liver cirrhosis (12).

Non-selective ß-blockers have been used for primary and secondary prophylaxis of variceal hemorrhage (13, 14), and there is much evidence that NSBBs reduce the risk of first and recurrent bleeding from esophageal varices. Less certain is to which degree and whether this also has an effect on survival. Furthermore, there is still uncertainty as to which patients respond to the administration of a NSBB. Also, there is now a body of evidence pointing to beneficial pleiotropic effects of NSBBs beyond their effect of lowering blood flow and blood pressure in the portal vein and its collaterals.

Hemodynamic changes in liver cirrhosis, catecholamines, their respective receptors and signaling

More than 60 years ago, it was observed that patients with liver cirrhosis have hyperdynamic circulation disorder, characterized by an increased cardiac index (CI) and a decreased systemic peripheral resistance (15–17). This disturbance increases with the extent of decompensation of liver cirrhosis. It is less dependent on the etiology. Especially in the abdomen, vasodilation occurs early as a result of portal hypertension, causing a shift of blood from the intrathoracic compartment into the splanchnic vasculature. Mediators for this phenomenon are vasodilators that act systemically and paracrine, especially nitric oxide (NO), but also other molecules such as carbon monoxide (CO), prostacyclines (PGI_2) or glucagon (1, 16, 18). One stimulus for formation of these molecules is believed to be vascular shear stress (19) in the splanchnic area (especially at onset of portal hypertension). Another is a subclinical chronic inflammation, of which it is increasingly discussed that a disturbed intestinal barrier and translation of pathogen-associated molecular patterns (PAMPs) from the gut into the body are the cause, together with an intestinal dysbiosis (20). The inhibitory effect of certain bile acids on vascular smooth muscle cell (VSMC) contraction may also play a role (21). The process is additionally maintained by an impaired VSMC response to vasoconstrictors, especially in decompensated cirrhosis (1, 22–24).

Adrenergic stimulation in liver cirrhosis

Around 40 years ago, the research group of Robert Schrier showed that plasma norepinephrine (NE) levels are significantly elevated in patients with decompensated liver cirrhosis as compared to controls (25). This is associated with water retention. By elegant investigations (head-out water immersion) they could show that it is mainly a reaction to a reduced arterial blood volume, i.e., vascular underfilling where intrathoracic baroreceptors react. The high plasma NE levels correlate significantly with vasopressin levels (26) and in cirrhosis with ascites they are associated with high plasma renin and aldosterone levels, as a consequence of an activated renin-angiotensin-aldosterone-system. Furthermore, high plasma vasoconstrictors correlate positively with the degree of portal hypertension (27). These plasma levels reflect a sympathetic overactivity induced by baroreceptor-stimulation, but may be also due to an overflow of local organ NE formation, such as in the kidney, liver, and heart. Furthermore, the central nervous system is involved. The contribution of the different organ compartments to the systematic plasma concentrations is difficult to differentiate. However, the activation of the baroreceptors is an essential source (28).

Adrenergic receptors

Catecholamines like norepinephrine and epinephrine, which–as stated above–increase with decreased liver function in cirrhosis, mediate their effect *via* adrenergic receptors which are G protein-coupled (GPCR). The effect of the sympathetic system depends on the expression of different receptors on the various cells and organs. They are categorized into two main groups: α and β receptors with nine subtypes (α_1 and α_2, with three subtypes each, as well as the $ß_1$, $ß_2$, and $ß_3$ receptors). All three ß-adrenergic receptors (ß-AR) are coupled on their cytoplasmic side to G_s proteins, and in the case of $ß_2$- and $ß_3$-AR also to G_i proteins. Comparing the potency of norepinephrine and epinephrine, epinephrine has a stronger effect on $ß_2$- and norepinephrine has a stronger effect on $ß_1$-AR. Both have about the same effect on α_1 receptors (29–31).

Although cardiac myocytes predominantly express $ß_1$-AR and peripheral vasculature or the bronchial system predominantly express $ß_2$-AR, most organs have a heterogeneous composition of ß-receptors. For instance,

ß$_1$-AR of the kidney are involved in renin secretion, whereas ß$_2$-AR directly influence sodium concentration in the tubular system *via* ion channels/transporters (32). ß$_3$-AR are expressed in a variety of human tissues such as skeletal muscle, atrium, heart, adipose tissue, brain or–to a great extent–in the urinary bladder (33–35). We found an up-regulated expression of ß$_3$-AR in arteries and the liver in the condition of liver cirrhosis (36).

Thus, with respect to the cardiovascular and intestinal systems, activation of ß$_1$-AR causes an increase in the frequency and contraction of the heart, whereas activation of ß$_2$-AR causes vasodilation. The motility of the intestine and gallbladder is decreased by ß$_2$-agonists.

While we know that the sympathetic system is activated in cirrhosis, we have little insight beyond systemic levels into the extent of activation at the cellular and organ levels across stages of cirrhosis. Even less is known about how the expression of the various adrenergic receptors change at the cellular level in the different organs during liver cirrhosis.

Signaling and ß-receptors

The contraction and relaxation of smooth muscle cells depends largely on the phosphorylation and dephosphorylation of myosin light chains (MLC), essential contractile proteins. Here, calcium homeostasis, regulated by its *trans*-membrane influx against efflux of potassium plays an important role. Thus, vascular smooth muscle contraction by activation of vasoconstrictor receptors, including α$_1$-AR or receptors for angiotensin-II result in contraction by promotion of MLC phosphorylation caused by three prototypical intracellular signaling pathways, shared by all contractile receptors (1).

Vascular ß-AR are coupled to G protein alpha subunit (Gαs) (see **Figure 1**; 37). Activation of Gαs by ß-adrenergic receptors causes intracellular adenylyl cyclase activation, subsequent cAMP production, and cAMP-dependent relaxation in smooth muscle cells or contraction in cardiomyocytes, by various mechanisms (34, 37). One major mechanism in smooth muscle cells is activation of protein kinase A (PKA) by cAMP, resulting in relaxation by decreasing cytosolic calcium concentrations and calcium sensitivity (see **Figure 1**; 38, 39). ß$_3$-AR mediate their effects probably mainly *via* Gαs in smooth muscle cells, but also *via* Gi and eNOS, at least in endothelial cells and cardiomyocytes (34). The latter leads to formation of cGMP and vasodilatation (34). Their vasodilatory effect may also be further induced *via* inhibition of Rho-kinase (36).

However, a number of other responses of smooth muscle cells may occur after activation of ß-AR by recruiting other G-proteins or non-G protein interaction partners, altering the membrane localization of the receptor. As a result, other intracellular signaling proteins are activated (40). Here, the phenomenon of ß-arrestin recruitment to activated GPCR is of clinical relevance with a transduction of signaling toward other pathways (41)–besides the receptor desensitization effect of ß-arrestin. Principles of this phenomenon have been described, especially for activation of ß$_2$-AR and angiotensin II receptors (1, 42). Evidence that ß-arrestin-mediated receptor regulation also applies to splanchnic vessels in liver cirrhosis is available, at least for angiotensin II receptors (24, 43, 44).

The different ß-AR blockers

About 60 years ago, ß-blockers were introduced for the treatment of systemic hypertension (45). Although all ß-blockers have an antihypertensive effect they differ in pharmacokinetics and pharmacodynamics, depending on their molecular structure (29). ß-AR blockers can be broadly divided into water-soluble and lipid-soluble agents, as well as into ß$_1$ selective and non-selective substances.

At high concentrations, the selective ß$_1$-effect is partially lost. Some ß-blockers have an additional agonistic effect on the ß-AR (intrinsic sympathomimetic activity, e.g., pindolol), whereas carvedilol has an additional antagonistic effect on α-receptors. Nebivolol also promotes NO formation (39; **Table 1**). Conventional NSBB or ß$_1$-AR blockers have only a minimal effect on ß$_3$-AR (34).

Propranolol and metoprolol are lipophilic. They are almost completely absorbed *via* the intestine and largely metabolized by the liver. Thus, their bioavailability is quite variable. They also have a short plasma half-life. Nevertheless, mainly due to the receptor binding, a dosage twice daily or–in other galenic forms–even once daily is sufficient.

Most of the trials in patients with liver cirrhosis have been performed with non-selective ß-blockers (NSBBs) propranolol, nadolol, timolol or carvedilol (**Table 1**).

Among the different ß-blockers, carvedilol shows a unique pharmacological profile, which is, as mentioned above, first reflected by antagonism of α$_1$-AR in addition to blockade of ß-AR (**Figure 2**). Antagonism of α$_1$-AR by ß-adrenergic ligands is not uncommon, but often requires unphysiologically high concentrations (46, 47). However, binding of carvedilol to ß- and α$_1$-AR occurs with similar affinities, and within ranges of plasma concentrations (see **Figure 2**; 48, 49). Plasma levels with daily doses of 12.5 and 25 mg range from 115 to 131 nM in healthy patients, and from 256 to 315 nM in patients with chronic renal insufficiency (50). Thus, antagonism of α$_1$-adrenoceptors by carvedilol occurs *in vivo*, especially in kidney dysfunction, even though its clinical relevance has been debated (37). In contrast to most other NSBBs, carvedilol may not only "block" ß-adrenergic receptors, but may even activate ß-arrestin-induced signaling, a behavior allowing classification as a "biased ligand" (37, 51). Functional outcomes of signaling *via* ß-arrestins are unknown in the context of cirrhosis and with respect to portal hypertension, splanchnic vascular cells or the heart in liver dysfunction. ß-arrestin dependent

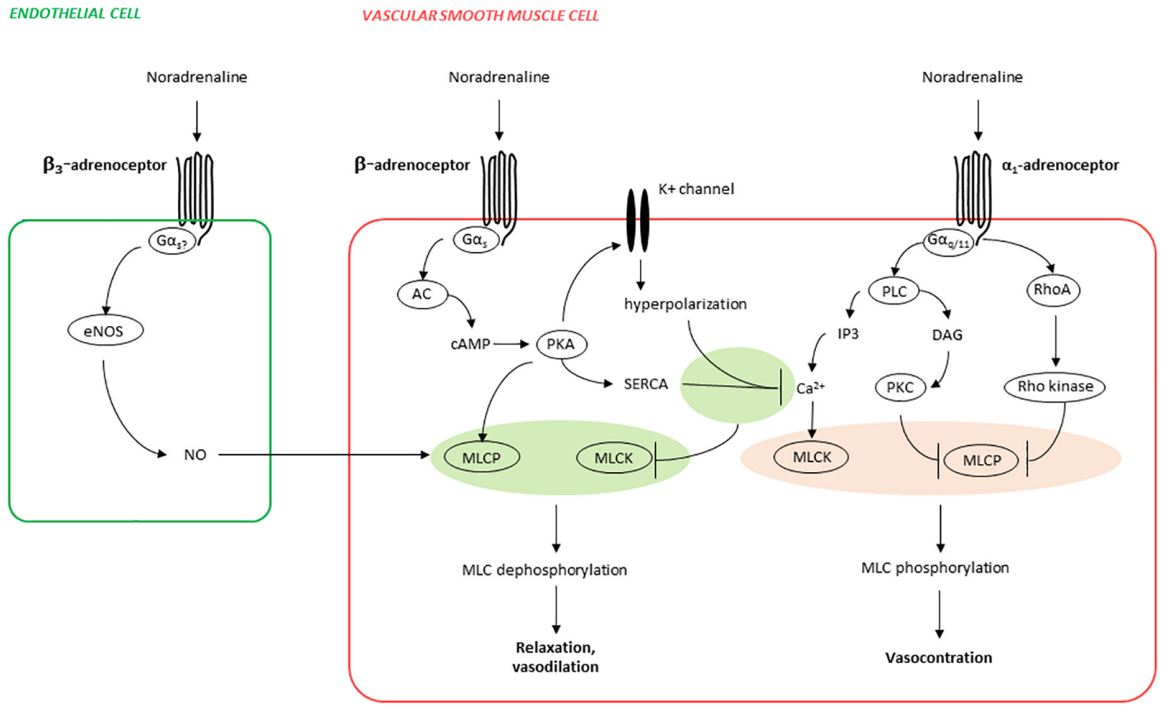

FIGURE 1
Mechanisms of ß-adrenergic vasorelaxation and α_1-adrenergic vasocontraction (simplified). Phosphorylation of myosin light chains (MLC) in smooth muscle cells is essential for contraction and is increased by MLC kinase (MLCK) and decreased by MLC phosphatase (MLCP), both being adversely regulated by α_1- and ß-adrenoceptors. Activation of α_1-adrenoceptors causes activation of phospholipase C (PLC), resulting in inositol-1,4,5-trisphosphate (IP3) formation, leading to increases in cytosolic calcium concentrations and finally to contraction by calcium-dependent MLCK activation. In parallel, MLC phosphorylation is promoted by deactivation of MLCP, caused by its inactivation by protein kinase C (PKC) activation by PLC-derived diacylglycerol (DAG), and in parallel by RhoA/Rho kinase. Activation of ß-adrenoceptors (predominantly $ß_2$-AR) causes activation of adenylyl cyclase (AC), subsequent cyclic adenosine monophosphate (cAMP) formation, and activation of the cAMP-dependent protein kinase A (PKA). PKA activates several potassium channels, resulting in hyperpolarization and finally in decreases of cytosolic calcium concentrations by closure of voltage-dependent calcium channels. In parallel, PKA-mediated decreases in cytosolic calcium are imparted by relocation of calcium from the cytosol to intracellular stores, resulting from PKA-mediated sarco/endoplasmatic Ca^{2+}-ATPase (SERCA) activation. Finally, PKA activates MLCP, leading to reduced MLC phosphorylation and relaxation, in addition to PKA-mediated decreases in cytosolic calcium. In endothelial cells, $ß_3$-adrenoceptors may activate endothelial nitric oxide synthase (eNOS), resulting in production of the vasodilator nitric oxide (NO), and NO-mediated vasorelaxation.

pathways include non-motoric functions, such as activation of mitogen-activated protein kinases (MAPK) with consequences on proliferation, differentiation, and growth of cells (37). See below!

It is evident that NSBBs lower cardiac index *via* ß1 blockade. But it is poorly studied what effect they have at the different vessel compartments in liver cirrhosis.

Beta-arrestin, biased signaling, and carvedilol

ß-arrestin-1 and -2 are ubiquitously expressed intracellular proteins that modulate the response to stimulation of GPCRs (52). By binding to phosphorylated GPCRs, they desensitize G-protein mediated signaling in the cell, partially through receptor endocytosis (53). In addition to this canonical role of ß-arrestin, current attention is increasingly focused on its function as a scaffold protein, which–internalized with other intracellular proteins–triggers further pathways as a cytoplasmic "signalosome", or directly by receptor GPCR binding, and thus inducing different patterns of signaling cascades in the cell. This phenomenon has been described particularly for activation of the angiotensin II receptor, but most likely also applies to other adrenergic receptors (1, 52, 54).

