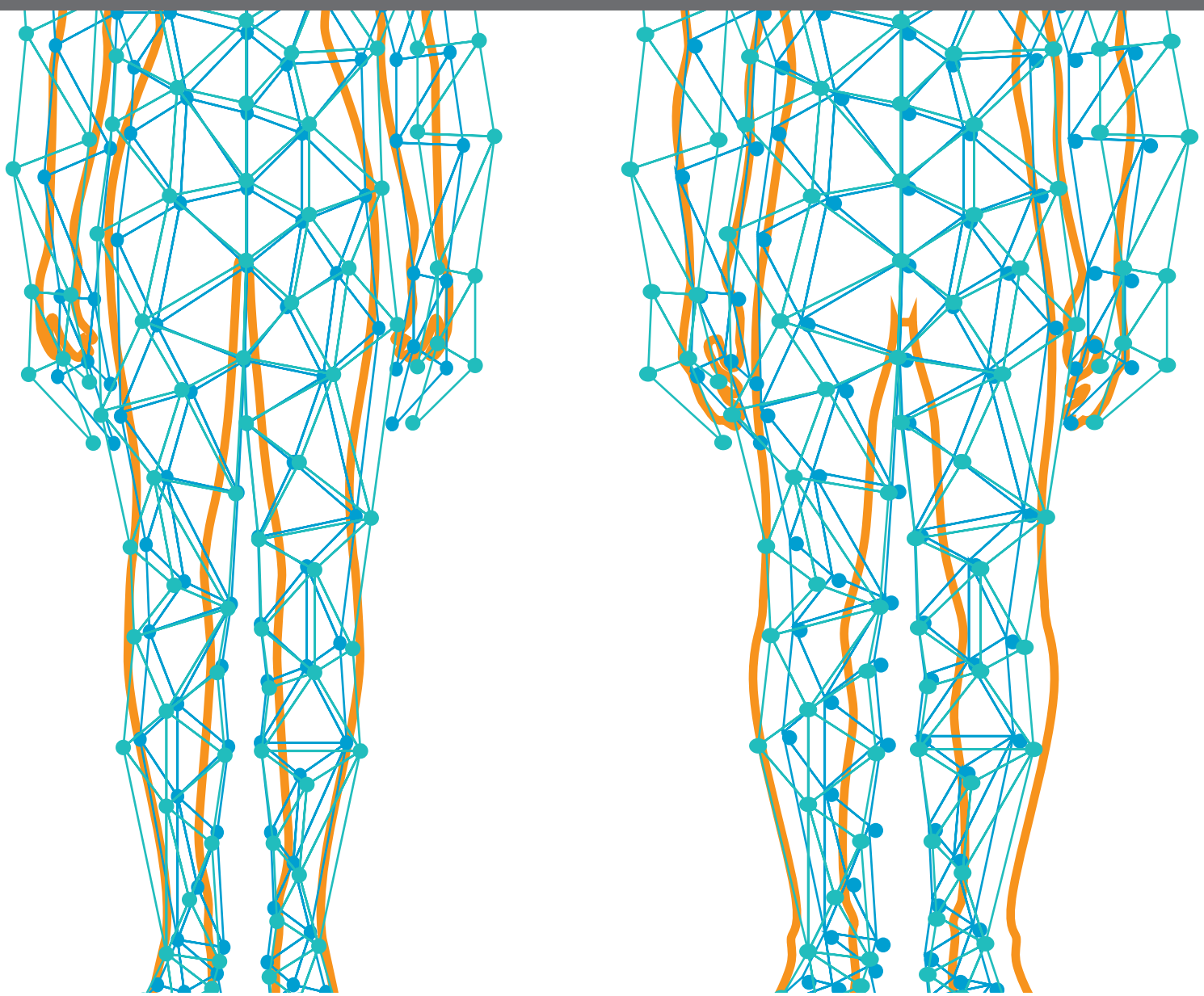
ACUTE-ON-CHRONIC LIVER FAILURE: NATURAL HISTORY, MECHANISM, AND TREATMENT

EDITED BY: Yu Shi, Yu-Chen Fan, Tao Chen and Cornelius Engelmann

PUBLISHED IN: Frontiers in Medicine



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88974-830-3
DOI 10.3389/978-2-88974-830-3

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

ACUTE-ON-CHRONIC LIVER FAILURE: NATURAL HISTORY, MECHANISM, AND TREATMENT

Topic Editors:
Yu Shi, Zhejiang University, China
Yu-Chen Fan, Shandong University, China
Tao Chen, Huazhong University of Science and Technology, China
Cornelius Engelmann, Charité Universitätsmedizin Berlin, Germany

Citation: Shi, Y., Fan, Y.-C., Chen, T., Engelmann, C., eds. (2022). Acute-On-Chronic Liver Failure: Natural History, Mechanism, And Treatment. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88974-830-3

Table of Contents

frontiers in Medicine

EDITORIAL
published: 11 March 2022
doi: 10.3389/fmed.2022.867436

Editorial: Acute-On-Chronic Liver Failure: Natural History, Mechanism, and Treatment

Yu Shi[1]*, *Yuchen Fan*[2] *and Tao Chen*[3]

[1] State Key Laboratory for Diagnosis and Treatment of Infectious, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Disease, National Clinical Research Center for Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, [2] Department of Hepatology, Qilu Hospital of Shandong University, Jinan, China, [3] Department and Institute of Infectious Disease, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Keywords: acute-on chronic liver failure, natural history, mechanism, prognosis, diagnostic criteria

Editorial on the Research Topic

Acute-On-Chronic Liver Failure: Natural History, Mechanism, and Treatment

Acute-chronic liver failure (ACLF) is a lethal syndrome due to the acute exacerbation of underlying chronic liver diseases (CLDs), which is characterized by multi-organ failure with high short-term mortality. ACLF is one of the three leading causes of death in patients with chronic liver diseases. Although the concept of ACLF was proposed on 1995 (1), the study of ACLF has undergone explosive growth since the development of the EASL-CLIF criteria in 2013 (2). However, the controversy persists, particularly in the diagnostic criteria of ACLF, which mainly arises from the different backgrounds of underlying CLDs in the East and the West (3). In the West, alcoholic cirrhosis and bacterial infection constitute the major types of underlying CLDs and acute precipitating events in ACLF patients (4). Extrahepatic organ failures are frequent, manifesting in the first organ involved. In contrast, hepatitis B virus (HBV)-related ACLF is most common in China (5). Patients with HBV-ACLF usually have compensated liver cirrhosis or advanced CLDs. Liver and coagulation failure usually come first, followed by extrahepatic organ failure (6, 7). The proposed EASL-CLIF criteria is enlarging the interest in Eastern ACLF, leading to a plethora of publications. Therefore, the appearance of this special collection is very timely.

The first topic of the special collection is about ACLF's diagnostic criteria which is a fundamental issue and a focus of controversy in this research field. One of the critical questions is the threshold of organ failure for ACLF diagnosis, which varies among different criteria. The APASL consensus applies a serum bilirubin level $\geq$ 5 mg/dL and INR $\geq$ 1.5 to define severe liver dysfunction in patients with underlying CLDs (8). A higher threshold value of serum bilirubin $\geq$ 12 mg/dL and INR $\geq$ 2.5 is utilized by EASL-CLIF criteria to define liver and coagulation failure in patients with de-compensated cirrhosis (2). And a serum bilirubin $\geq$ 12 mg/dL and INR $\geq$ 1.5 is used to define ACLF in patients with HBV-CLDs by the COSSH criteria (6). In our special collection, two articles investigated the relationship between the continuous changes of serum bilirubin and INR, and 90-day mortality in patients with CLDs and those hospitalized for acute decompensation (AD) or acute liver injury. Qiao et al. found that a significant correlation between bilirubin and death began to be evident only when serum bilirubin exceeded 12 mg/dl in patients without cirrhosis, while any increase in bilirubin in patients with cirrhosis was associated with risk of death. Wang Y. et al. found that an INR of 1.6/1.7 was the starting point of coagulation dysfunction with a rapid increase in mortality in patients with cirrhosis or with advanced fibrosis. And a 28-day LT-free

OPEN ACCESS

Edited and reviewed by:
Angel Lanas,
University of Zaragoza, Spain

***Correspondence:**
Yu Shi
zjushiyu@zju.edu.cn

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *01 February 2022*
Accepted: *07 February 2022*
Published: *11 March 2022*

Citation:
Shi Y, Fan Y and Chen T (2022) Editorial: Acute-On-Chronic Liver Failure: Natural History, Mechanism, and Treatment. Front. Med. 9:867436. doi: 10.3389/fmed.2022.867436

mortality of 15% was associated with an INR value of 2.1. In another study, Long et al. found that hepatic encephalopathy grades 3–4 were an independent risk factor for 28- and 90-day adverse outcomes in patients with acute-on-chronic liver diseases, and therefore could define brain failure in ACLF, regardless of the presence of cirrhosis. These three studies provide evidence for defining the threshold of organ failure for ACLF diagnosis and highlight that non-cirrhotic and cirrhotic ACLF may differ in the threshold of liver failure but share that of extrahepatic organ failure. On the other hand, in another article, Chen Y.-y. et al. extended the APASL criteria to patients with decompensated liver cirrhosis and identified a novel group of ACLF patients with short-term mortality similar to non-cirrhotic ACLF but higher long-term mortality (Xu et al.). Taken together, a basic question is raised by the four studies: do we require a unified diagnostic criteria or different criteria to identify patients with CLDs and high death risk?

ACLF is a lethal and highly dynamic disease. Liver transplantation can improve patient survival but is limited by the shortage of donor organs. The lack of other definite therapies puts the prediction of patients' prognosis in a very important position. Therefore, another Research Topic of this special collection is to develop a clinically useful tool to accurately predict the outcome of ACLF. Three articles reported their HBV-specific clinical prediction models, each was developed by multi-center data and verified by an external cohort with good discrimination and calibration (Chen J.-f. et al.; Yu et al.; Zhe-bin et al.). Importantly, a nomogram and online calculation tool was developed for the convenience of clinical use. Other articles explored the use of biomarkers, including testosterone and estradiol (Sun, Xu et al.), CD200R (Li Y. et al.), growth hormone (Wu D. et al.), neutrophil-to-lymphocyte ratio (NLR) (Liu et al.), and lymphocyte subsets (Li J. et al.), which may not only add to the performance of clinical prediction models, but also uncover some systemic features of ACLF in immunopathology and the endocrine system, and thereby provide clues for elucidating the pathophysiological mechanism. Another article developed a weighted score to predict the risk of invasive pulmonary aspergillosis (IPA), which is a lethal complication in patients with liver failure (Zhang et al.). However, all these clinical prediction scores and biomarkers should be tested further for their clinical utility in large cohorts and a real-world setting.