Increased ß-arrestin signaling has been associated with profibrotic diseases (55, 56). In liver cirrhosis, increased expression of ß-arrestin 2 was found in liver (57), gastric mucosa (58, 59), and splanchnic vessels (24)–both in humans and animal models. Contrary to this, shortage of ß-arrestin 2 in sinus endothelial cells (SEC) has been described for liver cirrhosis. This may be one explanation for hepatic deficiency of NO together with increased intrahepatic resistance in liver cirrhosis, since ß-arrestin 2 mediates NO formation in SEC (60). As concerns those findings, which describe a significantly increased ß-arrestin 2 in different whole tissue homogenates

TABLE 1 Non-selective ß-blockers used for therapy of portal hypertension.

Drug	$ß_1$–blockade potency ratio (propranolol = 1) (28)	Further effects	Daily oral dosage[#]
Carvedilol	10	α_1 blocking activity, biased signaling *via* ß-arrestin	Start with 1 × 6.25 mg (even lower) up to 2 × 6.25 mg
Nadolol	1.0		1 × 20–40 mg up to 1 × 160 mg
Propranolol	1.0		2 × 20 mg up to 2 × 80 mg
Timolol	0.6		1 × 10 mg up to 1 × 80 mg

[#]Resting heart rate 55–70 beats/min, systolic BP > 90 mm Hg.

in liver cirrhosis (24, 57, 58), we lack the exact assignment of which cells are involved and what increased expression of ß-arrestin in these cells causes functionally. Overexpression of ß-arrestin in splanchnic vessels in liver cirrhosis has been implicated as an explanation for the impaired vascular response to vasoconstriction (24).

Interestingly, it was then shown that patients, who respond hemodynamically to acute administration of an NSBB, express increased ß-arrestin in the stomach antrum mucosa (58, 61) and also have higher ß-arrestin serum levels (61). All these results are difficult to interpret in the absence of cell-level findings.

In the context of liver cirrhosis and alteration of ß-arrestin expression, it is interesting that carvedilol–in contrast to other NSBB–shows so-called biased signaling as mentioned above (62, 63). That is, in addition to the inhibitory effect on the G protein-dependent pathway, carvedilol induces an increased recruitment of ß-arrestin 2 by changing the conformation of the cytoplasmic portion of the receptor (64) and subsequent induction of signaling *via* ß-arrestin (51). Biased signaling has been associated with a cardioprotective effect in the cardiology literature (54, 65). As to liver cirrhosis, it would be very interesting to dissect how this alternative effect of carvedilol plays out on the cardiovascular system and other organs where an increased expression of this protein is already present.

According to the above findings, it could well be that ß-arrestin expression and binding to the ß-AR receptor (independent of carvedilol administration) increases with decompensation of liver disease, at least in the vasculature. This implies that carvedilol then acts differently with respect to a "biased-signaling" compared to individuals without liver disease (**Figure 2**). Thus, the functional effects of biased signaling induced by carvedilol are completely unclear in patients with liver cirrhosis to date.

ß-Arrestin is also thought to play a cancerogenic role, e.g., through a signal switch toward the wnt/beta-catenin pathway. However, despite the induction of ß-arrestin signaling, carvedilol may be protective against carcinogenesis (52, 66) at least in skin cancers (67). In animal experiments it inhibited nuclear translocation of ERK despite its effect on ERK phosphorylation (68). The effect of NSBB on HCC development is under debate (69, 70). Here, it would be interesting to have more specific data for carvedilol.

In summary, we need more information on carvedilol and its biased signaling effect in liver cirrhosis.

Non selective ß-AR blockers and their influence on pathomechanisms of liver cirrhosis

Two main pathogenic mechanisms are relevant for deterioration and acute decompensation of patients with liver cirrhosis, namely portal hypertension and systemic inflammation (71). There is evidence that gut-barrier dysfunction and changes in the microbiome with subsequent bacterial translocation also contribute to the latter (72). In the following, we address in more detail how NSBB might influence this pathomechanism.

The hemodynamic action of NSBB and portal hypertension

In their first study, published in the Lancet in 1980 (10), Lebrec and coworkers applied oral propranolol to eight patients with liver cirrhosis for 1 month at a dose that reduced heart rate by 25% (40–180 mg twice a day). Since then, this target reduction in heart rate has been used to dose propranolol in many centers. They showed that in all treated patients the gradient between blocked and free hepatic vein pressure (hepatic venous pressure gradient, HVPG), which–at least in alcoholic cirrhosis–correlates very well with portal pressure (73), decreased permanently. At the same time, cardiac index and hepatic blood flow (assessed by ICG method) decreased. From this, they concluded that propranolol acts *via* a reduction of splanchnic blood flow into the portal vein. None of these parameters changed in patients receiving placebo. Later they showed that there was poor correlation between the reduction in cardiac index (CI) and the decrease in HVPG. Therefore, a direct additional effect on the vessels in the splanchnic compartment was assumed. They postulated a vasoconstriction due "to unopposed alpha-adrenergic activity" as one of the factors reducing portal pressure (74).

However, a blockade of the vasodilatory effect mediated *via* the $ß_2$-AR in the splanchnic arteries could also play a role.

In Lebrec's first publication, the HVPG was reduced by 25% on average. Many other studies confirmed the portal

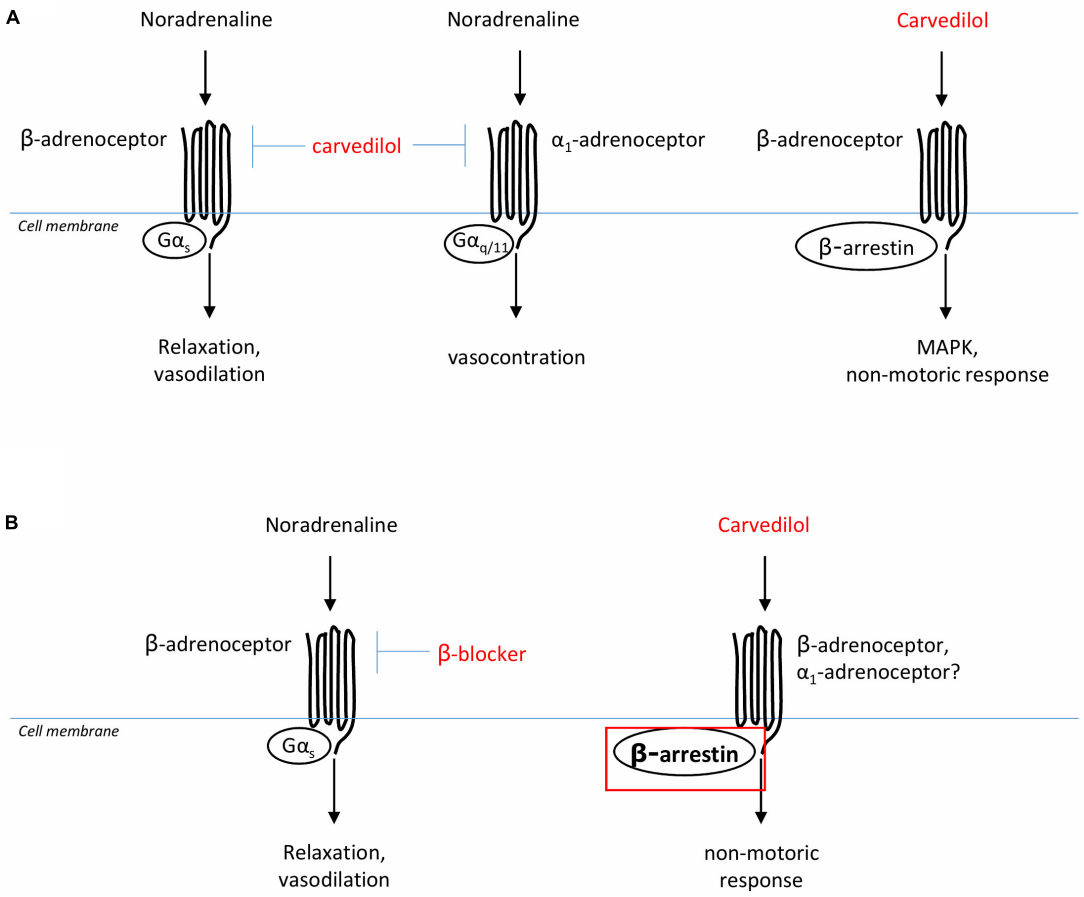

FIGURE 2
Presumed actions of ß-AR blockers on vascular smooth muscle cells. **(A)** Carvedilol is an adrenergic ligand, antagonizing ß- and α_1-adrenoceptors, and also inducing biased signaling by ß-arrestin. **(B)** In portal hypertension, ß-blockers are presumed to reduce splanchnic vasodilatation, and therewith portal tributary blood flow and portal pressure, by antagonism of ß-adrenoceptors. It could be speculated that in liver cirrhosis the biased signaling effect of carvedilol is even more pronounced due to up-regulation ß-arrestin in these patients.

pressure lowering effect of propranolol and other NSBB, but to a lesser extent. In the placebo-controlled studies (acute and chronic administration), the inhibitory hemodynamic effect of propranolol caused a reduction of HVPG from baseline between 10% and 22%, with a mean of about 15% (75–78). Carvedilol with its concomitant α_1-AR blocking effect decreased HVPG by a mean of 19% (79). For propranolol, there is only a loose correlation between dose or pulse reduction and relative reduction in HVPG (76, 78, 80). For example, we found a similar reduction of HVPG and response rate (HVPG reduction of at least 20% or below 12 mmHg) in two different placebo controlled trials with either a fixed dose of propranolol (20 mg b.i.d) or a dose aimed at reducing basal heart rate by 25% resulting in total daily dosages well beyond 100 mg/day (76, 81). These findings support more recent observations that low-dose administration of NSBB may be more beneficial than high-dose administration according to the previous standard (pulse reduction by 25%) (82). A dose dependency is more likely to be found for carvedilol (78, 83).

It has been shown that for an efficient reduction of the risk of bleeding from esophageal varices, certain thresholds (>20% HVPG reduction or an absolute reduction of HVPG to below 12 mm Hg) should be reached (see paragraph surrogate marker below). Some studies demonstrated that a relative reduction of the HVPG by >10% might have a beneficial effect on survival. But it remains to be seen whether such differences can be measured reproducibly and reliably in all centers. An adequate drop in HVPG is more common in patients with high portal pressures and hyperdynamic circulatory dysfunction as compared to patients with subclinical portal hypertension–at least in compensated cirrhosis (15). However, the correlation is not strong. A later study of the group found a more pronounced effect of NSBB on systemic hemodynamics (mainly cardiac index) in patients with decompensated cirrhosis than in compensated cirrhosis, while the portal pressure decrease was smaller in these latter patients (84).

There are a number of possible explanations for the poor correlation (85) between peripheral hemodynamic criteria

(CI, systemic blood pressure, pulse), the propranolol dose and the decrease in portal pressure measured by HVPG: variation in portosystemic shunts, different expression of ß-AR at the different end organs and/or different formation of vasoconstrictors in the respective patients with liver cirrhosis. In this context, it is interesting, that we found no correlation between portal venous blood flow and baseline HVPG in patients with liver cirrhosis (86). We also found that the contraction response of arteria hepatica and the portal vein of patients with liver cirrhosis to α_1-adrenergic stimulation is impaired compared with controls. While $ß_2$-stimulation showed differential effects, decreased relaxation of arteries, but increased relaxation of the portal vein in liver cirrhosis (22). One may speculate, that the $ß_2$-blocking effect of NSBBs acts differently in different vascular regions. In addition, it is unclear how other NO-forming stimuli modulate the effect of NSBBs in liver cirrhosis. NO-formation increases with Child-stage (87). To complicate matters further, there is no sound knowledge as to what extent hepatic dysfunction and concomitant chronic inflammation influence expression of adrenergic receptors, cellular signaling, and phenotype, as well as plasticity of their target cells. Due to the high catecholamine levels in liver cirrhosis, down-regulation of adrenoceptors has been discussed. On the other hand, we found an up-regulation of vasopressor receptors on the transcriptional (mRNA) level in human cirrhotic hepatic arteries as compared to controls (88). Also, protein expression of all three ß-AR was up-regulated in splanchnic arteries in cirrhotic animal models and also in human arteries ($ß_3$-AR) of patients with liver cirrhosis. Within the liver $ß_3$-AR (humans and rats) as well as $ß_2$-AR (rats) showed an increased protein expression in liver cirrhosis, but not $ß_1$-AR (36). However, we do not have sufficient information about the expression of these receptors on the different cell types, their membrane localization or the induction of the respective signaling cascades.

Furthermore, different hepatic drug metabolisms, drug interactions or change of protein binding (e.g., to albumin) of the drug have to be considered. Last but not least, the reproducibility of the HVPG determination, especially in less experienced centers, should also be taken into account when interpreting results of NSBBs on hemodynamics. Sex and etiology appear to be of minor importance for the effect of NSBB in liver cirrhosis (89).

Interestingly, propranolol keeps its portal pressure lowering effect after TIPS insertion (90). Addition of irbesartan to propranolol did not further reduce HVPG, but improved natriuresis (81). Adding statins may augment the HVPG lowering effect of NSBB (91, 92), although this was debated for carvedilol (93). Concomitant phosphodiesterase-5-inhibitors further reduce portal pressure and may improve erectile dysfunction at the same time (94).

NSBBs and inflammation

There is strong evidence in the cardiovascular literature that activation and recruitment of inflammatory cells is mediated by the adrenergic system, especially *via* $ß_2$-receptors (95), and that antihypertensive drugs (96) have a beneficial immunomodulatory effect.

The hemodynamic changes that occur with increasing decompensation in liver cirrhosis are accompanied by an activation of inflammatory cells in the sense of a chronic inflammatory syndrome with concomitant immune dysfunction (3, 97, 98). A translocation of microorganisms and/or associated molecules (PAMPs) from the intestine are blamed, besides stimuli from damaged tissue (DAMPs) (99). *Via* the activation of toll-like receptors (TLR) and the inflammasome, there is a release of cytokines (100). Direct and indirect evidence suggests that NSBB can favorably influence this situation. Their administration is associated with an improvement of the intestinal barrier, reduction of bacterial translocation and activation of the immune system as measured by plasma Il-6 levels (101). Furthermore, reduction of inflammatory biomarkers in case of NSBB therapy was associated with a longer survival. This, interestingly, showed only a loose non-significant association with HVPG drop (102). Also, the incidence of spontaneous bacterial peritonitis (SBP) decreases under NSBB (103) and NSBB favorably affect at least the short-term prognosis of patients with acute on chronic liver failure (ACLF) (104). This may be explained by effects on the intestinal motility (105), but also by a direct receptor-mediated effect on immune cell signaling (95, 106–109). On the other hand, at least in inflammatory models, $ß_2$-blockade has a proinflammatory effect on the kidney (32, 110). Thus, we still not fully understand how, where and when NSBBs favorably or possibly even unfavorably modulate inflammation in liver cirrhosis.