The third topic is to test novel therapies for this critical disease. One article reported encouraging results of a non-bioartificial liver support system (NB-ALSS) in treating patients with HBV-ACLF (Li J. et al.). The large, multi-center study enrolled 524 patients with HBV-ACLF and used paired analysis by propensity score matching to show that patients who received plasma exchange (PE)-based NB-ALSS had higher short-term and mid-term survival than those with standard medical therapy (SMT). Interestingly, further subgroup analysis revealed that PE-based NB-ALSS had the best efficacy in patients with ACLF grade 2 or a high MELD score of 30–40. The positive findings of NB-ALSS were in contrast with the futility of Western ACLF, despite of difference in devices. Another article performed a meta-analysis of the present literature on the use of granulocyte colony-stimulating factor (G-CSF) and demonstrated that G-CSF was not effective in the overall ACLF population. However, the subgroup analysis showed improved survival in Asian studies (Hou et al.). Although the findings of both articles are preliminary, a difference in pathophysiological mechanisms between Eastern and Western-defined ACLF can be speculated. For instance, there has been evidence that damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), while both involved in the development of systemic inflammation in ACLF, have differential contributions to the systemic innate immune derangement (9). Another important insight by the two articles lies in the potential presence of "efficacy threshold" and "treatment window" in treating ACLF, which indicates that risk stratification is critical in designing randomized controlled trials to test the efficacy of novel devices or drugs in the future. In addition to NB-ALSS, one article reported preliminary data of the efficacy of N-acetylcysteine in the treatment of HBV-ACLF, and its improvement of intrahepatic cholestasis and coagulation dysfunction of HBV-ACLF provides a new possibility for the treatment of HBV-ACLF (Wang M.-L. et al.). Another article studied the safety concern of the proton pump inhibitor (PPI) in ACLF (Sun, Ye et al.). The risk of PPI use in de-compensated cirrhosis has been debated, including the impact on risk of hepatic encephalopathy (HE), spontaneous bacterial peritonitis (SBP), and mortality. The article found that PPI use does not appear to increase mortality or the risk of HE and SBP in hospitalized de-compensated cirrhosis patients with and without ACLF.

The Research Topic also collected two up-to-date literature reviews. One summarizes the progress of systemic inflammation and immune cell paralysis and immunosuppression. The authors pointed out that the interaction between systemic inflammation and immunosuppression needs to be clarified. The review also provides insight into the specific precipitants of ACLF including HBV reactivation, extensive alcoholism, hepatitis E virus infection and flare-up of autoimmune hepatitis, complications including bleeding, hepatic encephalopathy (HE) and infections, and treatment options, in particular emerging stem-cell based therapies (Wu J. et al.). While the other review narrows on hepatitis E virus-related ACLF, mainly on immunological manifestations and mechanisms (Khanam and Kottilil).

Considering that ACLF is a highly complex syndrome in clinical phenotype and pathophysiological mechanisms, it is an apparent huge challenge to modify the clinical trajectory of this critical disease. Fortunately, broad interests on ACLF have been spiked both in the Eastern and Western scientific communities and many efforts have been put forward to establish diagnostic criteria, characterize natural history, and develop prediction tools and novel therapies. This Research Topic seeks to collect articles or reviews that provide either insights into the understanding of the disease or translational potential for better clinical care. Finally, we hope you enjoy reading this collection.

AUTHOR CONTRIBUTIONS

YS, YF, and TC conceived the idea. YS wrote the draft. YF and TC reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Ohnishi H, Sugihara J, Moriwaki H, Muto Y. [Acute-on-chronic liver failure]. *Ryoikibetsu Shokogun Shirizu.* (1995) 7:217–9.
2. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology.* (2013) 144:1426–37.e1429. doi: 10.1053/j.gastro.2013.02.042
3. Bajaj JS. Defining acute-on-chronic liver failure: will east and west ever meet? *Gastroenterology.* (2013) 144:1337–9. doi: 10.1053/j.gastro.2013.04.024
4. Arroyo V, Moreau R, Jalan R. Acute-on-chronic liver failure. *N Engl J Med.* (2020) 382:2137–45. doi: 10.1056/NEJMra1914900
5. Shi Y, Yang Y, Hu Y, Wu W, Yang Q, Zheng M, et al. Acute-on-chronic liver failure precipitated by hepatic injury is distinct from that precipitated by extrahepatic insults. *Hepatology.* (2015) 62:232–42. doi: 10.1002/hep.27795
6. Wu T, Li J, Shao L, Xin J, Jiang L, Zhou Q, et al. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure. *Gut.* (2018) 67:2181–91. doi: 10.1136/gutjnl-2017-314641
7. Li H, Chen LY, Zhang NN, Li ST, Zeng B, Pavesi M, et al. Characteristics, diagnosis and prognosis of acute-on-chronic liver failure in cirrhosis associated to hepatitis B. *Sci Rep.* (2016) 6:25487. doi: 10.1038/srep 25487
8. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. *Hepatol Int.* (2019) 13:353–90. doi: 10.1007/s12072-019-09946-3
9. Eppensteiner J, Kwun J, Scheuermann U, Barbas A, Limkakeng AT, Kuchibhatla M, et al. Damage- and pathogen-associated molecular patterns play differential roles in late mortality after critical illness. *JCI Insight.* (2019) 4:e127925. doi: 10.1172/jci.insight.127925

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.

frontiers in Medicine

ORIGINAL RESEARCH
published: 16 June 2021
doi: 10.3389/fmed.2021.692669

Derivation and Validation of a Nomogram for Predicting 90-Day Survival in Patients With HBV-Related Acute-on-Chronic Liver Failure

Jun-feng Chen[1†], Wei-zhen Weng[1†], Miao Huang[2], Xiao-hua Peng[3], Jian-rong He[4], Jing Zhang[1], Jing Xiong[1], Shao-quan Zhang[1], Hui-juan Cao[1], Bin Gao[1], Deng-na Lin[1], Juan Gao[1], Zhi-liang Gao[1,5,6] and Bing-liang Lin[1,5,6]*

[1] Department of Infectious Diseases, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, [2] Department of Nursing, Guangzhou Red Cross Hospital, Fourth Affiliated Hospital of Jinan University, Guangzhou, China, [3] Department of Gastroenterology, Seventh Affiliated Hospital of Sun Yat-sen University, Shenzhen, China, [4] Department of Obstetrics and Gynecology, Green Templeton College, University of Oxford, London, United Kingdom, [5] Guangdong Provincial Key Laboratory of Liver Disease, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, [6] Key Laboratory of Tropical Disease Control (Sun Yat-sen University), Ministry of Education, Guangzhou, Guangdong, China

OPEN ACCESS

Edited by:
Yu-Chen Fan,
Qilu Hospital, Shandong University, China

Reviewed by:
Hai Li,
Shanghai Jiao Tong University, China
Demin Yu,
Shanghai Jiao Tong University, China

***Correspondence:**
Bing-liang Lin
linbingl@mail.sysu.edu.cn

[†] These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 08 April 2021
Accepted: 17 May 2021
Published: 16 June 2021

Citation:
Chen J-f, Weng W-z, Huang M, Peng X-h, He J-r, Zhang J, Xiong J, Zhang S-q, Cao H-j, Gao B, Lin D-n, Gao J, Gao Z-l and Lin B-l (2021) Derivation and Validation of a Nomogram for Predicting 90-Day Survival in Patients With HBV-Related Acute-on-Chronic Liver Failure. Front. Med. 8:692669. doi: 10.3389/fmed.2021.692669

Background: Conventional prognostic models do not fully reflect the severity of hepatitis B virus (HBV)-related acute-on-chronic liver failure (ACLF). This study aimed to establish an effective and convenient nomogram for patients with HBV-related ACLF.

Methods: A nomogram was developed based on a retrospective cohort of 1,353 patients treated at the Third Affiliated Hospital of Sun Yat-sen University from January 2010 to June 2016. The predictive accuracy and discriminatory ability of the nomogram were determined by a concordance index (C-index) and calibration curve, and were compared with current scoring systems. The results were validated using an independent retrospective cohort of 669 patients consecutively treated at the same institution from July 2016 to March 2018. This study is registered at ClinicalTrials.gov (NCT03992898).

Results: Multivariable analysis of the derivation cohort found that independent predictors of 90-day survival were age, white blood cell (WBC) count, hemoglobin (Hb), aspartate aminotransferase (AST), total bilirubin (TBil), international normalized ratio, serum creatinine (Cr), alpha fetoprotein (AFP), serum sodium (Na), hepatic encephalopathy (HE), pre-existing chronic liver disease(PreLD), and HBV DNA load. All factors were included in the nomogram. The nomogram calibration curve for the probability of 90-day survival indicated that nomogram-based predictions were in good agreement with actual observations. The C-index of the nomogram was 0.790, which was statistically significantly greater than those for the current scoring systems in the derivation cohort ($P < 0.001$). The results were confirmed in the validation cohort.

Conclusions: The proposed nomogram is more accurate in predicting the 90-day survival of patients with HBV-related ACLF than current commonly used methods.

Keywords: hepatitis B virus, acute-on-chronic liver failure, nomogram, prognosis, MELD score, CLIF-C ACLF score, COSSH score, CLIF-C OF score

INTRODUCTION

Acute-on-chronic liver failure (ACLF) is the acute deterioration of liver function in patients with chronic liver diseases, and the condition progresses rapidly with a mortality rate of more than 50% (1). Liver transplantation is the only treatment that has been proven beneficial for ACLF (2). However, the number of liver donors is limited. As such, development of a simple and accurate prognostic method is necessary to that liver transplantation can be performed on patients with the greatest needed.

Model for end-stage liver disease (MELD) score is commonly used to evaluate patients with ACLF for transplantation (3). However, MELD score only takes into account total bilirubin (TBil), international normalize ratio (INR), and serum creatinine (Cr). Other important valuables such as age, hepatic encephalopathy (HE), and indexes of infection are not included, even though these indexes had been proven to be important for predicting outcomes (4, 5). Furthermore, studies have shown that MELD score does not fully reflect the severity of liver failure (6).

In recent years, the European Association for the Study of the Liver (EASL)-Chronic Liver Failure (CLIF) Consortium Organ Failure (CLIF-C OF) score and CLIF-C ACLF score have been proposed to determine the prognosis of ACLF patients in the West (4, 7). However, the CLIF-C OF score and CLIF-C ACLF score might not be the most appropriate models for predicting the outcomes of Eastern ACLF patients. First, Eastern and Western definitions of ACLF are different. In the West, ACLF is defined as the acute deterioration of patients with cirrhosis (7), while in the East ACLF is considered to develop in both chronic hepatitis and cirrhosis patients (8). In addition, cirrhosis has been confirmed to be an independent risk factor for ACLF (9). Second, most cases of ACLF in the West are associated with alcoholic hepatitis and bacterial infection (7), while in the East most are associated with reaction of hepatitis B virus (HBV) and alcohol abuse (8, 10).

In 2017, Li et al. (5) reported a new prognostic scoring system (Chinese Group on the study of Severe Hepatitis B, COSSH ACLF score) specifically for HBV-ACLF, and the method was superior to MELD, Model for end-stage liver disease with the addition of the Na level (MELD-Na), Child-Turcotte-Pugh (CTP), CLIF-C OF, and CLIF-C ACLF scores. However, the COSSH ACLF score system only includes the INR, TBil, age, Cr, HE, mean arterial pressure, and respiratory function. It is difficult to predict the complex progress of HBV-ACLF comprehensively, meanwhile, it is not convenient to use in clinical practice because of the need for complicated calculations. Thus, it would be necessary to establish an effective and more convenient prognostic scoring system for HBV-ACLF patients.

Nomograms are graphical depictions of predictive statistical models, and have advantages over traditional scoring systems with respect to predicting outcomes (11). Nomograms have been developed and used for various diseases, including liver failure (12, 13). They have also been developed to guide treatment allocation for critical diseases (14).

Thus, the purpose of this study was to develop a nomogram using clinical and laboratory factors to predict 90-day survival in patients with HBV-ACLF, and to compare the predictive value with traditional scoring methods.

MATERIALS AND METHODS

Study Design and Participants

This was a retrospective cohort study performed at a single center in southern China. Consecutive HBV-ACLF patients treated at the Third Affiliated Hospital of Sun Yat-sen University from January 2010 to June 2016 were included for model development (derivation cohort; $n = 1{,}353$). An second, independent cohort of HBV-ACLF patients with the same inclusion and exclusion criteria treated at the same institution from July 2016 to March 2018 were included to validate the model (validation cohort; $n = 669$).