Regarding the interaction of chronic inflammatory syndrome and administration of NSBBs, it is completely unclear to what extent inflammatory stimuli in liver cirrhosis influence the expression and function of ß-AR in different organs. Corresponding changes would of course have implications for the pharmacological effect of NSBB.

NSBBs, gut motility, and inflammation

In vitro studies on human colonic muscle strips could show that ß-AR are functionally expressed at the colon and that $ß_1$- and (less) $ß_2$-agonists lead to intestinal dilatation (111). In healthy subjects, ß-adrenergic agonists (isoprenaline as $ß_2$-agonist) delayed orocoecal transit and ß-blockers ($ß_1$-blocker and propranolol) accelerated it (112). Thus, one might conclude that activation of the adrenergic system in liver cirrhosis promotes constipation. The authors are

not aware of adequate clinical studies on the question as to what extent intestinal motility changes with increasing decompensation of liver cirrhosis. Such trials are presumably difficult to perform due to so many other influencing factors. Nevertheless, there is some evidence of impaired intestinal motility in liver cirrhosis (113). This provides the rationale for administration of NSBBs to alter intestinal dysmotility, presumably induced by adrenergic stimulation, with the aim to influence the bacterial translocation. On the other hand, there is increasing evidence that resident macrophages in the gut favorably influence inflammation *via* ß$_2$-mediated signaling (114, 115) and we do not know how intestinal dysbiosis, which has been demonstrated especially in alcoholic cirrhosis (116), affects neuroinflammatory processes in the intestine and whether NSBBs have a favorable or unfavorable effect on this.

Indications for NSBBs, results from randomized trials

After the use of NSBBs for prophylaxis of first variceal hemorrhage and prevention of recurrent hemorrhage was established, further studies were conducted on the administration of NSBB to prevent further decompensation of compensated cirrhosis. In the following, we review the studies on these three indications (prevention of decompensation, as well as prevention of first and recurrent hemorrhage) in terms of (a) hemorrhage and (b) survival.

Prevention of cirrhosis decompensation by NSBBs

It has long been an unanswered question whether starting the administration of NSSBs early in the course of liver cirrhosis can prevent later decompensation. Several years ago, Groszman and coworkers initiated a controlled trial to test the hypothesis, that application of NSBB in an early stage of liver cirrhosis (213 pts, over 90% Child A, around 60% hepatitis C, one fourth alcoholics, all patients without gastroesophageal varices) might prevent development of varices, bleeding or ascites, i.e., whether NSBBs can prevent signs of further decompensation (117). Timolol, a NSBB, was used. The average baseline HVPG was around 12 mmHg. Timolol dropped the heart rate by 17% and also HVPG, but non-significantly as compared to placebo. With respect to the primary endpoint (development of esophageal varices or hemorrhage from the collaterals) there was no distinction between groups (39% vs. 40%) during a median follow-up of more than 50 months. Furthermore, development of ascites and or encephalopathy (around 12% each) did not differ, nor did death rate (9% timolol group vs. 14% placebo).

Post-hoc analysis showed that patients with baseline HVPG < 10 mmHg, a decrease of HVPG > 10% or an increase < 10% developed significantly fewer primary endpoints irrespective of the trial group. Following these findings some centers consider a 10% drop (not 20%) in HVPG as an important prognostic threshold. See below!

In 2019, Spanish working groups again addressed the question of the extent to which NSBBs can prevent the decompensation of liver cirrhosis (118). 201 patients (one third of screened) were randomly assigned to NSBBs (propranolol, carvedilol) or placebo. Contrary to the first trial, only patients (somewhat more than 60% hepatitis C) with an HVPG > 10 mmHg were included. Response to standardized intravenous propranolol was tested at inclusion. Patients who did not show a drop in HVPG of >10% received carvedilol. The target dose was based on the reduction in heart rate (mean daily dose 95 mg for propranolol and 19 mg for carvedilol). The drop in HVPG from baseline at 1 year was higher with carvedilol (16%) than with propranolol (10%), although carvedilol was only given to iv propranolol non-responders. After a median follow-up of 37 months the composite endpoint (death, ascites, bleeding or overt encephalopathy) occurred in 16% of the ß-blocker group and 27% of the placebo group, mainly due to a reduction of ascites formation. The occurrence of decompensation correlated with a lack of drop in HVPG after 1 year. It is not entirely clear to what extent antiviral therapy for HCV-associated cirrhosis affected the outcome in the final stage of the trial, and overall there is little knowledge about the comedication in the groups. In the NSBB group 8% died, as did 11% of the placebo patients.

The results of these two studies suggest that patients with significant portal hypertension (HVPG > 10 mmHg) but not yet decompensated cirrhosis benefit–mainly regarding the formation of ascites–from treatment with NSBB, preferably carvedilol, provided there is a decrease of HVPG > 10%. It remains to be seen whether this applies to alcoholic cirrhosis, which is the major cause of cirrhosis, at least in Western countries.

Prevention of esophageal bleeding

First bleeding

One main indication for application of NSBB is prevention of first bleeding from varices. In the largest placebo-controlled trial (meta-analysis of individual data from 589 patients) it has been shown that the two-year bleeding rate is reduced from 35% (controls) to 22% in the propranolol/nadolol group (119). Thus, the number of patients needed to treat (NNT) is around eight to prevent one bleeding event.

According to a recently published Cochrane review (network meta-analysis of 60 controlled trials, 6,212 patients using NSBB, nitrates, sclerotherapy or ligation of varices) on primary prophylaxis of variceal bleeding in liver cirrhosis patients with varices, NSBBs significantly reduced occurrence of any variceal bleeding compared to no active intervention in patients with varices. NSBBs or ligation (9 RCTs) were almost equal. An additional positive effect was observed by adding ligation of varices (only one trial), but there were more serious adverse events in the banding groups as compared to monotherapy with NSBB. The evidence for these findings are classified as uncertain, mainly because of low number of cases in the individual trials and the quality of the studies (120).

A very recent time to event analysis with individual patient data from 11 RCTs comprising 1,400 patients found no difference in the first bleeding rate between NSBBs (with or without ligation) and ligation only, but a lower risk to develop ascites in patients receiving NSBBs (121).

Rebleeding

Since the first RCT (11) demonstrating the potential of NSBBs to prevent rebleeding from esophageal varices, NSBBs have become an inherent part for prophylaxis of rebleeding. Compared to placebo they reduce the rebleeding risk from 60–70% to 30–40% and combined with ligation to 25% (122). This is less than shunt procedures (5–10% rebleeding after TIPS), but does not carry the risk to augment or induce hepatic encephalopathy, an adverse event consistently shown after TIPS placement, at least in the elective situation (76, 123).

Non selective ß-AR blockers and ligation carry not only the disadvantage of a higher risk of further hemorrhage as compared to TIPS, but also the drawback not to influence the pathophysiology of salt and water retention leading to ascites, whereas TIPS–by shifting blood into the central compartment–decreases renin-angiotensin-aldosterone system (RAAS) activation and kidney salt reabsorption (124). At least one fifth of patients receiving NSBBs and ligation for prevention of rebleeding in the end was transferred to TIPS for refractory ascites (76, 123). In a recent meta-analysis, using individual patient data analysis in nearly 4,000 patients, TIPS proved superior to standard of care for rebleeding–which is in most patients ligation and NSBBs–and also with respect to further decompensation and even survival, the latter mainly due to the cohorts receiving pre-emptive TIPS after a variceal bleeding episode (125).

Most patients, who fail to respond adequately with HVPG to the administration of propranolol, respond to carvedilol. By this–compared to ligation–the primary bleeding rate can be significantly reduced (77). However, a Cochrane review (126) analyzed the randomized trials (10 RCT, 810 patients) comparing carvedilol with conventional NSBBs and found no difference with regard to primary and secondary bleeding rates and side effects, while a very recent meta-analysis (127) based on individual data showed improved survival compared to controls (ligation or placebo).

In summary, the combination of a NSBB along with ligation of varices remains the standard therapy to prevent recurrent variceal bleeding (128), although there is debate as to whether narrow-lumen TIPS, used directly in conjunction with the acute bleeding event might be the optimal recurrent bleeding prophylaxis independent from degree of decompensation and severity of bleeding, at least in patients beyond seven Child-Pugh points, mainly to protect patients from early rebleeding, which exerts an increased risk for death (129).

Effect of NSBBs on survival of patients with liver cirrhosis

In several more recent studies, bleeding has been found to be only a minor contributor to mortality. This may explain the rather low impact of NSBBs on mortality, despite their unquestioned beneficial effect on bleeding risk. Nevertheless, the question of other pleiotropic effects of NSBBs (besides the influence on variceal hemorrhage) arises, especially for those studies that show a prolongation of survival under NSBBs as compared to alternative approaches (see above).

Prevention of cirrhosis decompensation

Both studies investigating the effect of NSBB on decompensation of liver cirrhosis found no effect of NSBB on overall survival compared with the placebo group (117, 118).

Prevention of first bleeding

In the first meta-analysis (119) of individual patient data (589 pts from 4 RCT) 2-year-survival was similar (68% placebo vs. 71% NSBBs). A significant beneficial effect on survival in the setting of primary bleeding prophylaxis as compared to no active intervention was described in a very recent network-meta-analysis on 6,653 patients, however with only marginal differences when NSBBs were compared to variceal ligation with and without additional NSBBs (120). A competing risk meta-analysis of 11 RCT trials comprising individual data of 1,400 patients in the setting of prevention of bleeding from high risk varices showed that NSBBs alone or in combination with ligation achieved a better survival than ligation alone in patients with compensated cirrhosis, but not in patients with decompensated cirrhosis (121).

Prevention of rebleeding

The very first meta-analysis (130) on the value of the use of NSBBs in recurrent bleeding prophylaxis of variceal hemorrhage already showed that NSBBs as compared to no active intervention not only significantly decreased the risk of bleeding during the observation period of about 2 years (mean improvement 20%), but also prolonged survival to a minor degree (increase in survival rate during a follow-up of around 2 years from 67 to 75%). There was no difference in mortality between ligation alone and ligation plus NSBBs for prevention of rebleeding. It is difficult to deduce from a recent network meta-analysis (3,526 participants, 48 randomized trials) the effect of NSBBs on survival when compared to other active treatments (131). It does not appear that there is a major difference. Interestingly, the potential beneficial effect on survival might be independent of hemorrhage protection (132).

A beneficial effect on mortality in the situation of rebleeding prophylaxis has been shown to be predominantly limited to patients in whom NSBBs (propranolol or carvedilol) reaches a drop in HVPG of at least 10% (133–135). In this respect it is noteworthy that insertion of a covered TIPS, which achieves the most effective drop of portal pressure has a higher impact on survival than standard of care (ligation and NSBBs). However, this was mainly due to TIPS placement in early temporal relationship to bleeding (125), while elective TIPS does not improve survival compared to drugs (76, 123). It remains an open question in this setting, whether hemodynamic non-responders (HVPG) profit from continuation of NSBBs treatment with respect to survival (136).

NSBBs in cirrhosis: Controversies

NSBBs in decompensated liver cirrhosis with ascites

It has been shown that early administration of NSBBs can prevent ascites formation in some patients with compensated cirrhosis (118, 121, 137). But the question was raised by the Paris working group whether it is useful to give NSBBs to patients with refractory ascites (138). In a prospective case-only study, they found that patients receiving propranolol had a significantly shorter survival as compared to those without NSBBs. The vast majority of patients without NSBBs–otherwise comparable–had no esophageal varices in this study, a fact that later became a matter of debate. There is now a number of reviews that carefully analyze the existing literature, as to whether NSBBs are appropriate in severely decompensated liver cirrhosis (14, 139). Some evidence suggests that ß-blockers can/should also be given in patients with ascites and decompensated liver cirrhosis (140–142) under strict control of pulse, blood pressure and renal function using an adjusted lower dosage (143). Further reduction of the cardiac index (CI) in the presence of primarily already reduced cardiac function in the sense of cirrhotic cardiomyopathy is certainly unfavorable (144, 145). Systolic blood pressure <90 mmHg, elevated creatinine levels above >1.5 mg/dl (better > 1.3 mg/dl?) or an increase in creatinine value are contraindications to starting or continuing the administration of NSBBs (14). Some authors regard application of carvedilol with its more pronounced effect on visceral and systemic hemodynamics as being contraindicated in patients with marked ascites (146). In any case, NSBBs should be dosed carefully in patients with reduced CI.

Contraindications, side effects, duration of therapy, and adherence

In our own experience (76), nearly 10% of patients with cirrhosis had contraindications to NSBBs (such as refractory ascites, non-compliance, hepatic vein thrombosis, severe heart failure, or HRS type 1). In another randomized trial (147), 5% of eligible patients with liver cirrhosis had contraindications for propranolol. Complaints such as symptomatic hypotension, dizziness, impotence, and Raynaud symptoms occurred in nearly 70% of patients receiving propranolol, requiring withdrawal in 16% of the patients (of these 80% hypotension). In a controlled trial on early treatment of liver cirrhosis with NSBBs, 5 % of the screened patients had contraindications against NSBBs. Eight percent of patients discontinued NSBBs for side effects, as did 6% of the placebo patients (118). In another controlled trial on pre-primary variceal bleeding prophylaxis 18% of the patients had serious adverse events probably related to study medication (placebo 6%) such as bradycardia, fatigue, wheezing, claudication, and impotence (117). In a meta-analysis of eight RCT comprising 311 NSBB-patients in the setting of prophylaxis of first bleeding, side effects of NSBBs (mainly hypotension and breathlessness) required stopping treatment in 15% of the patients (148).

There is evidence that discontinuation of NSBB in patients with cirrhosis is associated with a high risk of rebleeding and that these patients may even have an increased mortality (147, 149, 150). Therefore, patients must be carefully selected for NSBBs, since it is aimed as life-long therapy.

Long-term drug application also concerns the assessment of compliance and adherence. Poor medication adherence is an important cause of inadequate treatment of long-term diseases (151). It is believed that 30–70% of hospital admissions in the US are due to non-adherence (152). The proportion of non-adherence to medications against hypertension, dyslipidemia, or diabetes is around 30% in the US (153).