Inclusion criteria for the study were based on the consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) 2014 (10), and Diagnostic and Treatment Guidelines for Liver Failure in China (15). The study inclusion criteria were: (1) ACLF, characterized by acute hepatic deterioration manifesting as jaundice (TBil $\geq 10\times$ the upper limit of normal, in micromoles per liter) and coagulopathy (INR $\geq$ 1.5, or prothrombin activity $<$ 40%), complicated within 4 weeks by ascites and/or encephalopathy in patients with previously diagnosed or undiagnosed chronic liver disease; (2) Positive serum HBV surface antigen (HBsAg) for more than 6 months; (3) Age $>$ 18 years. Exclusion criteria were: (1) Systemic or local malignancy; (2) HIV infection or other immunodeficiency disease; (3) hepatitis C virus (HCV) infection; (4) Marked organ dysfunction (e.g., renal dysfunction) not related to liver disease (detailed definitions are presented in the **Supplementary Material**); (5) Pregnancy or lactation; (6) Incomplete data or lost to follow-up; (7) Hospital stay $<$ 1 day.

All patients received comprehensive medical treatment as required, including nutritional supplementation, administration of human serum albumin (ALB), and appropriate treatment for complications such as infections (e.g., respiratory tract, urinary tract, biliary tract, digestive tract, and spontaneous peritonitis), HE, gastrointestinal bleeding (GB), and hepatorenal syndrome (HRS).

This study conformed strictly to the Ethical Guidelines of the 1975 Declaration of Helsinki. The study protocol was approved by the Ethics Committee on Clinical Trials of the Third Affiliated Hospital of Sun Yat-sen University. Due to the retrospective nature of the study, informed consent was waived. This study is registered at ClinicalTrials.gov (NCT03992898).

Follow-Up and Outcome Measures

Patients were followed up for 90 days, until death, or until liver transplantation. Survival and transplantation data were collected from medical records or by contacting the patient or their family members. The study endpoint was 90-day transplantation-free survival.

Potential Predictors

After enrollment, demographic and clinical and laboratory data were collected using the hospital information system. Data

collected were patient sex and age, precipitating event(s), blood pressure (BP), and pulse oximetry data. Laboratory data included white blood cell (WBC) count, hemoglobin (Hb) level, platelet (PLT) count, serum ALB, globulin, serum sodium (Na), alanine aminotransferase (ALT), aspartate aminotransferase (AST), TBil, INR, serum Cr, alpha fetoprotein (AFP), HBsAg, HBV e antigen (HBeAg), HBV e antibody (HBeAb), and HBV DNA. Antiviral treatments for HBV (nucleoside analogs, including lamivudine, adefovir, entecavir, telbivudine, and tenofovir) within 6 months prior to and during hospitalization were recorded. Complications of ACLF examined included ascites, HE, HRS, GB, and infection. Pre-existing chronic liver diseases (PreLD), including hepatitis and cirrhosis (detailed definitions are presented in the **Supplementary Material**), were recorded.

Model Derivation

Continuous variables were transformed into categorical variables based on clinical, routine cutoff points. The cutoff points for AFP and HBV DNA were based on quartile and median, respectively. Survival curves were produced using the Kaplan-Meier method, and compared by the log-rank test. Risk factors associated with 90-day transplantation-free survival were examined in the derivation cohort using Cox proportional hazards models. Variables with values of $P < 0.05$ in the univariate Cox regression analysis included in the multivariate analysis using backward stepwise selection (Entry: 0.05, Removal: 0.1). Data were presented with hazard ratios (HR) and 95% confidence intervals (CIs).

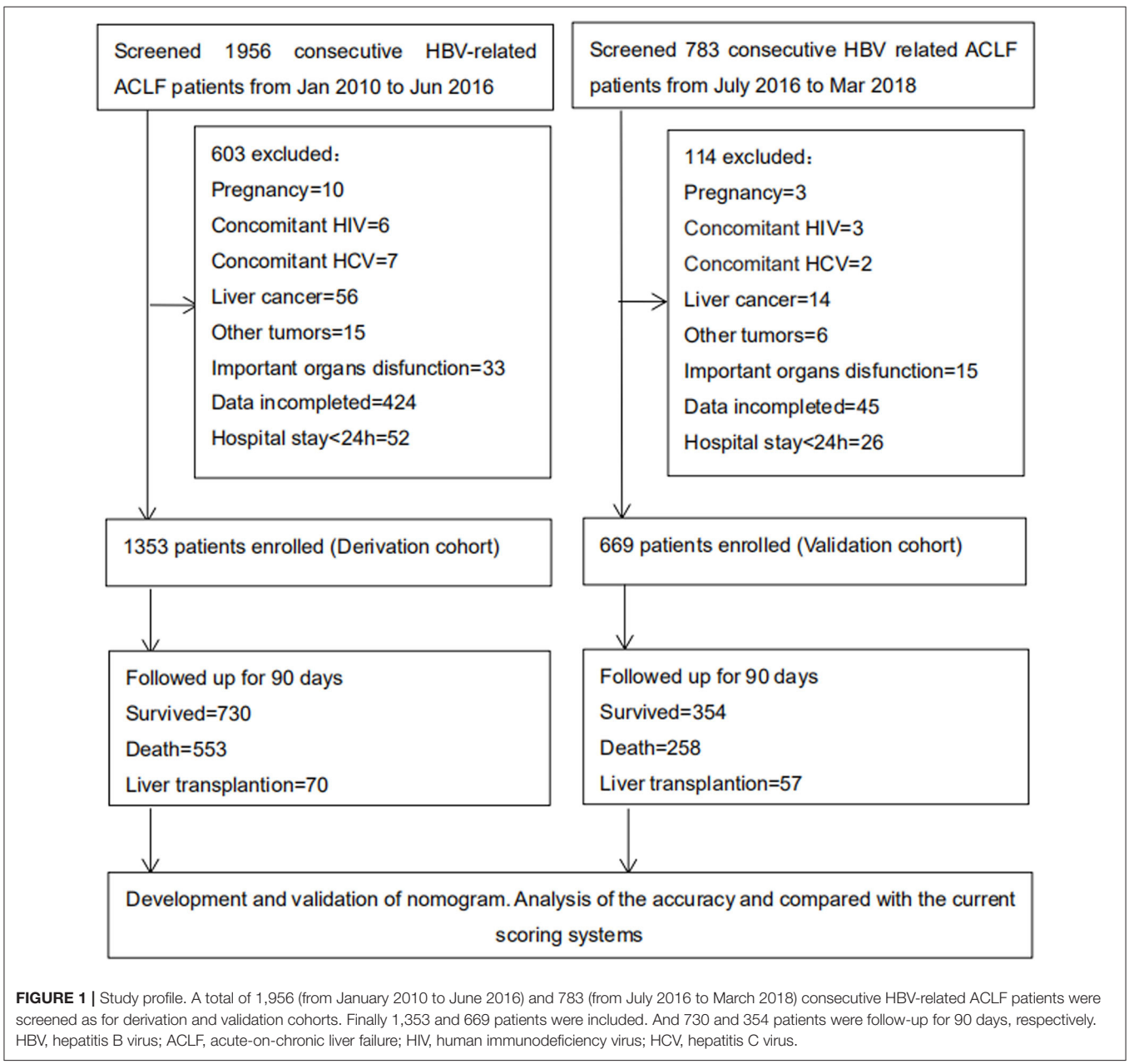


FIGURE 1 | Study profile. A total of 1,956 (from January 2010 to June 2016) and 783 (from July 2016 to March 2018) consecutive HBV-related ACLF patients were screened as for derivation and validation cohorts. Finally 1,353 and 669 patients were included. And 730 and 354 patients were follow-up for 90 days, respectively. HBV, hepatitis B virus; ACLF, acute-on-chronic liver failure; HIV, human immunodeficiency virus; HCV, hepatitis C virus.

TABLE 1 | Patient demographics and clinical characteristics†.

Characteristic	Derivation cohort (n = 1,353) (No. %)	Validation cohort (n = 669) (No. %)	P-value
Age, years			<0.001
18–29	132(9.8)	52(7.8)	
30–39	365(27.0)	141(21.1)	
40–49	399(29.5)	191(28.6)	
50–59	283(20.9)	161(24.1)	
≥60	174(12.9)	124(18.5)	
Sex			0.009
Male	1,221(90.2)	578(86.4)	
Female	132(9.8)	91(13.6)	
WBC, 10^9/L			0.587
<4	94(6.9)	55(8.2)	
4–10	950(70.2)	464(69.4)	
>10	309(22.8)	150(22.4)	
Hb, g/L			0.235
Male: <120, Female: <110	569(42.1)	308(46.0)	
Male:120–160, Female:110–150	764(56.5)	352(52.6)	
Male: >160, Female: >150	20(1.5)	9(1.3)	
PLT, 10^9/L			0.002
<100	521(38.5)	294(43.9)	
100–300	820(60.6)	360(53.8)	
>300	12(0.9)	15(2.2)	
ALT, U/L			0.520
<200	522(38.6)	269(40.2)	
200–799	452(33.4)	229(34.2)	
≥800	379(28.0)	171(25.6)	
AST, U/L			0.856
<200	607(44.9)	295(44.1)	
200–799	537(39.7)	274(41.0)	
≥800	209(15.4)	100(14.9)	
ALB, g/L			0.003
<28	200(14.8)	77(11.5)	
28–34.9	799(59.1)	371(55.5)	
≥35	354(26.2)	221(33.0)	
TBil, μmol/L			0.003
171–256.5	256(18.9)	147(22.0)	
256.6–342.0	287(21.2)	171(25.6)	
342.1–427.5	281(20.8)	149(22.3)	
427.6–513.0	242(17.9)	93(13.9)	
>513.0	287(21.2)	109(16.3)	
INR			0.365
1.5–1.99	372(27.5)	188(28.1)	
2.0–2.49	365(27.0)	194(29.0)	
2.5–2.99	233(17.2)	122(18.2)	
≥3.0	383(28.3)	165(24.7)	
Na, mmol/L			0.002
<135	416(30.7)	170(25.4)	
135–145	929(68.7)	487(72.8)	
>145	8(0.6)	12(1.8)	
Cr, μmol/L			0.805
<1ULN	1,248(92.2)	611(91.3)	
1–1.49ULN	67(5.0)	34(5.1)	
1.5–1.99ULN	20(1.5)	12(1.8)	
≥2.0ULN	18(1.3)	12(1.8)	
AFP, ng/ml			0.237
<15.19	338(25.0)	157(23.5)	
15.19–50.12	338(25.0)	180(26.9)	
50.13–149.83	339(25.1)	186(27.8)	
>149.83	338(25.0)	146(21.8)	
HBeAg			0.193
Positive	437(32.3)	197(29.4)	
Negative	916(67.7)	472(70.6)	
HBV DNA, IU/ml			0.289
<149,000	678(50.1)	352(52.6)	
≥149,000	675(49.9)	317(47.4)	
Pre-existing chronic liver diseases			0.011
Chronic hepatitis	575(42.5)	245(36.6)	
Cirrhosis	778(57.5)	424(63.4)	
Alcoholic liver disease			0.992
Yes	105(7.8)	52(7.8)	
No	1,248(92.2)	617(92.2)	
Potential precipitating events			<0.001
Bacterial infection	53(3.9)	38(5.7)	
Re-activation or flare of HBV	653(48.3)	394(58.9)	
Super-infection of hepatitis E virus	90(6.7)	43(6.4)	
Hyperthyroidism	27(2.0)	9(1.3)	
Hepatotoxic drugs	66(4.9)	41(6.1)	
Alcohol	73(5.4)	34(5.1)	
Unknown	391(28.9)	110(16.4)	
Hepatorenal syndrome			0.217
Yes	51(3.8)	33(4.9)	
No	1,302(96.2)	636(95.1)	
Hepatic encephalopathy			0.182
None	1,076(79.5)	555(83.0)	
Grade 1–2	234(17.3)	97(14.5)	
Grade 3–4	43(3.2)	17(2.5)	
Gastrointestinal bleeding			0.176
Yes	16(1.2)	13(1.9)	
No	1,337(98.8)	656(98.1)	
Infection			0.456
Yes	986(72.9)	477(71.3)	
No	367(27.1)	192(28.7)	
MELD score			0.054
<20	97(7.2)	68(10.2)	
20–30	928(68.6)	452(67.6)	
>30	328(24.2)	148(22.1)	