With respect to liver disease, in one study, 23% of patients showed poor adherence to NSBB intake for bleeding prophylaxis from esophageal varices (154). Other small intervention studies showed that 25% of patients with cirrhosis have poor medication adherence and just under 50% showed good adherence (155). Under the conditions of a randomized trial

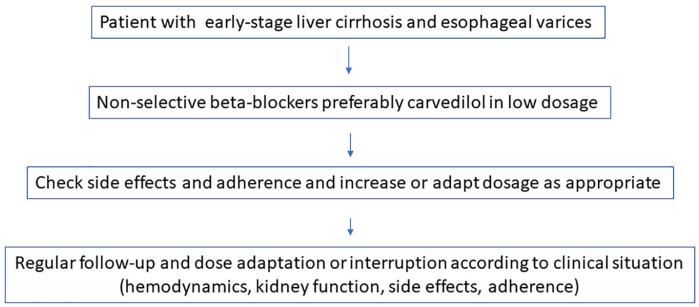

FIGURE 3
Non-selective ß-blocker (NSBB) in liver cirrhosis: The pragmatic approach.

9% were non-compliant within a period of 2 years (147). Analysis of a large US database found around 60% of patients with variceal bleeding and decompensated cirrhosis receiving NSBBs. Of those, only 8% showed consistent use (156). Targeted care to improve medication adherence can reduce the rate of emergency hospital admissions in cirrhotic patients (157, 158).

Prognostic markers and surrogates for endpoints

In the 1980s, it was shown that certain endoscopically definable criteria of varices (e.g., size or the so-called red color sign) are associated with higher blood pressure in the varices and their risk of bleeding (159–161). These endoscopic appearances have been used for decades to select patients, especially in primary prophylaxis of variceal hemorrhage with NSBB (162). As early as the 1960s, the Child classification was introduced and more or less modified over the years (163). Parameters from the Child classification were then combined with renal function (164). These systems are–with modifications–undisputed for the prediction of survival and also for selection for liver transplantation (128, 165). Their prognostic accuracy can be slightly improved by adding inflammatory parameters (166–168). To what extent these scores should be brought into the decision process for application of NSBBs is still in debate.

It is to the credit of Lebrec and his group (169), and later mainly Spanish and also Austrian working groups, to have introduced in a very consistent and careful way over the years the role of portal hypertension, measured as HVPG, for the prognosis of patients with liver cirrhosis, supported by many clinical studies (170, 171). Patients with an HVPG below 10 mmHg have a low risk of developing hepatic decompensation or death at least during the following 3–5 years. Patients with a HVPG > 20 mmHg have not only a high risk of early recurrent hemorrhage in case of variceal bleeding, but also a high risk of death (134, 172, 173). They benefit from the early enrollment for a TIPS in case of bleeding (173, 174). The decrease of HVPG > 20 % or to a value below 12 mm Hg, is a good criterion for protection against variceal hemorrhage, even ascites, and possibly for better survival (133, 134, 172, 175–178). According to some of these studies, the drop in HVPG of 10% is sufficient for the prognosis of prolonged survival. However, the value of this hemodynamic parameter as a surrogate for clinical end points in trials remains controversial. Among other reasons, because the measurement of HVPG is only performed by a limited number of centers in routine practice and because it is unclear how exactly other groups can measure HVPG (variability of measurement, but also intraindividual variability). More and more the measurement of liver or spleen stiffness is used for evaluation of portal hypertension (179), but its value to measure the response of portal pressure to NSBBs or to define clear thresholds is probably not sufficient.

Determination of pharmacokinetic parameters, including change of stereoselective metabolism in liver cirrhosis (180), are probably not of prognostic value for hemodynamic response to NSBBs. Studies on pharmacogenetics with respect to ß-AR gene polymorphisms and the action of NSBBs are sparse and inconclusive for patients with liver cirrhosis (181, 182).

Epilogue: Pragmatism or perfection?

Although NSBBs have been used for the prophylaxis of variceal bleeding for four decades, a number of questions remain unanswered, as we have explained above. This concerns the choice of NSSB or the question of whether and how the expression of ß-AR in the different organs changes in liver cirrhosis. It remains also completely unexplored, how ß-AR-dependent intracellular signaling cascades change in a cell-specific manner with decreasing liver function. While we have focused a lot on the cardiovascular system in terms of NSBBs, we know very little about how they act in the diseased liver, especially with respect to liver resistance to portal flow. It is unclear to what extent biased signaling *via* ß-arrestin, in the event of carvedilol administration, exerts on the liver or on the heart in patients with liver cirrhosis. NSBBs may also have a

double-edged effect on the immune system in liver cirrhosis. The question as to whether it is best to use NSBBs in a HVPG response-controlled manner remains open, and we do not know, whether patients who do not respond adequately with a drop in HVPG, will benefit at all from further administration of NSBBs. Also, we do not know to any great extent how the degree of liver cirrhosis or a change in albumin metabolism influence the effect of NSBBs. Last but not least, the standards for the optimal dose range of NSBBs in liver cirrhosis are also under discussion. Thus, there is much no man's land in questions about pharmacokinetics and pharmacodynamics and the use of NSBBs in patients with liver cirrhosis.

Are NSBBs a good long-term therapy in routine clinical practice, given that about 10% of patients have primary contraindications to NSBBs, almost 20% have to discontinue NSBBs because of side effects, and given that no more than 50% of this patient group shows adequate drug adherence, although lifelong therapy is necessary? This issue is relevant, considering that in Western countries, most patients now have metabolic cirrhosis (food and/or alcohol). One wonders whether these patients are really compliant for such a therapy. That may be an unfair assumption. Sufficient data is lacking in this regard. There is also insufficient data on quality of life under continuous treatment with NSBBs in patients with liver cirrhosis. More studies on combination therapy–e.g., NSBBs with statins, angiotensin II receptor blockers, phosphordiesterase-5-inhibitors–drugs that might work against chronic inflammation in cirrhosis, or even the combination of NSBBs with a narrow-lumen TIPS are also necessary.

Can we answer these open questions with rigor, through more perfection? By individualizing the choice of NSBB or combined treatment? By regular monitoring of the HVPG? By better informing the patient and controlling drug adherence? By assessing the quality of life of the patients (there are hardly any studies on this)? Certainly not immediately. But some of these questions are worth further clinical research to achieve more perfection in the treatment of patients with liver cirrhosis using NSBBs.

On the other hand, clinical action requires pragmatism, taking into account the evidence, based on the available studies present. Controlled trials (RCTs) provide the best unbiased information about the effect of an intervention in medicine. And there are a lot of RCTs with respect to NSBBs and liver cirrhosis. For the single patient, RCTs show the best possible choice of intervention, but they will never give the answer as to how the individual will respond. Under these circumstances it might be easiest to start NSBBs–preferably carvedilol–very early and at a low dose so that the patient complies with the therapy, with attention to pulse reduction, monitoring renal function and blood pressure at regular intervals, and to choose an alternative therapy in case of intolerance or lack of adherence or deterioration of kidney function (Figure 3). All this must be done in consideration of the other medications the patient needs. This pragmatism should, however, be accompanied by further research, which demands perfection. To this end we suggest further studies (Table 2).

TABLE 2 Proposals for further research–non-selective ß-blocker (NSBBs) and liver cirrhosis.

With respect to pharmacokinetics
- Distribution, metabolism, excretion in decompensated cirrhosis
With respect to pharmacodynamics
- Signaling at different organs and cells, dependent on etiology and stage of liver cirrhosis
- Pleiotropic effects on intestinal and immune system
- Modulation by genetic background
- Biased signaling effects of carvedilol on intrahepatic resistance and fibrosis (increase/decrease?)
With respect to individual behavior
- Adherence to NSBBs
With respect to concomitant drugs or interventions (TIPS, ligation)
- Additive, complementary, or neutralizing effects
With respect to dosage
- Optimal tradeoff between side effects and efficacy
With respect to selection of biomarkers under NSBBs–prediction of:
- Bleeding
- Ascites
- HCC
- Survival

Author contributions

TS wrote the first draft of the manuscript. MH, JT, and RS corrected and reformulated sections of the manuscript. RS supported editing the references. 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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Pathophysiology and management of liver cirrhosis: from portal hypertension to acute-on-chronic liver failure

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Cirrhosis transcends various progressive stages from compensation to decompensation driven by the severity of portal hypertension. The downstream effect of increasing portal hypertension severity leads to various pathophysiological pathways, which result in the cardinal complications of cirrhosis, including ascites, variceal hemorrhage, and hepatic encephalopathy. Additionally, the severity of portal hypertension is the central driver for further advanced complications of hyperdynamic circulation, hepatorenal syndrome, and cirrhotic cardiomyopathy. The management of these individual complications has specific nuances which have undergone significant developments. In contrast to the classical natural history of cirrhosis and its complications which follows an insidious trajectory, acute-on-chronic failure (ACLF) leads to a rapidly downhill course with high short-term mortality unless intervened at the early stages. The management of ACLF involves specific interventions, which have quickly evolved in recent years. In this review, we focus on complications of portal hypertension and delve into an approach toward ACLF.

KEYWORDS

portal hypertension, liver cirrhosis, HVPG, acute-on-chronic liver failure, chronic liver disease

1. Introduction

Cirrhosis is a major cause of morbimortality, constituting around 2.4% of global deaths (1). The natural history of cirrhosis has a progressive and dynamic course transitioning from a relatively stable state of compensated cirrhosis to an advanced stage of decompensated cirrhosis (2). Central to the dynamics of the transition is the degree of portal hypertension (PH) which serves as the primary driver of complications like the development of varices, ascites, renal dysfunction, hepatic encephalopathy (HE), hyperdynamic circulation, and cardiomyopathy (3, 4). While on the one hand, the stagewise progression of cirrhosis with worsening of PH delineates the conventional natural history of cirrhosis, another distinct syndrome marked by an acute deterioration of liver function with or without extrahepatic organ failures known as acute-on-chronic liver failure (ACLF) has opened up newer paradigms in PH over the last decade (5). This review explores newer insights into the pathophysiology of PH in cirrhosis and ACLF.

2. Basic pathophysiological mechanisms of development of PH

Central to the development of PH is the occurrence of resistance at any point in the portal venous system, leading to the effect of a pressure gradient. In patients with cirrhosis, this resistance level is at the level of hepatic sinusoids, which arises from a combination of structural (fibrosis, nodule formation) and functional alterations (6). The static or architectural changes behind the development of PH are driven by alterations in the interplay between hepatic stellate cells (HSCs) and liver sinusoidal endothelial cells (LSECs). In response to any injury or insult, HSCs are activated and lead to extracellular matrix formation and fibrogenesis, while LSECs undergo a phenotypic remodeling leading to capillarization of the sinusoids, thereby increasing intrahepatic resistance. Coupled with this, a dynamic component arising from myofibroblast contraction and decreased vasodilators like nitric oxide further accentuate the resistance pathway (6, 7). These two fundamental mechanisms lead to the progressive development of PH, leading to splanchnic vasodilation, neurohormonal disturbances, systemic vasodilatation, decreased mean arterial pressures (MAP), and an overall hyperdynamic state (8) (Figure 1). In combination with these, gut microbial alterations, increased intestinal permeability, and systemic inflammation act as both precipitants and perpetrators of worsening PH and further downstream complications (8) (Figure 2). In the following sections, we elaborate on the individual consequences of PH and their management.

3. Variceal hemorrhage

3.1. Development of varices and importance of hepatic venous pressure gradient

Resistance to portal blood flow and increased portal venous blood inflow result in the reversal of flow and formation of alternate blood flow channels between the portal and systemic circulation, which result in varices. The development of varices acts as a surrogate marker of PH and signifies clinically significant portal hypertension (CSPH). HVPG is the closest surrogate marker of actual portal pressure and PH, with the presence of PH being defined as an HVPG $>$ 5 mm Hg, while a value of $>$10 mmHg signifies CSPH (9) (Table 1). In patients with VH, an HVPG $>$ 20 mmHg (measured within 24 h after admission) is the best predictor of a poor outcome. A reduction in the HVPG $<$ 12 mm Hg or a reduction of more than 20% from the baseline value has been associated with a decreased risk of VH and improved survival (10). HVPG $>$ 20 mm Hg has been associated with a 5.21-fold likelihood of rebleeding, and reducing HVPG below this threshold using a vasoactive drug improves outcomes. Patients with HVPG $>$ 20 mmHg or $<$10% decline in HVPG (non-responders) on vasoactive medications increases the risk of rebleeding and have higher mortality (10). All patients presenting with VH should ideally undergo HVPG measurement, although access to the procedure at all centers is limited (11, 12). Patients with VH who have an HVPG $>$ 20 mmHg should be evaluated for an early transjugular portosystemic shunt (TIPSS) (13).

3.2. Risk factors for VH and risks associated with re-bleeding

VH from esophageal varices or gastric varices can result in high mortality (10–20% at 6 weeks) (3, 14). Other rare ectopic sites for VH ($<$5% of VH) are the rectum, duodenum, and post-surgical stomas. There are multiple risk factors for VH, including the larger size of varices ($>$5 mm), higher HVPG, higher grade of the child class, presence of red color signs (RCS) markings, active alcohol consumption, and presence of sepsis. There are also certain high-risk factors for re-bleeding, including a pressure gradient measured within 24 h of bleeding more than 20 mmHg, presence of large varices, age $\geq$ 60 years, renal failure, and severe initial bleeding (on admission, hemoglobin $<$ 8 g/dL) (11, 15).

3.3. Management of acute variceal bleeding

The management consists of controlling acute bleeding to prevent death and prevention of re-bleeding. Hemodynamic resuscitation is the initial treatment considering patient age, co-morbidities, ongoing blood loss, hemodynamic status, and other parameters. Fluid resuscitation should be cautious and restrictive to keep hemoglobin between 7 and 9 gm/dl, as overaggressive resuscitation can worsen PH and bleeding (16). INR-based corrections with fresh frozen plasma, factor VII transfusion, platelet, cryoprecipitate, or other blood products are not warranted (17, 18). Moreover, overzealous use of these products can be harmful due to the increase in PH due to volume overload or transfusion-related lung injury (14, 19). After gastrointestinal (GI) bleeding, blood acts as a culture media to grow infections; therefore, adequate purging should be done to prevent post-bleed sepsis, HE, ascites, or other complications of PH. Post-bleed sepsis can increase mortality; thus mandating the use of antibiotics during bleeding events as per local antibiograms. Currently, third-generation cephalosporins are recommended (ceftriaxone 1 gm IV every 24 h for 7 days) (20, 21). Vasoconstrictors should be started as early as possible in VH, along with proton-pump inhibitors. Vasoconstrictors should be continued for at least 2–5 days (15). Somatostatin, octreotide, and terlipressin are the recommended agents with comparable efficacy and safety (22).

Endoscopy-based endotherapy is definitive in managing VH and should be done within 12 h after hemodynamic resuscitation (23). Prokinetics (intravenous erythromycin) and anti-emetics should be given before the endoscopy for better visualization (24). Patients with altered mentation, severe sepsis, shock, and acidosis should be electively intubated before endoscopy. Endoscopic band ligation (EBL) is the definitive therapy for esophageal varices and gastro-esophageal varices (GOV) type 1. Endoscopic glue injection with cyanoacrylate glue remains the most used therapy for treating

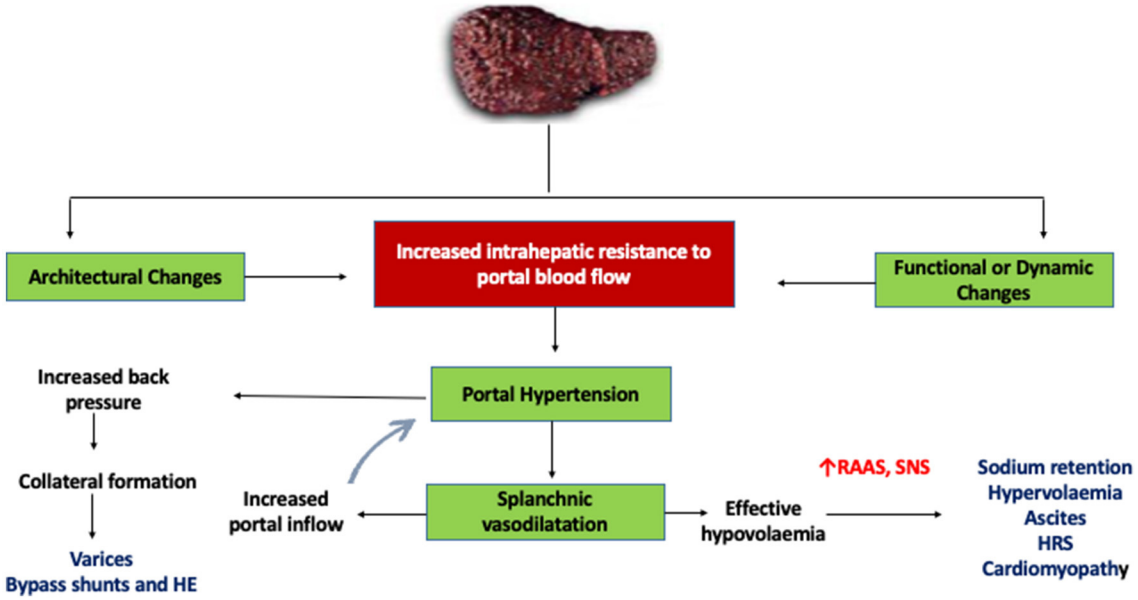

FIGURE 1
Mechanism of portal hypertension in cirrhosis. HE, hepatic encephalopathy; RAAS, renin-angiotensin-aldosterone system; SNS, sympathetic nervous system; HRS, hepatorenal syndrome.

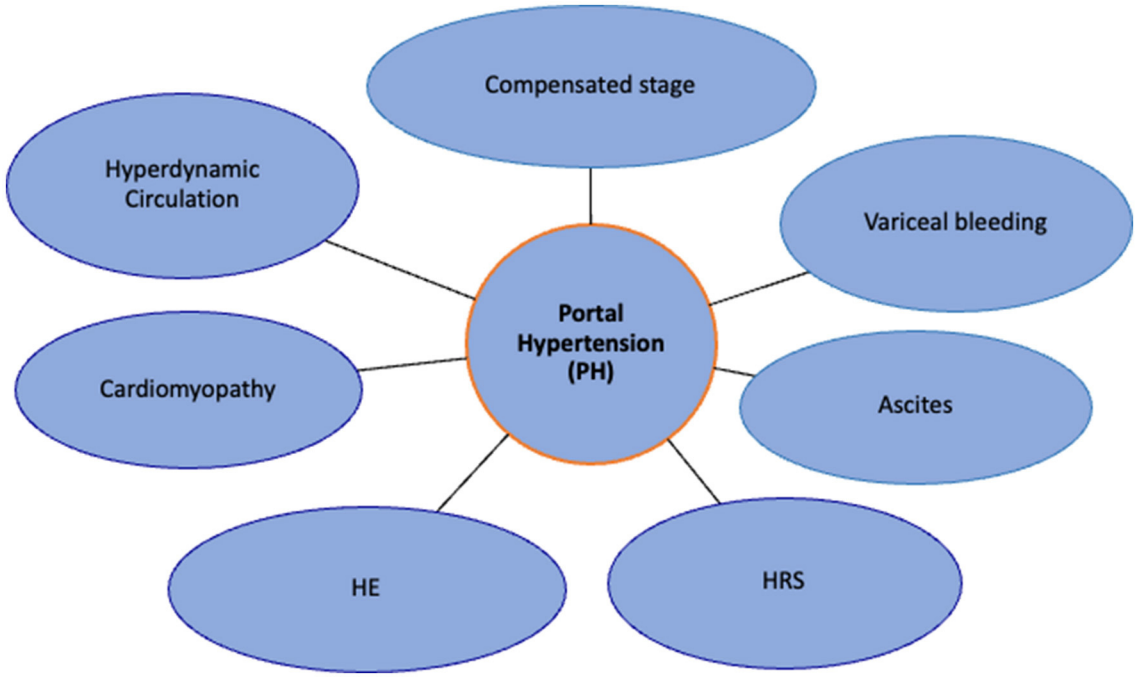

FIGURE 2
Effects of portal hypertension: migration from compensated stage to decompensated stage. HE, hepatic encephalopathy; HRS, hepatorenal syndrome.

bleeding from isolated gastric varices (IGV) and GOV type 2 (Figure 3). Tamponade with Sengastaken–Blakemore (SB tube) or Minnesota tube is usually considered a salvage modality in cases of refractory bleeding, often serving as a bridge to more definitive therapy such as TIPSS. The role of TIPSS in VH has been advocated as a pre-emptive modality (pre-emptive TIPSS) and a salvage modality (rescue TIPSS) (25). After stabilization, imaging studies (ultrasonography/computed tomographic scan) to rule out acute causes of PH like portal vein thrombosis (PVT) and hepatocellular carcinoma (HCC) should be performed (26).

3.4. Newer perspectives

An emerging concept proposed is identifying risk factors and possible avoidance of antibiotics in patients with well-preserved liver functions presenting with VH, however, prospective validation is needed (27, 28). Although the model for end-stage liver disease (MELD) is reasonable in predicting outcomes of patients with VH, a recent study reported MELD-Lactate to be superior in predicting mortality after VH (29, 30).

3.5. Primary prophylaxis of VH

Non-selective beta-blockers (NSBBs) or EBL are the treatments of choice to prevent VH (31). The use of NSBBs in PH is well-studied and has a pleiotropic mechanism. In addition to being economical to use, recent studies have demonstrated their pleiotropic effects, like preventing bacterial translocation, antioxidant properties, containing further non-bleed decompensations, and portal hypertensive gastropathy progression, as well as improving survival in ACLF (32–35). Adding another rate-controlling agent, ivabradine, to NSBB has shown some promising results, achieving better hemodynamics, reducing the incidence of acute kidney injury (AKI) and HE, and achieving a target heart rate (36). However, external validation of this merits consideration.

Gastric variceal bleeds account for ∼20% of total variceal bleeds, are more profuse, are predominantly flow-related rather than pressure-related, and have higher mortality. Primary prophylaxis for GOV-1 is similar to EV: with either NSBB or balloon/coil/plug-assisted retrograde transvenous obliteration (BRTO/PARTO/CARTO) of gastrorenal/lienorenal shunt for patients with a history of HE. For patients with high-risk (size > 20 mm or severe PHG or MELD > 17) GOV2/IGV1, it may be preferable to perform CARTO/PARTO if there is a gastro renal shunt. Otherwise, an endoscopic ultrasonography-guided coil with or without NSBB or prophylactic cyanoacrylate injection is suggested in addition to NSBB. For patients with low-risk GOV2/IGV1 (<10 mm), NSBBs would be sufficient (37).

TABLE 1 Hepatic venous pressure gradient and esophageal varices.

Event	HVPG (mm of Hg)
Formation of varices (CSPH)	>10
Bleeding from varices	>12
Relatively no chances of re-bleed	<16
Higher chances of re-bleed	>20
Early TIPS	>20

CSPH, clinically significant portal hypertension.

3.6. Newer perspectives

Emerging data have frequently advocated BRTO to be effective in managing gastric variceal bleeding. A recent Korean study shows that BRTO and endoscopic obliteration are equivalent in preventing gastric variceal bleeds compared to placebo (38). This retrospective study needs further validation.

3.7. Secondary prophylaxis

Propranolol first demonstrated its effectiveness in preventing recurrent esophageal variceal bleeding in 1980 (39). Later, carvedilol was introduced, which has a better profile than propranolol. The addition of carvedilol to EBL than propranolol to EBL can lead to better HVPG response (40). NSBB reduces and prevents death while waiting for liver transplantation (LT) in patients with refractory ascites (RA) and/or VH, but controversies in advanced decompensated patients with ascites remain (41). TIPSS is traditionally performed for patients with refractory

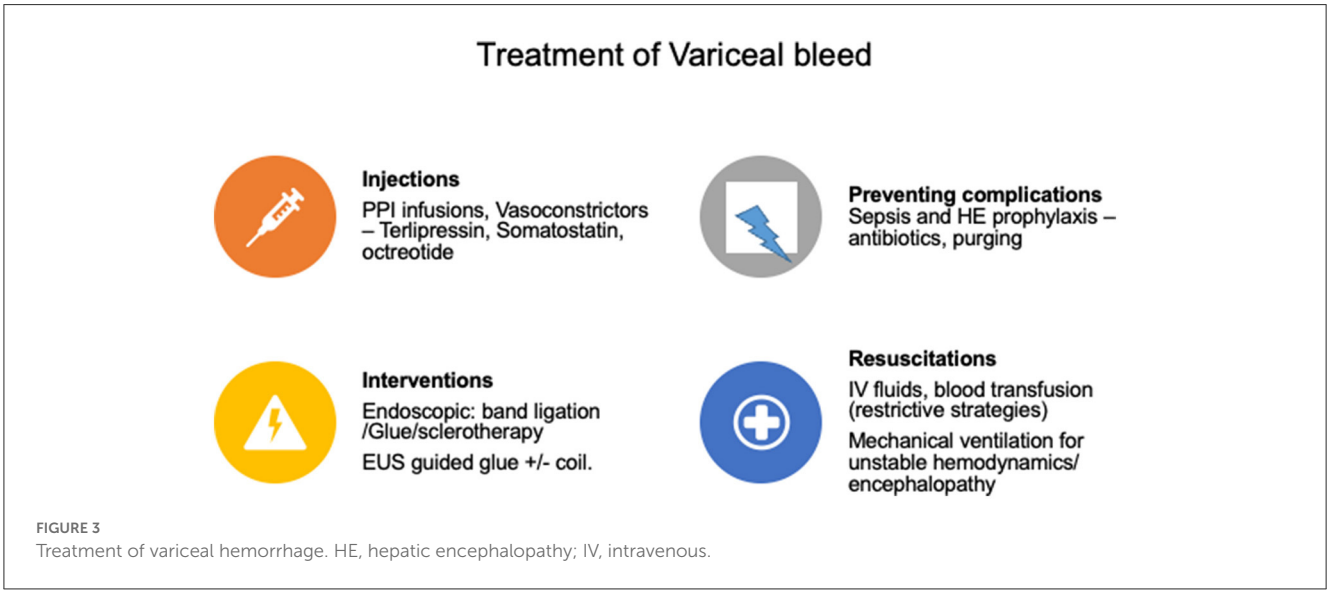

FIGURE 3
Treatment of variceal hemorrhage. HE, hepatic encephalopathy; IV, intravenous.

bleeding who fail EBL + NSBB. A recent study on early TIPSS (stent placement within 5 days of variceal bleed) has shown significant mortality benefits with a substantial reduction in the recurrence of variceal bleeding without increasing the risk of HE (42). In gastric variceal bleeding, TIPSS has been shown to prevent gastric variceal re-bleeding in patients with high HVPG (43, 44). BRTO, where the target flow is selectively occluded, is more effective than TIPSS in preventing re-bleeding from fundal varices as the bleed is flow-related than pressure-related and is associated with improved survival (45).

3.8. Newer perspectives

EUS-guided glue injection with or without coiling is safe and effective in primary and secondary prophylaxis of gastric varices bleeds (46, 47). Recent studies suggest performing TIPSS with BRTO in patients with recurrent variceal bleeding and spontaneous portosystemic shunts (SPSS) to prevent HE (48). The feasibility and cost-effective analysis of such procedures require further evaluation.

4. Ascites

Ascites is the most common complication of cirrhosis, and PH develops in ~85% of the cases (49, 50). To differentiate from other causes of ascites, ascitic fluid analysis is recommended, including serum-ascites albumin gradient (SAAG). SAAG value $\geq$ 1.1 g/dL has 97% sensitivity for PH as a cause of ascites (51). As discussed earlier, hepatic resistance and PH result in backflow and accumulation of vasodilatory substances, which results in intrahepatic vasoconstriction and peripheral vasodilation, including splanchnic vasodilation, which results in hypoperfusion of the renal system, even when the patient is euvolemic or hypervolemic (52). This state of relative hypovolemia due to vasodilation results in the activation of the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS), leading to salt and fluid retention (50). This leads to the retention of blood and a significant rise in blood volume leading to filtration from the liver surface and the mesenteric vessels. High hydrostatic pressure, low oncotic pressure (hypoalbuminemia), and increased vascular permeability contribute to increasing filtration through mesenteric vessels. The resorptive capacity of the peritoneum and lymphatics cannot counteract these mechanisms (53). Any inflammation or infection of the peritoneum can affect peritoneal resorption. Dysregulation of these can lead to an increase in ascitic fluid formation.

4.1. Management of ascites

The foremost important part of the treatment of ascites is sodium restriction (salt intake of <5 g) and the judicious use of diuretics. A combination of two diuretic classes (aldosterone antagonists and loop diuretics) is better tolerated and more effective than sequential treatment (i.e., first aldosterone antagonists followed by loop diuretics) (54). Use of albumin replacement and increased oral protein intake helps ascites mobilization. A recent pilot study has shown that early use of midodrine for a short course can control ascites better than diuretics alone, with a lesser occurrence of diuretic complications (55).