(Continued)

TABLE 1 | Continued

Characteristic	Derivation cohort (n = 1,353) (No. %)	Validation cohort (n = 669) (No. %)	*P*-value
MELD-Na score			0.023
<20	81(6.0)	56(8.4)	
20–30	818(60.5)	421(62.9)	
>30	454(33.6)	192(28.7)	
CTP score			0.021
5–6	0(0)	0(0)	
7–9	245(18.1)	150(22.4)	
10–15	1,108(81.9)	519(77.6)	
Antivirus drug			<0.001
None	125(9.2)	39(5.8)	
LAM	59(4.4)	4(0.6)	
ADV	7(0.5)	0(0.0)	
ETV	1,052(77.8)	556(83.1)	
TDF	26(1.9)	46(6.9)	
Ldt	27(2.0)	3(0.4)	
Combination therapy	57(4.2)	21(3.1)	

†Clinical and biochemical data were expressed as No. (%).
‡Alcohol liver disease is defined according to the guideline of prevention and treatment for alcoholic liver disease (2018, China) (18).
WBC, white blood cell; Hb, hemoglobin; PLT, platelet; ALT, alanine aminotransferase; AST, glutamic-oxaloacetic transaminase; ALB, albumin; TBil, total bilirubin; INR, international normalized ratio; Na, serum sodiun; Cr, serum creatinine; AFP, alpha fetal protein; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; MELD, model for end-stage liver disease; MELD-Na, Model for End-Stage Liver Disease with the addition of the Na level; CTP, Child-Turcotte-Pugh; LAM, lamivudine; ADV, adefovir dipivoxil; ETV, entecavir; TDF, tenofovir; Ldt, telbivudine.

A nomogram (HBV-ACLF nomogram) was formulated based on the results of multivariable Cox regression analyses. The performance of the HBV-ACLF nomogram was evaluated by Harrell's concordance index (C-index), and assessed by comparing nomogram-predicted vs. observed Kaplan-Meier estimates of survival probability; bootstraps with 1,000 resamples were applied to these analyses.

Model Validation

The total points of each patient in the validation cohort were calculated according to the established HBV-ACLF nomogram, and then Cox regression in this cohort was performed using the total points as a factor. Finally, the C-index and calibration curve were derived based on the regression analyses.

External Validation of Current Scoring Systems

Comparisons between the HBV-ACLF nomogram and MELD score, MELD-Na score, and CTP score were performed in the derivation and validation cohorts, and with CLIF-C OF, CLIF-C ACLF, and COSSH ACLF scores in the validation cohort. Comparisons were performed with the rcorrp.cens function in the Hmisc package in R software (16). Comparisons were tested using the C-index: a greater C-index indicates more accurate prognostic stratification.

TABLE 2 | Multivariable analysis of the derivation cohort†.

	90 Days' survival		
Variable	HR	95%CI	*P* value
Age, years			<0.001
18–29	Reference		
30–39	1.60	1.04–2.45	0.031
40–49	1.72	1.33–2.24	<0.001
50–59	1.81	1.44–2.28	<0.001
≥60	2.58	2.05–3.25	<0.001
WBC, 10^9/L			0.007
<4	Reference		
4–10	0.78	0.57–1.08	0.134
>10	1.18	0.93–1.50	0.174
Hb, g/L			0.007
Male: <120, Female: <110	Reference		
Male:120–160, Female:110–150	1.31	1.08–1.59	0.006
Male: >160, Female: >150	1.74	0.93–3.25	0.081
AST, U/L			<0.001
<200	Reference		
200–799	1.36	1.11–1.67	0.003
≥800	1.75	1.38–2.22	<0.001
TBil, μmol/L			<0.001
171–256.5	Reference		
256.6–342	1.35	1.01–1.81	0.046
342.1–427.5	1.44	1.13–1.83	0.003
427.6–513	1.88	1.50–2.36	<0.001
>513	1.70	1.39–2.07	<0.001
INR			<0.001
1.5–1.99	Reference		
2.0–2.49	1.15	0.88–1.51	0.316
2.5–2.99	1.97	1.57–2.48	<0.001
≥3.0	2.41	2.02–2.89	<0.001
Na, mmol/L			0.052
<135	Reference		
135–145	0.81	0.68–0.98	0.026
>145	1.44	0.66–3.15	0.360
Cr, μmol/L			<0.001
<1ULN	Reference		
1–1.49ULN	0.98	0.87–1.65	0.899
1.5–1.99ULN	1.84	1.32–3.63	0.027
≥2.0ULN	4.65	2.00–5.72	<0.001
AFP, ng/ml			<0.001
<15.19	Reference		
15.19–50.12	0.87	0.70–1.08	0.198
50.13–149.83	0.68	0.55–0.83	<0.001
>149.83	0.48	0.38–0.62	<0.001
HBV DNA, IU/ml			0.005
<149,000	Reference		
≥149,000	1.32	1.09–1.60	0.005
Pre-existing chronic liver diseases			0.001

(Continued)

TABLE 2 | Continued

Variable	90 Days' survival		
	HR	95%CI	*P* value
Chronic hepatitis	Reference		
Cirrhosis	1.38	1.13–1.69	0.001
Hepatic encephalopathy			<0.001
None	Reference		
Grade 1–2	1.58	1.29–1.95	<0.001
Grade 3–4	2.82	1.90–4.18	<0.001

CI, confidence interval; HR, hazard ratio; WBC, white blood cell; Hb, hemoglobin; AST, glutamic-oxaloacetic transaminase; TBil, total bilirubin; INR, international normalized ratio; Na, serum sodiun; Cr, serum creatinine; AFP, alpha fetal protein; HBV, hepatitis B virus.
†Hazard ratios estimated by Cox proportional hazards regression. All statistical tests were two-sided.

All related programs that are part of R and were used for creating the nomogram are described in detail in the **Supplementary Material**. Statistical analyses to identify risk factors for 90-day transplantation-free survival were performed using SPSS version 22.0 for Windows (SPSS Inc., Chicago, IL). The HBV-ACLF nomogram was computed with the rms package in R, version 3.6.1 (http:// www.r-project.org/) (17). All statistical tests were two-sided, and *P*-values of < 0.05 were considered to be statistically significant.

RESULTS

Profile and General Characteristics at Baseline

A total of 1,956 and 783 consecutive HBV-ACLF patients were screened as for inclusion in the derivation and validation cohorts, respectively. Of these, 603 and 114, respectively, were excluded. Thus, 1,353 patients were included in the derivation cohort, and 669 patients were included in the validation cohort (**Figure 1**). Patient baseline clinical and laboratory data of the derivation and validation cohorts are shown in **Table 1** (Continuous data prior to transformation into categorical variables are shown in **Supplementary Table 1**).

Survival Rate and Risk Factors Associated With 90-Day Transplantation-Free Survival in the Derivation Cohort

The 90-day transplantation-free survival rate of patients in the derivation cohort was 54.0%. Univariate analysis indicated that age, WBC count, Hb, PLT, AST, ALB, TBil, INR, Na, Cr, AFP, HBeAg, HBV DNA, HRS, GB, HE, infection, and PreLD were associated with 90-day transplantation-free survival (see **Supplementary Table 2**). Multivariable analyses demonstrated that age, WBC count, Hb, AST, TBil, INR, Cr, Na, AFP, HBV DNA, HE, and PreLD were independent risk factors for 90-day transplantation-free survival (**Table 2**).

Establishing HBV-ACLF Predictive Nomogram

The HBV-ACLF nomogram to predict 90-day transplantation-free survival of patients with HBV-related ACLF was developed using the variables of age, WBC count, Hb, AST, TBil, INR, Cr, Na, AFP, HBV DNA, HE, and PreLD (**Figure 2**). The calibration plots for the 90-day transplantation-free survival rate showed optimal agreement between nomogram prediction and actual observation in the derivation cohort (**Figure 3A**).

Nomogram Validation

The 90-day transplantation-free survival rate of the validation cohort was 52.9%. The C-index of the HBV-ACLF nomogram for predicting 90-day transplantation-free survival in the validation cohort was 0.793 (95% CI: 0.770–0.817). A calibration curve showed good agreement between predicted 90-day transplantation-free survival using the nomogram and observed survival based on the Kaplan-Meier method (**Figure 3B**).

Comparison of Predictive Accuracy Between the Nomogram and CTP, MELD, and MELD-Na Scores in the Derivation and Validation Cohorts

The predictive results for 90-day transplantation-free survival of the HBV-ACLF nomogram and CTP, MELD, and MELD-Na scores are shown in **Table 3**. In the derivation cohort, the C-index of HBV-ACLF nomogram was 0.790 (95% CI: 0.773–0.807), which was greater than the C-index of the CTP score (0.627; 95% CI: 0.606–0.647, $P < 0.001$), MELD score (0.717; 95% CI: 0.697–0.736, $P < 0.001$), and MELD Na score (0.709; 95% CI: 0.689–0.728, $P < 0.001$).

In validation cohort, the C-index of HBV-ACLF nomogram was 0.793 (95% CI: 0.770–0.817). This value was greater than the C-index of the CTP score (0.629; 95% CI: 0.598–0.659, $P < 0.001$), MELD score (0.712; 95% CI: 0.682–0.741, $P < 0.001$), and MELD-Na score (0.715; 95% CI: 0.686–0.744, $P < 0.001$).

The C-index of the CLIF-C OF score was 0.727 (95% CI: 0.701–0.754, $P < 0.001$), of the CLIF-C ACLF score was 0.746 (95% CI: 0.720–0.772, $P = 0.001$), and of the COSSH ACLF score was 0.762 (95% CI: 0.737–0.787, $P = 0.002$) (**Table 3**). All C-index values were less than that of the HBV-ACLF nomogram.

DISCUSSION

Acute-on-chronic liver failure is a severe disease that results in multiple organ failure, and has a high short-term mortality. An accurate predictive model is necessary to make clinical decision and prioritize patients for liver transplantation. In this study we developed a nomogram to predict 90-day transplantation-free survival that included age, WBC count, and 10 other risk factors. The HBV-ACLF predictive nomogram developed exhibited better predictive accuracy than current conventional and mainstream models including CTP, MELD, MELD-Na, CLIF-C OF, CLIF-C ACLF, and COSSH ACLF scores.