RA: A weight loss of <0.8 kg over 4 days in a patient with cirrhosis on intensive diuretic therapy for at least 1 week is termed diuretic-resistant ascites, provided the urinary sodium is less than the sodium intake/day (56). Furosemide 160 mg/day and spironolactone 400 mg/day are considered for intensive diuretic therapy. Diuretic-resistant ascites is a rare event, especially in Asian countries, as the recommended full dose of diuretics (160 mg of furosemide and 400 mg of spironolactone) is rarely reached as most patients develop adverse events with higher recommended doses, which is called diuretic-intractable ascites (4, 55). This diuretic intolerance in the Asian population is due to a higher incidence of sarcopenia, poor muscle reserve, and a higher occurrence of diuretic-related complications, including renal injury and electrolyte imbalances (4). Before labeling a patient as refractory to therapy, hepatocellular carcinoma, portal vein thrombosis, and infection of the peritoneum [sepsis, spontaneous bacterial peritonitis (SBP)/Non-SBP/tuberculosis] should be ruled out. An elevated ascitic fluid protein content of more than 2–2.5 g/dl is suggestive of tuberculous ascites (57). Moreover, the higher incidence of tuberculosis in Asian countries can occur in immuno-compromised cirrhosis patients without manifesting classical signs and symptoms. Therefore, adenosine deaminase (ADA) and gene x-pert (tuberculosis nucleic acid testing) analysis of ascitic fluid is suggested in all patients with cirrhosis with difficulty uncontrolled ascites before labeling them as RA, especially in tuberculosis-endemic countries (4). LT is the best and ideal treatment option for patients with RA. Large-volume paracentesis (LVP > 5 L) with albumin infusion (8 gm/L of ascites removed) is the recommended therapy to relieve the symptoms. However, LVP is associated with the risk of paracentesis-induced circulatory dysfunction (PICD), which is mitigated with concomitant albumin usage. In a network meta-analysis, midodrine was reported as superior to albumin in preventing PICD (55, 58, 59). NSBBs are contraindicated in patients with RA requiring LVP due to compromised cardiac performance (60). Midodrine, an alpha-1 agonist, is beneficial in RA as it increases urine sodium loss and urinary volume (61, 62). By reducing endotoxemia, rifaximin may offer an additional benefit in RA (63). Tolvaptan is beneficial in ascites control with survival benefit (64). However, tolvaptan has a black box warning as it can cause or precipitate bleeding episodes by platelet aggregation inhibition and depleting vitamin-K-dependent clotting factors and has a risk of liver injury (65). Therefore, its use should be cautious and restrictive to patients of grade 3 ascites/RA with refractory hyponatremia and should be used for the shortest duration possible (51). Terlipressin, the most used drug in hepatorenal syndrome (HRS) and RA, helps in ascites control by mobilizing ascites and increasing renal perfusion, glomerular filtration rate (GFR), and urinary sodium excretion (66). Long-term albumin administration in patients with ascites improves survival, decreases hospitalization, and reduces overt HE, ascites, SBP, and non-SBP infections (67). TIPSS is a valuable therapy in RA and has been found to increase transplant-free survival (68). Careful selection of patients for TIPSS

after a proper cardiac evaluation is recommended. A patient with age < 70 years with preserved liver function tests and low severity scores (MELD < 18 and Child score < 8) without any history of HE in the preceding 6 months are candidates suitable for TIPSS (Figure 4).

4.2. Newer perspectives

The automated low-flow ascites pump (ALFA) system, a novel device that transfers ascites from the peritoneal cavity to the urinary bladder, is effective in patients with RA (69). However, it is not universally available, complicated to use, and has higher adverse events; therefore, its use is currently limited (69). ANSWER trial reported the beneficial effects in terms of survival of long-term albumin infusions in patients with decompensated cirrhosis (70). Although results have been contradictory from two recent large trials, future research with more clearly defined selection criteria and endpoints may streamline the use of long-term albumin in ascites (70, 71). Sodium-glucose co-transporter 2 inhibitors (SGLT2I) increase sodium and glucose excretion in the urine and decrease renin secretion, showing significant improvement in ascites besides glycemic control in a few small studies (72, 73). Major side effect is an increased risk of urinary infections. Further prospective studies are needed in cirrhosis patients with RA for SGLT2I. Patients with RA and poor quality of life required long-term abdominal drains/catheters as a palliative measure. Although deemed to have an increased risk of infections, preliminary studies have shown good technical success and low rates of life-threatening infections providing options for home-based care (74, 75).

5. Renal dysfunction: acute kidney injury and hepatorenal syndrome

HRS, a functional renal failure, is a potentially reversible renal injury in patients with cirrhosis and ascites due to decreased renal blood flow (76). An increase in serum creatinine by ≥0.3 mg/dl within 48 h or an increase of >50% from baseline value with or without a decrease in urinary output < 0.5 ml/kg for >6 h in patients with cirrhosis and ascites in the absence of other evident cause for acute renal injuries such as proteinuria, shock, or nephrotoxins is termed HRS-AKI (76).

Recently, there has been a suggestion for a change in terminology, with previous terms like HRS-1 and HRS-2 being replaced by more physiologic HRS-AKI, HRS-acute kidney disease (AKD), and HRS-chronic kidney disease (CKD). The estimated incidence of HRS is around 18% at 1 year and 39% at 5 years and is associated with an inferior median survival of ≤3 months without a transplant (51, 56).

Although several medical management options remain in HRS, LT is the definitive therapy. Vasoconstrictors (terlipressin, octreotide in combination with midodrine and noradrenaline) and albumin infusion are the cornerstones of the treatment of HRS. The crux of HRS therapy still revolves around an attempt to rule out other causes (infections, glomerular disease, shock, and acute tubular necrosis) concomitant with volume expansion with albumin for 48 h followed by initiation of vasoconstrictors. Terlipressin remains the most effective vasoconstrictor, with an infusion strategy of administration associated with lesser adverse events (77, 78). Patients with HRS who have not responded to therapy and have persistently low GFR (i.e., <25 ml/min) for more than 1.5 months and/or dialysis dependence are candidates for simultaneous liver-kidney transplantation (SLKT) (56). Recurrent episodes of HRS or renal insult lead to the development of HRS–CKD. The development of CKD in cirrhosis is a poor prognostic marker in both pre- and post-transplant settings (79). Risk factors of HRS-AKI progression to HRS-CKD are terlipressin non-response, high MELD score, albuminuria, recurrent AKI episodes, and high baseline serum cystatin (80). Management of HRS-CKD is unclear and needs further studies. Although treatment with terlipressin, diuretics in case of fluid overload, vaptans in case of hyponatremia, midodrine, and TIPSS with a high risk of HE are some options, SLKT is the definitive treatment (81, 82).

5.1. Newer perspectives

The use of TIPSS in patients with HRS-CKD has been recently shown to improve renal function with excellent control of ascites across stages of CKD (83). Recent studies suggest frailty as a predictor of HRS-AKI (84). It is unknown whether branched-chain amino acid (BCAA) supplementation reduces the development of HRS-AKI. With the approval of terlipressin in the US setting, exciting research is expected, with initial data advocating early initiation of terlipressin at lower grades of AKI being associated with improved survival (85).

6. Hepatic encephalopathy

HE is a neuropsychiatric manifestation related to severe liver disease. HE in a patient with acute liver failure is termed type A, while those related to shunts are termed type B, and those with cirrhosis are termed type C. HE is graded as per West-Haven criteria. HE can be covert [minimal HE (MHE) and Grade I HE], which needs to be identified with the help of specialized neuropsychological tests. Covert HE is reported among 80% of patients with advanced liver disease, while overt HE is reported among 40% (86). Overt HE can be new onset, episodic, with an interval between episodes of >6 months, or recurrent, where further episode occurs within 6 months. Persistent HE refers to an uncommon entity with no resolution of HE. Refractory HE (lack of response after treatment of precipitants and on treatment with lactulose and rifaximin for 48 h) is an uncommon but serious condition and requires active investigation into hidden precipitating events (i.e., portosystemic shunt) and requires alternative diagnosis to be ruled out (87). Important alternative causes include septic encephalopathy (23%), alcohol withdrawal, seizure, dyselectrolytemia, metabolic disorders, drugs/toxins (7%), intracranial structural lesions (5%), psychiatric disorders (1%), and multiple causes together (8%) (88).

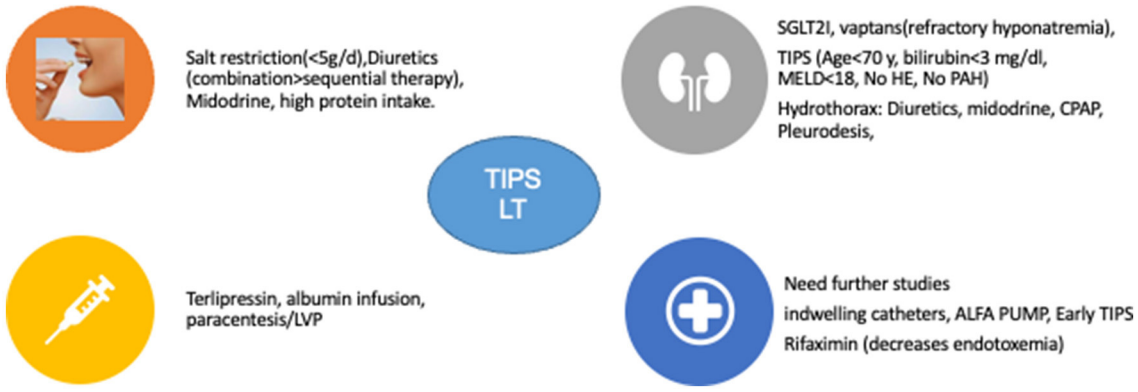

FIGURE 4
Treatment of ascites. SGLTA2I, Sodium-Glucose Co-transporter 2 inhibitors; MELD, model for end-stage liver disease; HE, hepatic encephalopathy; PAH, pulmonary arterial hypertension; TIPSS, transjugular intrahepatic portosystemic shunts; CPAP, continuous positive airway pressure; LVP, large volume paracentesis; LT, liver transplantation.

6.1. Pathophysiology of HE and effect of ammonia

Alterations in neurotransmission and brain–blood barrier coupled with persistent neuroinflammation and oxidative stress, apart from GABA-ergic or benzodiazepine pathway abnormalities, lead to disruptions in brain energy and blood flow, causing HE. Disturbed ammonia metabolism is the central and most studied event in HE, with complex multimodality mechanisms. In brief, as liver failure progresses, concentrations of ammonia increase which exerts its systemic effects and neurotoxicity through multiple pathways, including astrocyte swelling, inflammation, oxidative stress, mitochondrial permeability alterations, alteration in energy kinetics, and membrane potential alterations (89). Despite this implicating pathophysiological basis, no direct correlation has been established between the severity of HE and ammonia concentrations. However, it is imperative to state that in the presence of a normal ammonia level, the diagnosis of HE is almost always an exclusion.

A venous ammonia level of >55 μmol/L is 47% sensitive and 78.3% specific to diagnose HE (90). Other studies have identified a blood ammonia level cutoff of >133 μg/dl as a diagnostic of HE. Arterial ammonia is an excellent surrogate marker for the severity of HE in ACLF in advanced stages, and an ammonia level above 140 μg/dl at baseline or at any time point in first week with grades III–IV HE serves as a poor prognostic marker for 28- and 90-day survival (91). Venous NH3 is more variable; therefore, arterial ammonia measurements are used (91, 92).

Spontaneous portosystemic shunts (SPSS) should be actively looked for, especially in recurrent/refractory HE and where liver diseases are not advanced (e.g., MELD < 15). SPSS shunts are noted in 10–20% of patients with cirrhosis and PH. SPSS is a "release valve," a compensatory mechanism to reduce the portal pressure and bypass normal liver flow. More than 90% of patients with large SPSS have enlarged spleen, hepatic atrophy, and thrombocytopenia (93). Identification of these shunts is essential as these need to be ligated at the time of liver transplant, or else the patient can have persistent HE, even after liver transplant.

6.2. Management strategies in HE

Correct identification of the precipitant is the key to the management of HE. Non-absorbable disaccharidases (lactulose/lactitol) are the first-line therapy. Adding polyethylene glycol to non-absorbable disaccharidases leads to earlier, sustainable improvement in HE with survival benefits (94). Studies have shown a positive role of rifaximin and intravenous L-ornithine L-aspartate (LOLA) in overt HE management (95, 96).

Diet and calorie requirements must be met, especially for patients with altered mentation who cannot take orally. Adequate calories (35–45 kcal/kg/day) and protein (1–1.5 gm/kg/day) are essential to improve overall nutritional status. BCAA may be beneficial as they are metabolized in muscle and brain and promote protein synthesis, suppress protein catabolism, and act as gluconeogenesis substrates (97). Rifaximin is an oral antibiotic with minimal absorption (<0.4%), broad-spectrum activity against enteric bacteria, excellent tolerability, no significant drug interactions, and no dose adjustment requirement in hepatic or renal dysfunction (98). The evidence for using rifaximin in HE needs close attention. The most robust evidence for rifaximin is as an add-on agent to lactulose in HE recurrence. However, high-quality evidence does not support its use as monotherapy

for treating an episode of HE and direct comparative trials with non-absorbable disaccharides.

When used in conjunction with lactulose, rifaximin is effective in HE improvement, mortality reduction, and reduction in length of hospital stay (99). Zinc is a co-factor of urea cycle enzymes, and zinc deficiency has been reported to precipitate HE, thereby mandating the use of zinc supplements in HE (100). Although few studies have reported improvement in HE with probiotics, it is currently not FDA-approved (101).

6.3. Newer perspectives

Ammonia-lowering agents (Phenylacetate, Phenylbutyrate, and Sodium Benzoate) and drugs affecting neurotransmission (flumazenil and bromocriptine) have been reported to be effective but are rarely used. Recent trials have demonstrated the efficacy of L-ornithine L-aspartate in critically ill patients with HE (102, 103). CARTO/PARTO of SPSS is an excellent modality for patients with HE (104). The side effects of shunt occlusion include worsening of esophageal varices (19–46%), new onset varices in 6%, and new/worsening ascites in 14% of cases. Fecal microbiota transplantation (FMT) or intestinal microbiota transplantation is a feasible and safe option for patients with recurrent or persistent HE (105). By modulating the gut flora favorably, FMT restores the altered gut–liver–brain axis. The role of human albumin infusions in the management of HE has been controversial. However, in a recent randomized controlled trial, of outpatients with cirrhosis, prior HE, and current MHE, albumin infusions improved cognitive function and quality of life (106). Along similar lines, a systematic review indicates a possible beneficial effect of albumin in overt HE (107).