In addition to common risk factors associated with survival in patients with liver disease, such as TBil, INR, Cr, Na, and HE

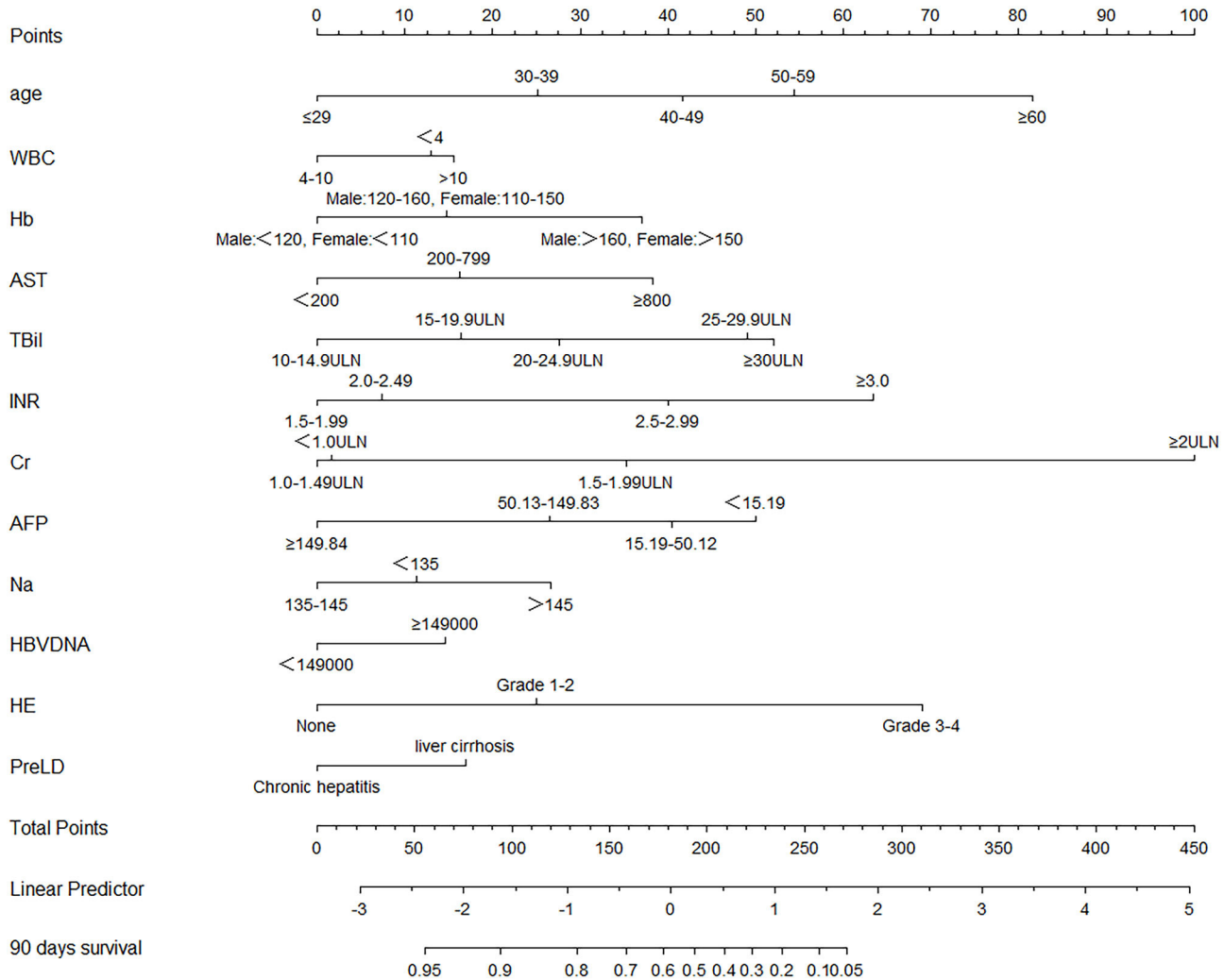


FIGURE 2 | Nomogram, including age, WBC count, Hb, AST, TBil, INR, Cr, AFP, Na, HBV DNA, HE, and PreLD for 90-day transplantation-free survival in HBV-related ACLF patients. The nomogram allows the user to obtain the probability of 90 days transplantation-free survival corresponding to a patient's combination of covariates. As an example, locate the patient's TBil and draw a line straight upward to the "Points" axis to determine the score associated with that TBil. Repeat the process for each variable, and sum the scores achieved for each covariate, and locate this sum on the "Total Points" axis. Draw a line straight down to determine the likelihood of 90 days transplantation-free survival. WBC, white blood cell; Hb, hemoglobin; AST, glutamic-oxaloacetic transaminase; TBil, total bilirubin; INR, international normalized ratio; Cr, serum creatinine; AFP, alpha fetal protein; Na, serum sodium; HBV, hepatitis B virus; HE, hepatic encephalopathy; PreLD, pre-existing chronic liver diseases; ACLF, acute-on-chronic liver failure.

(6, 9, 19), the nomogram developed in this study included age, AST, AFP, PreLD, HBV DNA, WBC count, and Hb. Among them, AFP is an important indicator of regeneration ability of liver cells. Patients with high AFP levels had a better prognosis than those with low levels, which is similar to the results reported by Singh et al. where a rising AFP level was considered to be associated with translation-free survival (20). Aspartate aminotransferase mainly exists in the mitochondria of cells, and an elevation of AST indirectly reflects mitochondrial damage, and may indicate more serious damage than elevation of ALT. Age was included in our model as in other models (4–6, 9), and likely reflects the risks of comorbidities which increase with age.

In our study, cirrhosis was considered a risk factor for evaluating the prognosis of HBV-ACLF patients. Patients with cirrhosis before the onset of ACLF had worse outcomes than those without cirrhosis, which is consistent with results reported by Wu et al. (9) The regeneration ability of liver cells is insufficient in patients with cirrhosis (21), and the patients with cirrhosis in our study had a greater risk of complications (e.g., HE, HRS, and infection; see **Supplementary Table 3**) than patients without cirrhosis. Moreover, the cirrhosis patients had worse liver function than the chronic hepatitis patients (see **Supplementary Table 3**), which may lead to a higher risk of a poor outcome. Therefore, the inclusion of cirrhosis as a risk

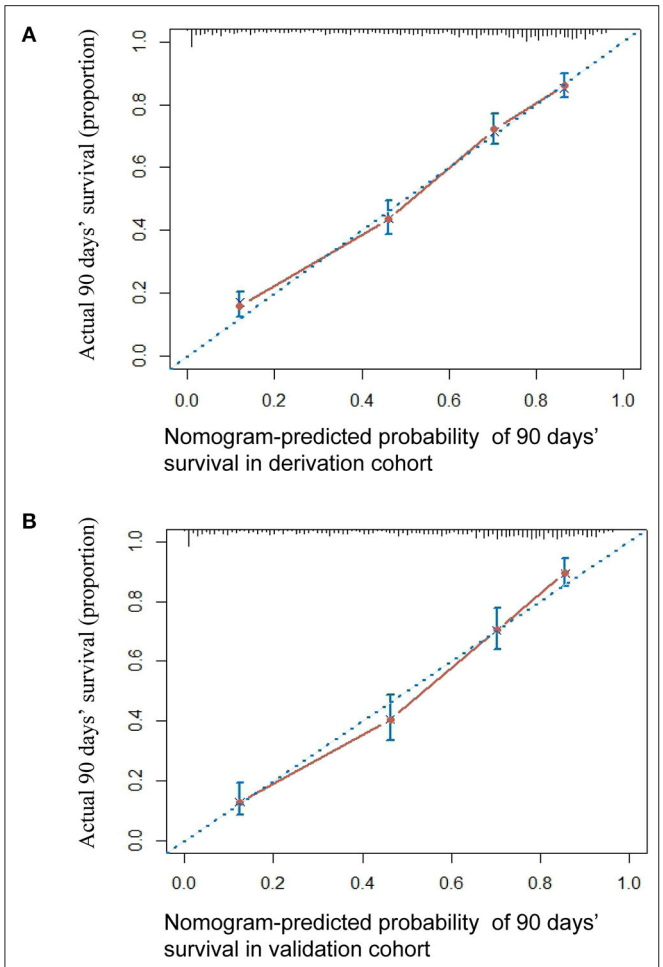


FIGURE 3 | The calibration curve of nomogram for predicting 90-day transplantation-free survival in the derivation cohort **(A)** and in the validation cohort **(B)**. Actual probability of 90-day transplantation-free survival is plotted on the y-axis; nomogram-predicted probability of 90-day transplantation-free survival is plotted on the x-axis.

TABLE 3 | The C-index of HBV-related ACLF-Nomogram and different scoring systems for prediction of 90 days' survival in the derivation and validation cohorts.

Factor	Derivation cohort		Validation cohort	
	C-index (95%CI)	*P*-value†	C-index (95%CI)	*P*-value†
HBV-ACLF-Nomogram	0.790 (0.773,0.807)		0.793 (0.770,0.817)	
MELD	0.717 (0.697,0.736)		0.712 (0.682,0.741)	
MELD-Na	0.709 (0.689,0.728)		0.715 (0.686,0.744)	
CTP	0.627 (0.606,0.647)		0.629 (0.598,0.659)	
CLIF-C OF			0.727 (0.701,0.754)	
CLIF-C ACLF			0.746 (0.720,0.772)	
COSSH ACLF			0.762 (0.737,0.787)	
HBV-ACLF-Nomogram vs. MELD		<0.001		<0.001
HBV-ACLF-Nomogram vs. MELD-Na		<0.001		<0.001
HBV-ACLF-Nomogram vs. CTP		<0.001		<0.001
HBV-ACLF-Nomogram vs. CLIF-C OF				<0.001
HBV-ACLF-Nomogram vs. CLIF-C ACLF				0.001
HBV-ACLF-Nomogram vs. COSSH ACLF				0.002

C-index, concordance index; HBV, hepatitis B virus; ACLF, acute on chronic liver failure; CI, confidence interval; MELD, model for end-stage liver disease; MELD-Na, model for end-stage liver disease with the addition of the Na level; CTP, child-Turcotte-Pugh; CLIF-C OF, CLIF-consortium organ failure score; CLIF-C ACLF, CLIF-consortium acute-on-chronic liver failure score; COSSH ACLF, Chinese group on the study of severe hepatitis B acute-on-chronic liver failure score.

†P-values are calculated based on normal approximation using function rcorrp.cens in Hmisc package.

factor in our model suggested that the nomogram was an appropriate model for Eastern ACLF patients.

HBV DNA load was included in the HBV-ACLF nomogram. We divided ACLF patients into two groups using the median value of the HBV DNA loads (149,000 IU/ml), and patients with loads above the median had a worse prognosis than patients with values below the median. To our knowledge, this is the first time that a scoring system included HBV DNA load. Recently, some studies have indicated that infection is an important factor that leads to the development, and promotes the progression of ACLF (22). The peripheral blood WBC count is a very important indicator of infection, and was included in the nomogram developed in this study. Other studies have indicated that WBC count is predictive of survival in patients with ACLF (4, 23).

The predictive accuracy of the HBV-ACLF nomogram was better than that of CTP, MELD, MELD-Na scores in both the derivation and validation cohort. CTP, MELD, and MELD-Na scores are primarily used to determine the prognosis of cirrhosis patients (3). However, important indexes associated with outcomes such as age, AFP, and infection are not included in these models. HBV-ACLF represents a complex condition that is different from liver cirrhosis in many aspects, such as short-term and long-term survival (24). The nomogram is more accurate than the other methods presumably because it includes more known risk factors.

The CLIF-C OF, CLIF-C ACLF, and COSSH ACLF scoring systems include evaluation of respiratory function using PaO_2/FiO_2 or SpO_2/FiO_2. However, at our center routine evaluation of respiratory function of ACLF patients with pulse oximetry or arterial blood gas analysis was not begun until July 2016. As such, CLIF-C OF, CLIF-C ACLF, and COSSH ACLF scores could only be calculated for patients in validation cohort. Based on the C-indices, the HBV-ACLF nomogram demonstrated better predictive accuracy than the other three methods. This suggests that the HBV-ACLF nomogram is more suitable for evaluating HBV-ACLF patients in Asia diagnosed by

APASL and Chinese guideline criteria and reducing the risk of local bleeding due to arterial blood collecting. We can speculate the reasons for this finding. First, the main cause of ACLF in Asia is HBV infection while in the West is alcohol abuse. Disease progression and outcomes of HBV-induced liver disease and alcohol-induced liver disease are different. Second, non-cirrhosis patients are included in the Eastern diagnostic criteria, while in EASL and AASLD criteria the presence of cirrhosis is a requirement for the diagnosis of ACLF. Third, although the COSSH ACLF scoring system exhibited a good predictive ability (C-index = 0.762) for HBV-ACLF in our study, it does not include the variables of Na, AFP, HBV DNA, and the PreLD (cirrhosis or chronic hepatitis). These variables were proven to be important risk factors influencing the prognosis of patients in this study, and in prior studies (9, 10).

Five related studies had been carried out to evaluate the prognosis of ACLF patients with nomogram (13, 25–28). The relatively small number of cases in these studies led to some important risk factors not being included in the models (such as liver cirrhosis, HBV DNA load, etc.). In addition, four out of five studies only compared the nomograms with conventional models, but not with the current mainstream prognostic scoring systems, such as CLIF-C ACLF score and COSSH ACLF score (13, 25, 27, 28). The model of this study was based on the large cohort data, which could reduce the bias caused by insufficient sample size. At the same time, this study also compared the prediction model with the existing mainstream scoring systems. These are the advantages of this study.

This study had several limitations that should be considered. Firstly, specific patient comorbidities were not included in the nomogram development. Severe comorbidities can affect patient survival. Although study has demonstrated that comorbidities are correlated with the survival of cirrhosis patients (29), it is difficult to create categorized variables and to quantify risk because of the diversity of comorbidities. Secondly, this is a retrospective study and subject to all standard limitations associated with this format. For example, a total of 448 patients were lost to follow-up (without outcome data), which may cause bias for the results. Thirdly, The data were obtained exclusively from one center. Thus, the results need to be validated in other data sets.

In conclusion, we developed and validated a nomogram for predicting 90-day transplantation-free survival in patients with HBV-ACLF. The nomogram is more accurate than current predictive models, including CTP, MELD, MELD Na, CLIF-C OF, CLIF-C ACLF, and COSSH ACLF scores, and is very user-friendly. The nomogram may be useful for allocating medical resources in patients with HBV-ACLF; however, to generalize the use of this nomogram validation with data from other institutions is required.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because because of patients' privacy. Requests to access the datasets should be directed to linbingl@mail.sysu.edu.cn.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee on Clinical Trials of the Third Affiliated Hospital of Sun Yat-sen University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

B-lL and J-fC designed the study, J-fC and J-rH performed the analysis and interpretation of the data. J-fC, W-zW, MH, X-hP, S-qZ, H-jC, JZ, JX, BG, D-nL, and JG participated in the data collection and follow-up of patients, B-lL and Z-lG provided financial support for this work, J-fC and B-lL wrote and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by the following funding sources: National Science and Technology Major Project of China (2018ZX10302204, 2017ZX10203201003); National Natural Science Foundation of China (81901940, 82070612). The Guangzhou Major Project in collaborative innovation of industry (1561000157).

ACKNOWLEDGMENTS

We thank all the patients participated in this research.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.692669/full#supplementary-material

REFERENCES

1. Garg H, Kumar A, Garg V, Sharma P, Sharma BC, Sarin SK. Clinical profile and predictors of mortality in patients of acute on-chronic liver failure. *Digest Liver Dis.* (2012) 44:166–71. doi: 10.1016/j.dld.2011.08.029
2. Chan AC, Fan ST, Lo CM, Liu CL, Chan SC, Nq KK, et al. Liver transplantation for acute-on-chronic liver failure. *Hepatol Int.* (2009) 3:571–81. doi: 10.1007/s12072-009-9148-8
3. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Koserg CL, et al. A model to predict survival in patients with end-stage liver disease. *Hepatology.* (2001) 33:464–70. doi: 10.1053/jhep.2001.22172

4. Jalan R, Saliba F, Pavesi M, Amoros A, Moreau R, Ginès P, et al. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. *J Hepatol.* (2014) 61:1038–47. doi: 10.1016/j.jhep.2014.06.012
5. Wu TZ, Li J, Shao L, Xin JJ, Jiang LY, Qian ZQ, et al. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure. *Gut.* (2018) 67:2181–91. doi: 10.1136/gutjnl-2017-314641
6. Peng Y, Qi X, Tang S, Deng H, Li J, Ning Z, et al. Child-Pugh, MELD and ALBI scores for predicting the in-hospital mortality in cirrhotic patients with acute-on-chronic liver failure. *Expert Rev Gastroent.* (2016) 10:971–80. doi: 10.1080/17474124.2016.1177788
7. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology.* (2013)144:1426–37. doi: 10.1053/j.gastro.2013.02.042
8. Sarin SK, Kedarisetty CK, Abbas Z, Amarapurkar D, Bihari C, Chan AC, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL) 2014. *Hepatol Int.* (2014) 8:453–71. doi: 10.1007/s12072-014-9580-2
9. Wu SJ, Yan HD, Zheng ZX, Shi KQ, Wu FL, Xie YY, et al. Establishment and validation of ALPH-Q score to predict mortality risk in patients with acute-on-chronic hepatitis B liver failure. *Medicine.* (2015) 94:e403. doi: 10.1097/MD.0000000000000403
10. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Mahtab MA, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL): an update. *Hepatol Int.* (2019)13:353–90. doi: 10.1007/s12072-019-09946-3
11. Kattan MW, Scardino PT. Evidence for the usefulness of nomograms. *Nat Clin Pract Urol.* (2007) 4:638–9. doi: 10.1038/ncpuro0968
12. Tang LQ, Li CF, Li J, Chen WH, Chen QY, Yuan LX, et al. Establishment and validation of prognostic nomograms for endemic nasopharyngeal carcinoma. *J Natl Cancer Inst.* (2016) 108: djv291.doi: 10.1093/jnci/djv291
13. Shi KQ, Cai YJ, Lin Z, Dong JZ, Wu JM, Wang XD, et al. Development and validation of a prognostic nomogram for acute-on-chronic hepatitis B liver failure. *J Gastroen Hepatol.* (2016) 32:497–505. doi: 10.1111/jgh.13502
14. Cheung WY, Renfro LA, Kerr D, de Gramont A, Saltz LB, Grothey A, et al. Determinants of early mortality among 37,568 patients with colon cancer who participated in 25 clinical trials from the adjuvant colon cancer endpoints database. *J Clin Oncol.* (2016) 34:1182–9. doi: 10.1200/JCO.2015.65.1158
15. Liver Failure and Artificial Liver Group, Chinese Society of Infectious Diseases, Chinese Medical Association; Severe Liver Diseases and Artificial Liver Group, Chinese Society of Hepatology, Chinese Medical Association. Diagnostic and treatment guidelines for liver failure. *Chin J Hepatol.* (2019) 27:18–26. doi: 10.3760/cma.j.issn.1007-3418.2019.01.006
16. Frank EHJ. *Harrell Miscellaneous*. *R Package version 3.9-2.* Available online at: http://CRAN.Rproject.org/package Hmisc (accessed October 7, 2015).
17. Frank EHJ. *Rms: Regression Modeling Strategies. R Package version 3.4-0.* Available online at: http://CRAN.Rproject.org/package rms (accessed October 7, 2015).
18. Li YM, Fan JG, on behalf of the National Workshop on Fatty Liver and Alcoholic Liver Disease, Chinese Society of Hepatology, Chinese Medical Association, Fatty Liver Disease Expert Committee. Chinese Medical Doctor Association. Guidelines of prevention and treatment for alcoholic liver disease (2018, China). *J Digest Dis.* (2018) 34:641–9. doi: 10.1111/1751-2980.12687
19. Shi KQ, Zhou YY, Yan HD, Li H, Wu FL, Xie YY, et al. Classification and regression tree analysis of acute-on-chronic hepatitis B liver failure: seeing the forest for the trees. *J Viral Hepatitis.* (2016) 24:132–40. doi: 10.1111/jvh.12617
20. Singh S, Hynan LS, Rule JA, Lee WM. Changes in alpha-foetoprotein and Gc-globulin in relation to outcomes in non-acetaminophen acute liver failure. *Liver Int.* (2019) 39:2368–73. doi: 10.1111/liv.14216
21. Ouyang GX, Liu JY,Wang P, Ren Y, Yi P, Zhou Q, et al. Multiple factors affect the regeneration of liver. *Rev Assoc Med Bras.* (2018) 64:791–8. doi: 10.1590/1806-9282.64.09.791
22. Sargenti K, Prytz H, Nilsson E, Kalaitzakis E. Predictors of mortality among patients with compensated and decompensated liver cirrhosis: the role of bacterial infections and infection-related acute-on-chronic liver failure. *Scand J Gastroenterol.* (2015) 50:875–83. doi: 10.3109/00365521.2015.1017834
23. Li H, Chen LY, Zhang NN, Li ST, Zeng B, Pavesi M, et al. Characteristics, diagnosis and prognosis of acute-on-chronic liver failure in cirrhosis associated to hepatitis B. *Sci Rep.* (2016) 6:25487. doi: 10.1038/srep25487
24. Jalan R, Pavesi M, Saliba F, Amoros A, Fernandez J, Holland-Fischer P, et al. The CLIF Consortium Acute Decompensation score (CLIF-C ADs) for prognosis of hospitalised cirrhotic patients without acute-on-chronic liver failure. *J Hepatol.* (2015) 62:831–40. doi: 10.1016/j.jhep.2014.11.012
25. Lin S, Chen J,Wang M, Han L, Zhang H, Dong J, et al. Prognostic nomogram for acute-on-chronic hepatitis B liver failure. *Oncotarget.* (2017) 8:109772–82. doi: 10.18632/oncotarget.21012
26. Gao F, Zhang Q, Liu Y, Gong G, Mao D, Gong Z, et al. Nomogram prediction of individual prognosis of patients with acute-on-chronic hepatitis B liver failure. *Digest Liver Dis.* (2019) 51:425–33. doi: 10.1016/j.dld.2018.08.023
27. Gong J, Zhou W, Xiao C, Jie Y, Zhu S, Zheng J, et al. A nomogram for predicting prognostic value of inflammatory biomarkers in patients with acute-on-chronic liver failure. *Clin. Chim. Acta.* (2018) 478:7–12. doi: 10.1016/j.cca.2017.12.018
28. Chen RC, Wang XD, Dong JZ, Lin Z, Wu JM, Cai YJ, et al. A MELD-based nomogram for predicting 3-month mortality of patients with acute-on-chronic hepatitis B liver failure. *Clin Chim Acta.* (2017) 468:195–200. doi: 10.1016/j.cca.2017.03.005
29. Jepsen P, Vilstrup H, Lash TL. Development and validation of a comorbidity scoring system for patients with cirrhosis. *Gastroenterology.* (2014) 146:147–56. doi: 10.1053/j.gastro.2013.09.019

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.

frontiers in Medicine

ORIGINAL RESEARCH
published: 23 June 2021
doi: 10.3389/fmed.2021.704452

Different Effects of Total Bilirubin on 90-Day Mortality in Hospitalized Patients With Cirrhosis and Advanced Fibrosis: A Quantitative Analysis

Liang Qiao[1,2†], Wenting Tan[3†], Xiaobo Wang[4†], Xin Zheng[5†], Yan Huang[6†], Beiling Li[7†], Zhongji Meng[8], Yanhang Gao[9], Zhiping Qian[10], Feng Liu[11,12], Xiaobo Lu[13], Jia Shang[14], Junping Liu[14], Huadong Yan[15], Wenyi Gu[1,2], Yan Zhang[1,2], Xiaomei Xiang[3], Yixin Hou[4], Qun Zhang[4], Yan Xiong[5], Congcong Zou[5], Jun Chen[6], Zebing Huang[6], Xiuhua Jiang[7], Sen Luo[8], Yuanyuan Chen[8], Na Gao[9], Chunyan Liu[9], Wei Yuan[10], Xue Mei[10], Jing Li[12], Tao Li[12], Rongjiong Zheng[13], Xinyi Zhou[13], Jinjun Chen[7], Guohong Deng[3], Weituo Zhang[16‡] and Hai Li[1,2*‡]*