7. Hyper-dynamic circulation

As discussed earlier, an imbalance between vasodilators and vasoconstrictor occurs in PH and leads to hepatic vasoconstriction and peripheral vasodilation, which leads to hyperdynamic circulation, which is a very close mimic of the septic state. This is also known as "Hepsis" (108). In cirrhosis, immunological mechanisms are compromised, leading to a state of cirrhosis-associated immune dysfunction (CAID), predisposing patients with cirrhosis to the development of sepsis, which leads to an increase in pathogen-associated molecular patterns (PAMPs) and cytokines (tumor necrosis factor-α, interleukin-1β) and other vasodilators including nitric oxide. Consequently, a cycle of preferential splanchnic vasodilatation leading to the activation of vasoconstrictive systems along with central hypovolemia and cardiovascular dysfunction leads to a gradual development of the hyperdynamic syndrome and multiple organ dysfunctions (50, 109, 110). Treatment is targeted on these fundamental mechanisms. Still, so far, no single agent has been found to take care of all these aspects, and multimodality management addressing underlying pathophysiology is advocated.

7.1. Newer perspectives

Obeticholic acid (OCA) has been used in several liver diseases, including non-alcoholic steatohepatitis, primary biliary cholangitis, and primary sclerosing cholangitis (111). OCA has been reported to effectively reduce intrahepatic vascular resistance and improve PH in pre-clinical models (112). A recent study showed the beneficial effects of curcumin in cirrhotic rats with PH due to its antifibrotic, vasoactive, and anti-angiogenesis actions (113). Curcumin counteracts the hyperdynamic circulation of cirrhosis by inhibiting endothelial nitric oxide synthetase (eNOS) activation and reducing mesenteric angiogenesis by blocking the vascular endothelial growth factor (VEGF) pathway. However, the current evidence is too premature to recommend these drugs.

8. Cirrhotic cardiomyopathy

Hyperdynamic syndrome in patients with cirrhosis and PH leads to persistently activated compensatory mechanisms, activation of RAAS, and SNS, which results in tachycardia, increase in cardiac output, and reduction in systemic vascular resistance and MAP. This phenomenon, over time, results in cardiac dysfunction, described as "cirrhotic cardiomyopathy (CCM)." Altered contractile response to stress, abnormalities in electrophysiologic transmission, and diastolic dysfunction are the characteristic features of CCM in the absence of any evident cardiac disease (114). These can be in compensated form and result in symptoms only in case of stress (e.g., volume overload and post-TIPSS). Dyspnea and exertional fatigue due to pulmonary edema is the most common manifestation. Some other complications include overt heart failure, pulmonary hypertension, arrhythmias, pericardial effusion, and cardiac thrombus formation. The proposed pathophysiological mechanisms include aberrant beta-adrenergic signaling, increased endocannabinoid activity, alterations in Na^{+}/Ca^{2+} exchanger, and the negative inotropic effect of nitric oxide and carbon monoxide (115, 116).

CCM is associated with an increased risk of complications (including RA, HRS, and impaired response to stressors), leading to poor quality of life, increased morbidity, and mortality. A targeted heart rate reduction using ivabradine can improve cardiac filling and output (114). CCM is potentially reversible with LT, provided other pathological diseases of cardia are ruled out (114). There have been some contradictory viewpoints about the effect of CCM on disease severity, with one study showing the lack of association of CCM with the severity of PH or liver dysfunction and age being the predominant determinant of CCM (117). Further studies resolve the contradictory observations that are required. Treatment of CCM is non-specific and supportive and rests on minimizing the treatment and interventions which can aggravate CCM (118). LT should be considered for well-optimized stable CCM patients and good performance status (119). Management of heart failure is similar to non-cirrhotic patients, including salt and fluid restriction, use of diuretics, and afterload reduction. Cardiac glycosides are not effective in cirrhotic patients (120). The studies on NSBB are conflicting. β-blocker can reduce prolonged QT intervals with some improvement in electromechanical uncoupling

but with a reduction in cardiac output, which can be detrimental (121, 122).

8.1. Newer perspectives

Targeted heart rate reduction to improve cardiac filling and thereby improve the cardiac output with ivabradine can be tried in sinus rhythm patients (114). Potassium-Canrenoate can reduce the left ventricular wall thickness and left ventricular diastolic dysfunction (LVDD) in patients with Child A cirrhosis (122). These require further randomized controlled trials before universal recommendation.

9. ACLF

9.1. Basic pathophysiological mechanisms and clinical outcomes in ACLF

Decompensation in cirrhosis is a dynamic process, and patients can transition in the Child stage between A and C, depending upon the type and number of decompensation. Therefore, decompensation can be an index/first event or a recurrent event after recovery from the first event. In some cases, it becomes very severe to cause hepatic or extrahepatic organ failures/organ dysfunctions and is identified as ACLF, which heralds high short-term mortality of over 15% at 28 days with organ dysfunctions/organ failures (123). It is a state of dysregulated inflammation with a potential for reversibility, and it is different from acute liver failure and acutely decompensated cirrhosis (91, 124).

Controversies exist between the definition and diagnostic criteria between east and west, but the central theme of the disease revolves around high short-term mortality (Figure 5). The two prominent definitions for ACLF are the Canonic by the European Association for the Study of Liver (EASL) and the Asian Pacific Association for Study of Liver (APASL) definition (Table 2). A large electronic database study reported significant discordance between APASL and EASL definitions (125). The incidence rate of ACLF as per APASL definition was 5.7 per 1,000 person-years, and the incidence rate of ACLF as per EASL definition was 20.1. Mortality was higher in EASL-identified ACLF than APASL identified (125). The median bilirubin level in the EASL-ACLF cohort was 2.0 mg/dL implying preserved liver function in EASL-ACLF. EASL and APASL criteria do not measure the same entity, and there is no uniformity in the ACLF definition. However, APASL ACLF is easier to use in clinical practice as it requires very few liver-specific laboratory variables (INR and bilirubin) and clinical history of ascites and/or encephalopathy.

9.2. Key pathophysiological interplays in ACLF

Systemic Inflammatory Response Syndrome (SIRS) and sepsis are the keys to the development of ACLF, which is caused by gut dysbiosis, leaky gut, increased intestinal translocation of viable bacteria, and PAMPs (110). In the initial phases of cirrhosis, lamina propria is the predominant site of inflammation in the gut, where it is contained with localized vasodilation, but as the disease progresses, there is the involvement of deeper structures leading to a leaky gut. Inflammation becomes pronounced as bacterial translocation occurs, along with products of bacterial metabolism and damage-associated molecular patterns (DAMPs) from the diseased liver. These changes occur rapidly and mostly coincide with a burst of systemic inflammation, SIRS, which is usually triggered by a precipitating event (126). Prostaglandin (PG) E2 and PGE2-EP4 pathway-mediated monocyte dysfunction are the predominant factors for immunosuppression in ACLF and lead to inflammation-related mitochondrial dysfunction (127, 128). Therefore, the overall pathogenesis is characterized by an initial cytokine burst presenting as SIRS, progression to compensatory anti-inflammatory response system (CARS), and associated immune paralysis, which leads to sepsis and multi-organ failure (Figure 6).

9.3. ACLF and acute decompensation

Acute decompensation (AD) of chronic liver disease refers to a sudden worsening of the condition of a previously compensated or decompensated cirrhotic patient due to an acute event that may present with hepatic (jaundice, ascites, and HE) or non-hepatic (VH, AKI, or sepsis) failure, up to 3 months of acute insult (91). ACLF is a distinct syndrome from "AD" due to intense systemic inflammation in ACLF. The precipitant for AD can be hepatic or non-hepatic (129). Mortality in patients with AD (<30% at 3 months) is lower than in those with ACLF (91). Management of AD and ACLF is quite similar, and LT would be the treatment of choice.

9.4. Precipitating events in ACLF

Since ACLF is triggered by an acute insult and has a potential for reversibility, identifying precipitating events is crucial so that targeted treatment can be instituted for better outcomes. Bacterial infections and active alcohol intake are the most common precipitating event in the west. In contrast, hepatitis B reactivation, followed by active sepsis and alcohol intake, is the most frequent precipitating event in the eastern world. However, no precipitating event may be found in about 40% of cases (129). In Asia, 1.8–5.7% of precipitating events are drugs related, which present as drug-induced liver injury (DILI) (130, 131). Acute viral hepatitis like hepatitis A, E, and other hepatotropic viruses can cause AD in ACLF. In addition, the flare of autoimmune hepatitis (AIH) can frequently be the precipitating event in female patients. Patients with AIH-related ACLF present histological features typical of AIH, including perivenulitis, lymphoid aggregates, and massive hepatic necrosis (132). The development of VH in patients with ACLF is an independent predictor of mortality (133). Acute hepatic venous outflow tract obstruction (HVOTO) or PVT can present as ACLF as per APASL guidelines (91). The underlying etiology of cirrhosis needs to be established in patients with ACLF presenting for the first time for appropriate management and prognostication.

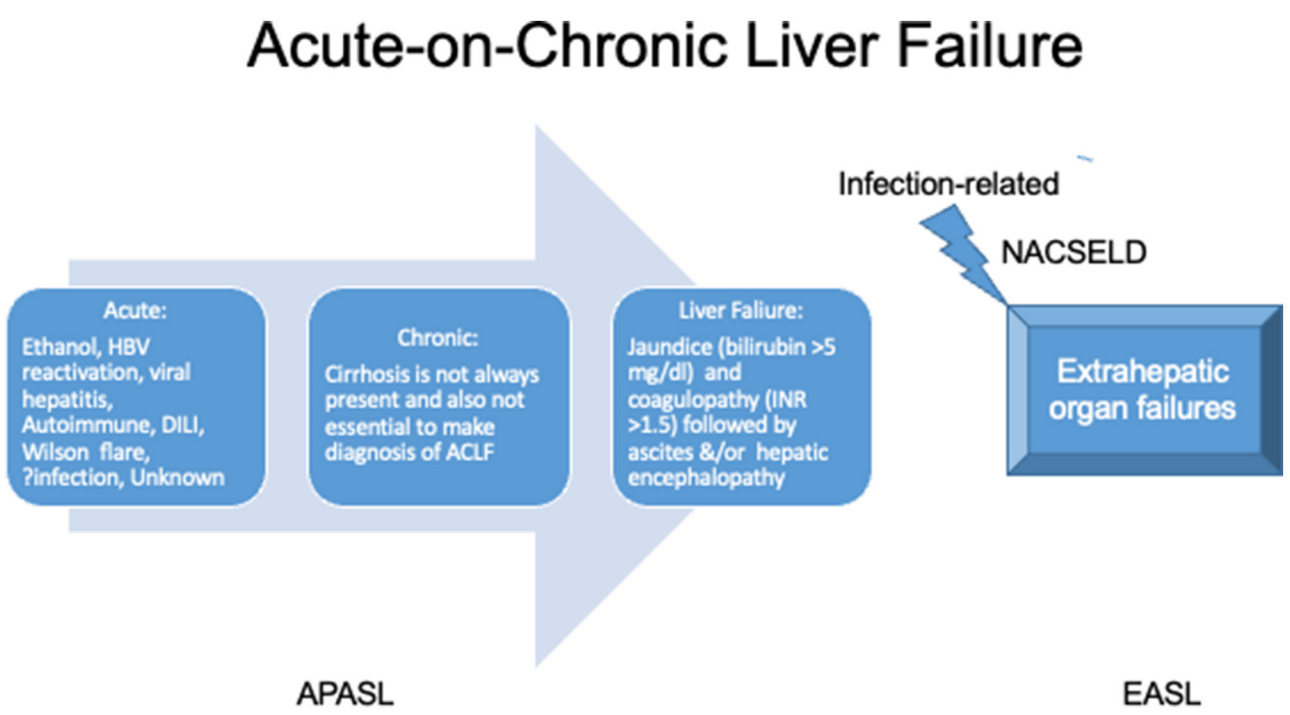

FIGURE 5
Definition of acute-on-chronic liver failure. APASL, Asian pacific association for the study of liver; EASL, European association for the study of the liver; NACSELD, North American Association for Study of Liver Diseases; INR, international normalized ratio; DILI, drug-induced liver injury.

TABLE 2 Differentiating two major definitions of acute on chronic liver failure (ACLF).

	APASL-ACLF	EASL CLIF consortium
Differences in key definition	Presence of an acute hepatic insult which manifests as jaundice with coagulopathy (INR > 1.5), and gets complicated within 4 weeks by ascites and/or encephalopathy in a patient with previously known or unknown chronic liver and has an intrinsically high 4-week mortality	Development of an acute deterioration of pre-existing chronic liver disease usually due to a precipitating event and leading to a high 4 week mortality due to multisystem organ failure.
Duration between insult and liver failure	4 weeks	Up to 12 weeks
What constitutes acute insult?	Only hepatic insults (alcoholic hepatitis, hepatotropic viruses, DILI, AIH)	Both hepatic and extrahepatic insults like infection and sepsis
How to define chronic liver disease?	Any chronic liver disease which is known or unknown which may or may not amount to cirrhosis (excludes previously decompensated cirrhosis)	Only patients with pre-defined cirrhosis including those with past history of decompensation
Sepsis	Is not considered as an acute event but may be a consequence of ACLF	A primary acute precipitant of liver failure and also may be a consequence
Variceal bleed as a precipitant	No consensus	Yes
Is reversibility defined?	Yes and is central to the definition	Not a clearly described
Disease severity score associated with definition	AARC	CLIF SOFA
Mortality described by definition	AARC-1:12.7%; AARC Grade 2:44.5% AARC Grade 3: 85.9%	ACLF Grade 1:22%; ACLF Grade 2: 33%; ACLF Grade 3:73%

APASL, Asia-Pacific Association for the Study of Liver; AARC, APASL-ACLF Research Consortium; CLIF, Chronic liver failure consortium; DILI, Drug-induced liver injury; AIH, Auto-immune hepatitis.

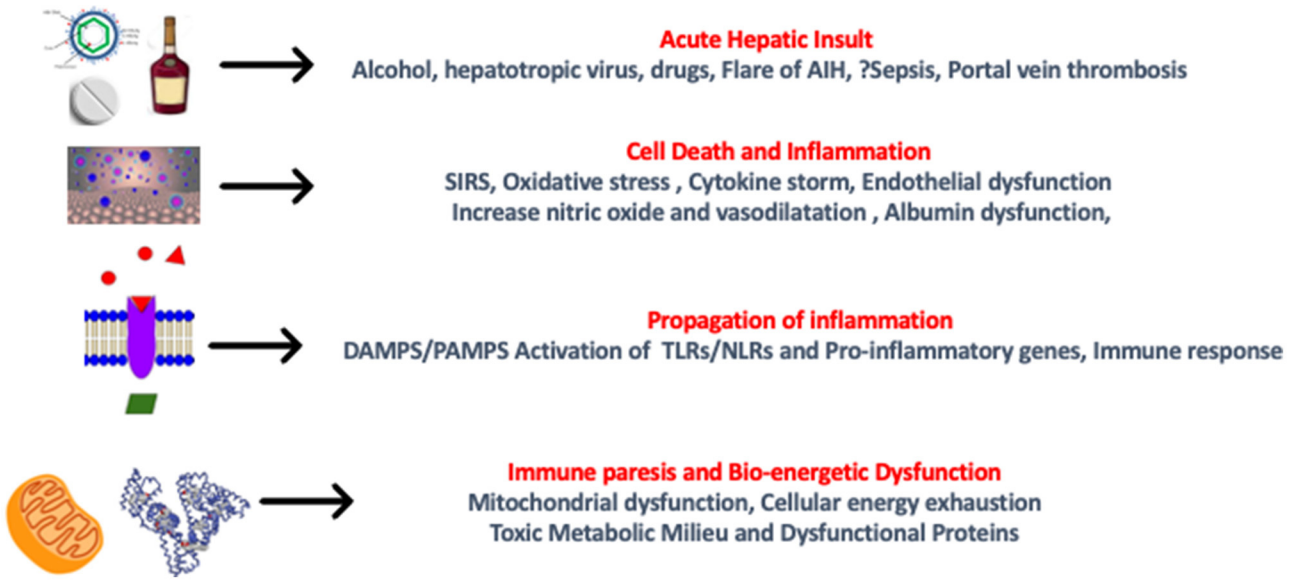

FIGURE 6
Pathophysiological derangements in acute-on-chronic liver failure. AIH, autoimmune hepatitis; SIRS, systemic inflammatory response syndrome; DAMPS, damage-associated molecular patterns; PAMPS, pathogen-associated molecular patterns; TLR, toll-like receptors; NLR, neutrophil-to-lymphocyte ratio.

9.5. Grade of ACLF

Organ failure (OF) includes both liver and extrahepatic organs. OF/organ dysfunction is the diagnostic hallmark of ACLF. CLIF-EASL grade is defined based on OF. *Grade-1* ACLF: only organ failure (renal, liver, coagulation, circulatory, or lung) that is associated with a serum creatinine level of 1.5–1.9 mg/dL; *Grade-2* ACLF: a combination of any 2 OFs. *Grade-3* ACLF: a combination of any 3 or more OFs (134). Conversely, the APASL definition is based on a dynamic score calculation known as the AARC score (91). AARC score between 5 and 7 is considered as APASL ACLF grade-1; 8–10 as AARC-2; and those with scores between 11 and 15 are AARC grade-3. Prognosis between the grades varies significantly, with grade 1 being a potentially recoverable group with a 28-day mortality of only 12.7%, and grade 3 needs immediate interventions to improve outcomes, with mortality at 28 days at around 85.9%.

9.6. SIRS, sepsis, ACLF, and LT

Liver failure predisposes to infections, and bacterial infections remain the most common cause of diseases in ACLF (135, 136). Infections are associated with severe inflammatory storms, high morbidity, cost, poor clinical course, and 4-fold high mortality (137). Sepsis is more likely associated with concomitant multi-organ involvement and poor prognosis (137). Frequency of infections in hospitalized cirrhotic patients ranges from 32 to 34% and increases with hospitalized cirrhotic patients with GI bleeding to 45%. The most common sites of first infections are SBP in 22–25%, urinary tract infection (UTI) in 20–28%, and pneumonia in 8–15% (137–139). Among pathogens, gram-negative (*E. coli* and *Klebsiella* spp.) are most frequent, followed by gram-positive (*Streptococcus pneumonia* and *Staphylococcus aureus*) and fungi (135).

Sepsis is an exaggerated inflammatory response to infection. SIRS in a patient with infection was required to identify sepsis (140). It is challenging to differentiate SIRS from sepsis due to the pre-existing hyperdynamic circulation in patients with cirrhosis and ACLF. Sepsis-3 criteria (rise of sofa score by 2 points) has been reported to be accurate in identifying sepsis in patients with cirrhosis. Furthermore, recent studies have suggested using fever and qSOFA scores to identify sepsis at the bedside (110, 141, 142). LT is the definitive therapy for ACLF. Early identification of those requiring LT or those who will have the resolution is the key to prolonging the survival of a patient with ACLF. Most hospitalized patients with ACLF have a clear prognosis between 3 and 7 days in either direction (143). Therefore, the concept of a transplant window period has been proposed by APASL and EASL (143, 144). Although early LT is associated with improved survival, such strategies are difficult in Asian settings where living donor liver transplantation is frequent, and the acceptance of LT is poor (145). In a large multination study of more than 1,000 patients who required LT, only 4% underwent LT (144).

9.7. Mechanisms of infections and organ failure

Damaged hepatocytes in liver diseases become dysfunctional and cause impaired protein synthesis, which leads to immune dysfunction. Disruption of gut homeostasis with altered gut

permeability increases the translocation of bacterial products, and persistent low-grade inflammation leads to non-response of the immune cells leading to immune exhaustion (146). Hepatocyte damage generates more DAMPs and PAMPs, which activate pattern recognition receptors and cytokine burst and hepatocyte death (147). OF results from simultaneous ongoing processes such as immune dysfunction, hemodynamic derangement, excessive CARS, and the exhaustion and dysfunction of critical innate and adaptive immune system cells. According to previous studies, one of the theories advocates both pro-inflammatory and anti-inflammatory responses occurring early and simultaneously, manifesting initially by an early, dominant, hyperinflammatory phase of fever, shock, and hypermetabolism, which then evolves over several days into a more protracted immunosuppressive late stage (148, 149). According to the second theory, there is an upregulation of genes of the innate immune response and a downregulation of genes of the adaptive immune response, leading to inflammation driven by the innate immune system with resultant organ dysfunction and failure (150).

9.8. Management options in ACLF

Nutritional rehabilitation is one of the cornerstones of the management of ACLF. A target of 1.5–2.0 g protein/kg per day and 35–40 kcal/kg per day with carbohydrate-predominant late-evening snacks is recommended for patients with advanced cirrhosis. Regular screening and clinical examination of patients with ACLF may help identify the infection and organ failures early. Antibiotics should be part of ACLF management irrespective of sepsis/SIRS status due to the high risk of infection-related complications, which can mimic liver failure. Albumin infusions can prevent organ dysfunction in patients with SBP. However, the evidence to support its use in non-SBP infections and ACLF is limited. Terlipressin and albumin have been demonstrated to be beneficial in patients with ACLF (151, 152). FDA has recently approved terlipressin for HRS-AKI but has restricted its use in patients with ACLF-grade 3 due to the risk of pulmonary overload and ischemic adverse events (152–154). Specific treatments are available as antiviral strategies in HBV reactivation, steroids for severe alcoholic hepatitis and AIH, withdrawal of offending drugs for DILI, and chelators and plasma exchange (PE) for Wilson's disease. PE has been shown to improve systemic inflammation and reduce OF development in ACLF (155). It offers significant survival benefits over other liver support systems and could be a preferred modality of liver support for ACLF patients. FMT is safe in a small study and was associated with improved short-term and medium-term survival of alcohol-related ACLF (156). LT has been shown to have excellent results in ACLF except in patients with high grades of respiratory or circulatory failure (157). The survival benefits of LT in ACLF have been shown convincingly in a large systematic review involving 22238 LT recipients, with worse outcomes only being reported in the subgroup of ACLF 3 when compared to 30791 non-ACLF recipients (158). The grade of ACLF on days 3–7 determines the outcomes of patients, and as such, patients, particularly those with advanced grades, merit early transplant consideration and listing of potentially viable candidates (143). However, such an early listing (<7 days) is impractical in resource-limited settings, while LT remains the therapy of choice as non-transplanted patients with ACLF have dismal survival of 8% at 1 year compared to 80% in those who undergo LT (159). Determination of timely access to LT facilities within the "window to transplant" is essential, beyond which LT is possibly a futile effort. Several areas need further research, including uniformity in definition and non-transplant measures to improve outcomes. Identifying futility is an important aspect of listing ACLF patients for LT. Some of the indicators of futility include patients with ≥4 organ failures, CLIF-C score > 64 at day 3–7, ACLF grade 2/3 patients with either active GI bleed, controlled sepsis for <24 h, high vasopressor support (3 mg/h), PaO2/FiO2 (P/F) ratio < 150, active drug abuse; infections by MDROs or invasive fungal infections, high cardiac risk, and significant comorbidities (143, 159–161).

9.9. Newer perspectives

9.9.1. Acute event and ACLF

Since the central concept of ACLF revolves around acute precipitation, the identification of acute precipitants is of key importance in the management of ACLF. There remain differences between the east and the west regarding the type of precipitants, with bacterial infections being the most common in the West while alcohol and hepatotropic viruses are common in Asia. In ∼2–16% of the patients, no precipitant is identified (162). In this context, there has been recent interest in the identification of uncommon precipitants like cytomegalovirus as potential acute precipitants in ACLF in the background of a state of immune dysfunction in ACLF with CMV positivity in up to 24% of the cases (163). Similarly, drug-induced liver injury has been more frequently recognized with a large cohort of 3,132 patients with ACLF, having DILI as the precipitating event in 10.5% out of which the most common were complementary and alternative medications (71.7%) (164). However, therapeutic treatment of DILI is elusive and serves as an important area for future research. Recently, coronavirus disease (COVID-19) has been added to the list of precipitants of ACLF, which can be modified by vaccination (165–169). Surgical interventions (hepatic and non-hepatic) have also been investigated as precipitants of ACLF, with 24.5% developing ACLF in a cohort of 369 patients, with potential determinants being advanced age, hyponatremia, baseline bacterial infection, and abdominal non-hepatic surgery (170). Patients undergoing TIPSS, if sarcopenic, are at an increased risk of developing ACLF and consequent increased risk of hepatic encephalopathy and mortality (171). Interestingly, surgical interventions in patients who already have ACLF has also been studied and propensity-matched against TIPSS, with elective surgery being an independent predictor of worse outcomes and a recommendation to avoid elective surgery in those with ACLF and CLIF-C AD score of ≥50 (172).

9.9.2. Sarcopenia and ACLF

The impact of sarcopenia as an independent predictor for mortality in patients with decompensated cirrhosis has been well-studied. The reported prevalence of sarcopenia in ACLF based on

CT skeletal muscle index is 55.6% but was not found to be an independent predictor of mortality after adjusting for inherent liver dysfunction (173). However, it is important to note that based on a preliminary retrospective analysis, sarcopenia appears to co-relate with the severity or grade of ACLF as well as is an important predictor of post-transplant 1-year survival (174). Use of novel bedside methods of sarcopenia assessment, like muscle ultrasound techniques in this critically ill cohort of ACLF, appears a promising research subject (175).

9.9.3. Therapeutics, transplantation, and ACLF

Even a modest volume of paracentesis (<5 L) is associated with an increased risk of PICD in patients with ACLF, wherein midodrine is comparable to albumin in preventing PICD (176). While prophylaxis with norfloxacin effectively prevents infection in recovering patients of ACLF, a combination of low-dose corticosteroids with low-volume PE has been shown to improve short-term survival in ACLF in a small trial (135, 177).

Identification of prognostic models for predicting outcomes for LT in ACLF, especially in those with the highest grade of ACLF, is the need of the hour. Mortality prediction systems are central to ACLF, with artificial intelligence-based models being shown to be better than standard prognostic scores (178). A simplified prognostic model comprising age, pretransplant arterial lactate, leucocyte count, and respiratory failure and referred to as the TAM model (transplantation for ACLF-3 model) has been proposed. The model classifies a cutoff at 2 points to distinguish between a high-risk group (score $>$ 2) and a low-risk group (score $\leq$ 2) with a 1-year survival of 8.3 vs. 83.9%, respectively (179). The score has been further validated to stress the importance of downstaging and stabilizing patients with ACLF before transplant, with those with a downstaged favorable TAM score having a significantly higher post-LT survival rate than those with static or incremental TAM score (88 vs. 70%) (180). Despite evolving data on the success of LT in ACLF, there remain variations and inequalities in both prioritization and access to LT in this subgroup which calls for increasing interdisciplinary interactions and awareness (181). Establishing a balance adjusting for the success of LT and resource utilization is imperative as LT in ACLF has also been shown to be highly resource-consuming with regard to healthcare use and costs (182).

9.9.4. Prevention of ACLF and recompensation in ACLF

The field of ACLF has seen rapid developments and a plethora of research in the recent past. On the preventive aspect, exposure to statins and a decrease in von Willebrand factor (after NSBB therapy) have been shown to prevent subsequent ACLF development (183, 184). Rifaximin, in a recent retrospective study, has been shown to reduce clinical complications and progression to ACLF in patients with severe AH (185). Sepsis is a common precipitant of ACLF through the LPS-TLR4 pathway (186). Recombinant alkaline phosphatase (recAP), may reduce the risk of organ dysfunction by dephosphorylating the endotoxins and containing hepatic TLR4 expression (186, 187). Resatorvid (TAK-242) is a small-molecule inhibitor of TLR4 and is being utilized for the prevention of organ failures. Yak-001, an orally administered, non-absorbable, synthetic microporous carbon, has a high adsorptive capacity for bacterial products, lipopolysaccharides, and pro-inflammatory cytokines. Yak-001 was found to be safe and effective in reducing endotoxemia and inflammatory mediators (188). DIALIVE, a novel liver dialysis device that replaces dysfunctional albumin and removes pathogen-associated and damage-associated molecular patterns, has been shown to improve outcomes in patients with ACLF (189, 190).

10. Conclusion

Early identification of the severity of PH and addressing downstream complications is central to the management of cirrhosis. Each complication merits detailed redressal, and overall management demands a holistic approach. ACLF needs to be identified early in the course with the institution of specific therapies. Newer modalities such as plasmapheresis and FMT have promising results. LT remains the definitive care in both advanced cirrhosis and ACLF.

Author contributions

AK and RJ made the study design and concept. RJ, AK, and AR prepared the initial draft. Figures by MP and KK. MS, PR, and DR provided the technical support. AK and MP critically reviewed and edited the final manuscript. All authors approved the final 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

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