[1] Department of Gastroenterology, School of Medicine, Ren Ji Hospital, Shanghai Jiao Tong University, Shanghai, China, [2] Key Laboratory of Gastroenterology and Hepatology, Shanghai Institute of Digestive Disease, Chinese Ministry of Health, Shanghai Jiao Tong University, Shanghai, China, [3] Department of Infectious Diseases, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China, [4] Center of Integrative Medicine, Beijing Ditan Hospital, Capital Medical University, Beijing, China, [5] Department of Infectious Diseases, Tongji Medical College, Institute of Infection and Immunology, Union Hospital, Huazhong University of Science and Technology, Wuhan, China, [6] Hunan Key Laboratory of Viral Hepatitis, Department of Infectious Diseases, Xiangya Hospital, Central South University, Changsha, China, [7] Hepatology Unit, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China, [8] Department of Infectious Disease, Taihe Hospital, Hubei University of Medicine, Shiyan, China, [9] Department of Hepatology, The First Hospital of Jilin University, Changchun, China, [10] Department of Liver Intensive Care Unit, Shanghai Public Health Clinical Centre, Fudan University, Shanghai, China, [11] Tianjin Institute of Hepatology, Nankai University Second People's Hospital, Tianjin, China, [12] Department of Infectious Diseases and Hepatology, The Second Hospital of Shandong University, Jinan, China, [13] Infectious Disease Center, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, China, [14] Department of Infectious Diseases, Henan Provincial People's Hospital, Zhengzhou, China, [15] Department of Infectious Diseases, Hwamei Hospital, Ningbo No. 2 Hospital, University of Chinese Academy of Sciences, Ningbo, China, [16] Clinical Research Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China*

Introduction: Total bilirubin (TB) is a major prognosis predictor representing liver failure in patients with acute on chronic liver failure (ACLF). However, the cutoff value of TB for liver failure and whether the same cutoff could be applied in both cirrhotic and non-cirrhotic patients remain controversial. There is a need to obtain the quantitative correlation between TB and short-term mortality *via* evidence-based methods, which is critical in establishing solid ACLF diagnostic criteria.

Methods: Patients hospitalized with cirrhosis or advanced fibrosis (FIB-4 > 1.45) were studied. TB and other variables were measured at baseline. The primary outcome was 90-day transplantation-free mortality. Multi-variable Cox proportional hazard model was used to present the independent risk of mortality due to TB. Generalized additive model and second derivate (acceleration) were used to plot the "TB-mortality correlation curves." The mathematical (maximum acceleration) and clinical (adjusted 28-day transplantation-free mortality rate reaching 15%) TB cutoffs for liver failure were both calculated.

OPEN ACCESS

Edited by:
Yu-Chen Fan,
Shandong University, China

Reviewed by:
Yu Shi,
Zhejiang University, China
Yubao Zheng,
Third Affiliated Hospital of Sun Yat-sen University, China

***Correspondence:**
Hai Li
haili_17@126.com
Weituo Zhang
weituozhang@126.com

†These authors have contributed equally to this work and share first authorship

‡These authors have contributed equally to this work

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *03 May 2021*
Accepted: *14 May 2021*
Published: *23 June 2021*

Citation:
Qiao L, Tan W, Wang X, Zheng X, Huang Y, Li B, Meng Z, Gao Y, Qian Z, Liu F, Lu X, Shang J, Liu J, Yan H, Gu W, Zhang Y, Xiang X, Hou Y, Zhang Q, Xiong Y, Zou C, Chen J, Huang Z, Jiang X, Luo S, Chen Y, Gao N, Liu C, Yuan W, Mei X, Li J, Li T, Zheng R, Zhou X, Chen J, Deng G, Zhang W and Li H (2021) Different Effects of Total Bilirubin on 90-Day Mortality in Hospitalized Patients With Cirrhosis and Advanced Fibrosis: A Quantitative Analysis. Front. Med. 8:704452. doi: 10.3389/fmed.2021.704452

Results: Among the 3,532 included patients, the number of patients with cirrhosis and advanced fibrosis were 2,592 and 940, respectively, of which cumulative 90-day mortality were 16.6% (430/2592) and 7.4% (70/940), respectively. Any increase of TB was found the independent risk factor of mortality in cirrhotic patients, while only TB >12 mg/dL independently increased the risk of mortality in patients with advanced fibrosis. In cirrhotic patients, the mathematical TB cutoff for liver failure is 14.2 mg/dL, with 23.3% (605/2592) patients exceeding it, corresponding to 13.3 and 25.0% adjusted 28- and 90-day mortality rate, respectively. The clinical TB cutoff for is 18.1 mg/dL, with 18.2% (471/2592) patients exceeding it. In patients with advanced fibrosis, the mathematical TB cutoff is 12.1 mg/dL, 33.1% (311/940) patients exceeding it, corresponding to 2.9 and 8.0% adjusted 28- and 90-day mortality rate, respectively; the clinical TB cutoff was 36.0 mg/dL, 1.3% (12/940) patients above it.

Conclusion: This study clearly demonstrated the significantly different impact of TB on 90-day mortality in patients with cirrhosis and advanced fibrosis, proving that liver failure can be determined by TB alone in cirrhosis but not in advanced fibrosis. The proposed TB cutoffs for liver failure provides solid support for the establishment of ACLF diagnostic criteria.

Keywords: liver failure, cutoff, quantitative analyse, short-term mortality, total bilirubin

INTRODUCTION

The disease burden of acute on chronic liver failure (ACLF) is enormous. It affects 10–35% hospitalized patients with cirrhosis (1–4) and a considerable part of patients with non-cirrhotic chronic liver diseases (5, 6). ACLF is associated with sharply increased risk of short-term mortality (4, 6–10) and heavy financial costs (11, 12). The presence of organ failure is a prerequisite for the diagnosis of ACLF (4, 13, 14). In East Asia, where hepatitis B virus (HBV) infection is highly endemic (15, 16), liver failure is the most common type of organ failure (10, 17). Therefore, a clear definition of liver failure is essential in ACLF diagnosis in HBV high-endemic areas.

Though high total bilirubin (TB) has been confirmed to be strongly associated with poor liver preservation and high short-term mortality (7, 13, 18, 19), there are fierce controversies between the East and the West on the application of TB in the diagnosis of liver failure. The European Association for the Study of the Liver - Chronic Liver Failure (EASL-CLIF) defined liver failure using TB alone (TB > 12 mg/dL) (4, 20). While in Asia, liver failure was defined more frequently with a combination of TB and international normalized ratio (INR), such as the Asia-Pacific Association for the Study of the Liver (APASL) criteria (TB > 5 mg/dL and INR > 1.5) (21) and the Chinese Group on the Study of Severe Hepatitis B (COSSH) criteria (TB > 12 mg/dL and INR > 1.5) (6). The inequality was mainly due to whether patients with non-cirrhotic chronic liver diseases included in the respective study (2, 4, 6, 14, 22).

The key to resolving these controversies on the definition of liver failure is to clarify whether the liver failure of cirrhosis and non-cirrhotic chronic liver diseases (mainly advanced fibrosis) can be diagnosed by the same criteria. Given the absence of studies evaluating this specific issue, we aimed to describe the quantitative correlation between the baseline TB level and the 90-day mortality among hospitalized patients with cirrhosis and advanced fibrosis, respectively. The detailed and intuitive presentation of this correlation is crucial to define liver failure and establish the solid ACLF diagnostic criteria.

MATERIALS AND METHODS

Study Design, Setting, and Oversight

We combined the data from two prospective observational cohorts of the Chinese AcuTe on CHronic LIver FailurE (CATCH-LIFE) study: the CATCH-LIFE investigation cohort (NCT02457637) involving 14 hospitals throughout China, enrollment occurred from January 2015 to December 2016; and the CATCH-LIFE validation cohort (NCT03641872), involving 13 hospitals throughout China, enrollment occurred from September 2018 to March 2019. All participants were followed-up for at least 90 days. The study protocols and baseline characteristics have been described in detail elsewhere (23, 24). The medical ethics boards of Shanghai Renji Hospital (the lead center of the CATCH-LIFE study), approved the study [ethics code: (2014)148 k and (2016)142 k]. Written informed consent were obtained from every participant or his/her legal surrogates before enrollment.

Study Population

Eligible patients were required with cirrhosis or advanced fibrosis hospitalized with at least one of the following criteria: (1) with acute decompensations (ADs), including overt ascites (25), gastrointestinal bleeding, hepatic encephalopathy (HE) (26), bacterial infection, or jaundice (TB > 5 mg/dL)] within 1

month before enrollment; (2) with acute liver injury, including alanine aminotransferase (ALT), or aspartate aminotransferase (AST) >3 upper limitation of normal level or total bilirubin (TB) >2 upper limitation of normal level within recent 1 week before enrollment.

Patients with cirrhosis and advanced fibrosis were divided into two groups for comparison. Cirrhosis was diagnosed according to the signs of dysmorphia and relation to liver fibrosis and portal hypertension in the imaging examination (computerized tomography, magnetic resonance imaging or abdominal ultrasound) after enrolment. The cirrhotic patients with a history of decompensation at least 1 month ago are defined to have decompensated cirrhosis, and other cirrhotic patients without any history of decompensation (including the patients hospitalized due to the first episode of decompensation events) are defined to have compensatory cirrhosis. Non-cirrhotic patients with a fibrosis-4 (FIB-4) score (27) < 1.45 were excluded to rule-out those who with merely mild or no fibrosis.

Exposure and Outcomes

The primary exposure was TB level (mg/dL), which was measured at admission. Other clinical data were measured and collected at admission as well, including demographics (age, sex), etiology of underlying liver diseases (HBV related, alcohol related or others), complications (type of ADs), laboratory findings and severity scores [the model of end-stage liver diseases [MELD] (18), MELD-Sodium (28), and Child-Turcotte-Pugh (29)].

The primary outcome was the 90-day mortality (transplantation-free). The patients who received liver transplantation (LT) within 90 days were excluded to present the correlation between baseline TB level and patients' natural 90-day prognosis.

Statistical Analysis

Continuous variables were summarized by mean and standard deviation (SD) or median or inter-quartile range (IQR) based on their distribution. Categorical variables were summarized by frequency and proportion [with 95% confidence interval (CI)].

The correlation between TB and 90-day mortality were analyzed using a multivariable Cox proportional hazard (COXPH) model, adjusting for important risk factors and potential confounders, including age, sex, etiologies of underlying chronic liver diseases, presence or absence of overt ascites, gastrointestinal bleeding and bacterial infection, the grades of HE, the level of INR, creatinine, ALT, and serum sodium. The risk of 90-day mortality was expressed as a continuous variable with hazard ratios (HRs) calculated per mg/dL increment of TB, and as a categorical variable based on different range of TB levels as following: <2, 2–5, 5–8, 8–12, 12–16, 16–20, and >20 mg/dL; the patients with lowest TB (<2 mg/dL) were taken as the reference.

The non-lineal relationship in 90-day mortality over the range of TB level (0–50 mg/dL) was plotted as a "TB-mortality correlation curve." The estimated mortality corresponding to the TB values in the curves was adjusted for above listed confounding factors by the generalized additive model (GAM) (30) to present the independent impact of TB on mortality. Spline (31) was taken as connection function in GAM and the smoothing parameters were chosen to optimize the Akaike Information Criterion. Second derivative (acceleration) (32) of TB to mortality was used to describe the non-lineal relationship.

Finally, based on the multivariable adjusted TB-mortality correlation curves, the TB values that correspond to the inflection points (maximum of acceleration) of the curves would be taken as the mathematical cutoff. Additionally, the TB value that correspond to adjusted 28-day transplantation-free mortality reaching 15% (the definition of EASL-CLIF) (4) would be calculated as well and taken as clinical cutoff for liver failure.

The analysis above mentioned will be performed in patients with cirrhosis and advanced fibrosis, respectively to compare the characteristics of them. The software environment R 4.0.0 and MATLAB 2016b were used for the analysis. The R package *gam* was used for the GAM fitting and the MATLAB tool box *curve fitting* was used for curve fitting and second derivative calculation. All tests were 2-sided with $\alpha = 0.05$.

RESULTS

Enrollment and Baseline Characteristics

A total of 3,532 patients were included in this analysis (**Figure 1**). Among them, 2,592 (73.4%) patients had cirrhosis (including 1,532 compensated and 1,060 decompensated), and 940 (26.6%) patients had advanced fibrosis. Patients underwent LT within 90 days ($n = 246$), with non-cirrhosis chronic liver diseases and a FIB-4 score < 1.45 ($n = 178$), or with missing TB values ($n = 5$), or FIB-4 score ($n = 9$) were excluded. All patients included finished the 90-day follow-up. The cumulative 90-day transplantation-free mortality of the patients with cirrhosis and advanced fibrosis were 16.6% (430/2592) (**Table 1**) and 7.4% (70/940), respectively (**Table 2**). Demographic, clinical characteristics and 90-day transplantation-free mortality of patients with cirrhosis and advanced fibrosis are presented in **Tables 1**, **2**, respectively according to different TB level. The pattern of missing data can be found in **Figure 2.**

Any Increase of TB Independently Increased the Mortality Risk in Cirrhosis

In patients with cirrhosis, any increase in TB, either expressed as a continuous variable or categorical variable, was the risk factor of 90-day mortality in all univariable and multivariable-adjusted analysis. The full-adjusted HR was 1.060 (95% CI, 1.051–1.069) per 1 mg/dL increase of TB (**Table 3**). Categorical-variable analysis showed that compared with the cirrhotic patients with normal TB (<2 mg/dL), the HRs were significantly higher in patients within any other categories of elevated TB ($p < 0.001$ for all). Moreover, the HRs were positively correlated with the TB level of patients p (for trend = 0.002).

The Mortality Risk Increased only if TB Is >12 mg/dL in Advanced Fibrosis

In patients with advanced fibrosis, though as a continuous variable, higher TB was also independently associated with 90-day mortality overall [adjusted HR of 1.080 per 1 mg/dL increase of TB, (95% CI, 1.049–1.112)]. Interestingly, categorical-variable

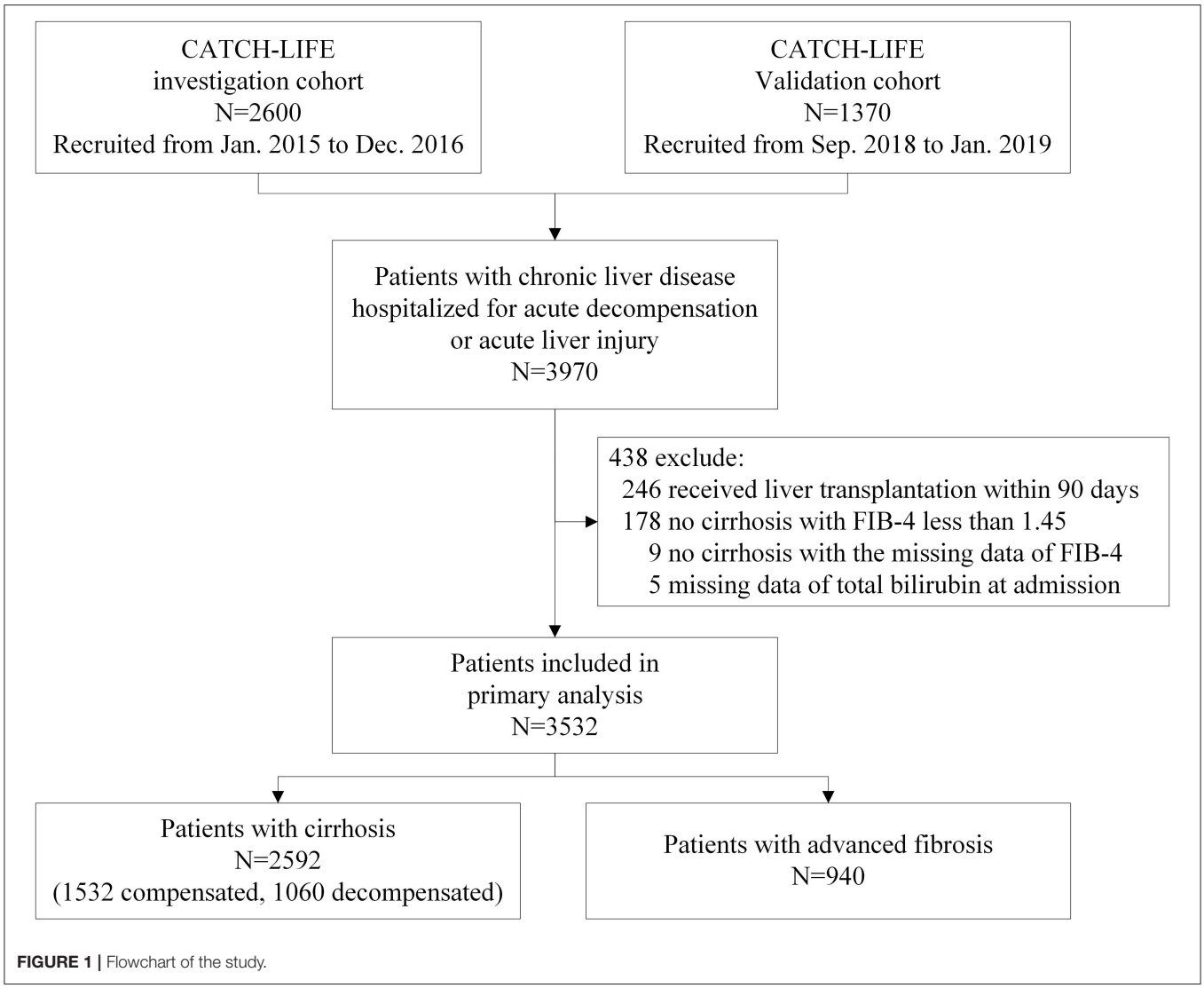


FIGURE 1 | Flowchart of the study.

analysis demonstrated that these effects were primarily driven by TB over 12 mg/dL (**Table 4**), any TB categories <12 mg/dL was not the independent risk factor of mortality. Besides the qualitative *p*-values, it can be found that compared to the HRs of the patients with TB of 2–5 mg/dL [0.802 (95% CI, 0.130–4.956), $p = 0.812$], 5–8 mg/dL [1.443 (95% CI, 0.237–8.803), $p = 0.691$], and 8–12 mg/dL [1.866 (95% CI, 0.401–8.681), $p = 0.426$], the HR in patients with TB of 12–16 mg/dL markedly elevated to 4.186 [(95% CI 1.070–16.371), $p = 0.040$], suggesting a threshold effect within the range.

Liver Failure Can Be Defined by TB Alone in Cirrhosis

Through the GAM and spline, we intuitively plotted the relationship between TB and multivariable-adjusted 90-day transplantation-free mortality. Both the TB-mortality correlation curves of cirrhosis and advanced fibrosis were monotonically increasing. However, the rate of change of 90-day transplantation-free mortality per mg/dL of TB was not a constant, implying the existence of non-lineal effects.

The TB-mortality correlation curve of cirrhosis (**Figure 3A**) was S-shaped and can be roughly divided into three parts by two inflection points (TB of 14.2 and 24.8 mg/dL). The two points, respectively correspond to the maximum and minimum values (peak and valley in **Figure 3B** of the second derivative (acceleration) of the mortality relative to the change in TB. To be specific, when TB was between 0 and 14.2 mg/dL, the mortality acceleratingly increases with TB. When TB was between 14.2 and 24.8 mg/dL, the absolute mortality brought by per mg/dL increase of TB is the largest. When TB exceeds 24.8 mg/dL, the increase of mortality slows down, showing a saturation effect.

The mathematical TB cutoff was 14.2 mg/dL, with 23.3% (605/2592) patients exceeding the cutoff. The corresponding adjusted 28- and 90-day transplantation-free mortality were 13.3 and 25.0%, respectively. Moreover, the clinical TB cutoff was 18.1 mg/dL **Figure 4A**, with 18.2% (471/2592) patients exceeding the cutoff. The corresponding adjusted 90-day transplantation-free mortality was 32.7%. If take the TB of 24.8 mg/dL (the valley in **Figure 3B**) as a cutoff, 10.3% (268/2592) patients have TB higher than the cutoff, corresponding to

TABLE 1 | Comparison of baseline characteristics of patients with cirrhosis based on different level of total bilirubin (mg/dL) at admission.

Variable	0<TB≤2 *N* = 839	2<TB≤5 *N* = 601	5<TB≤8 *N* = 215	8< TB≤12 *N* = 220	12<TB≤16 N = 172	16<TB≤20 *N* = 139	20<TB *N* = 406
Demographics							
Age, mean (SD)	54.4 (11.4)	52.2 (11.0)	51.1 (11.4)	50.8 (11.4)	49.0 (11.1)	48.1 (10.4)	48.3 (11.3)
Gender, No. (%)	553 (65.9)	439 (73.0)	149 (69.3)	161 (73.2)	124 (72.1)	112 (80.6)	351 (86.5)
Etiology, No. (%)							
HBV	486 (57.9)	366 (60.9)	131 (60.9)	123 (55.9)	118 (68.6)	96 (69.1)	307 (75.6)
Alcoholic	101 (12.0)	78 (13.0)	29 (13.5)	36 (16.4)	16 (9.3)	12 (8.6)	27 (6.7)
Others	252 (30.0)	157 (26.1)	55 (25.6)	61 (27.7)	38 (22.1)	31 (22.3)	72 (17.7)
Complications, No. (%)							
Ascites	481 (57.3)	369 (61.4)	131 (60.9)	138 (62.7)	104 (60.5)	98 (70.5)	285 (70.2)
Gastrointestinal bleeding	342 (40.8)	99 (16.5)	26 (12.1)	13 (5.9)	10 (5.8)	4 (2.9)	20 (4.9)
Bacterial infection	134 (16.0)	128 (21.3)	58 (27.0)	75 (34.1)	58 (33.7)	44 (31.7)	196 (48.3)
Hepatic encephalopathy							
Not overt	805 (95.9)	564 (93.8)	197 (91.6)	205 (93.2)	161 (93.6)	136 (97.8)	350 (86.2)
Grade 2	25 (3.0)	26 (4.3)	10 (4.7)	9 (4.1)	10 (5.8)	2 (1.4)	37 (9.1)
Grade 3	7 (0.8)	6 (1.0)	8 (3.7)	4 (1.8)	0 (0.0)	1 (0.7)	14 (3.4)
Grade 4	2 (0.2)	5 (0.8)	0 (0.0)	2 (0.9)	1 (0.6)	0 (0.0)	5 (1.2)
Laboratory results, median (IQR)							
Hemoglobin, g/L	97.0 (75.0, 118.0)	113.0 (95.0, 128.0)	105.0 (88.5, 122.0)	110.2 (89.0, 131.9)	115.5 (96.8, 129.0)	118.0 (107.2, 132.0)	119.0 (103.0, 133.4)
White blood cell, 10^9/L	3.9 (2.6, 5.5)	4.1 (3.0, 6.0)	4.8 (3.4, 6.4)	5.3 (3.7, 7.0)	5.7 (4.1, 7.8)	6.1 (4.7, 8.1)	7.1 (5.0, 10.1)
Platelet, 10^9/L	76.0 (51.0, 122.0)	64.0 (42.9, 98.0)	71.0 (46.0, 106.5)	76.0 (48.9, 118.5)	80.5 (54.0, 123.2)	92.0 (63.5, 128.0)	85.0 (58.0, 121.0)
International normalized ratio	1.3 (1.1, 1.4)	1.5 (1.3, 1.6)	1.6 (1.4, 1.9)	1.7 (1.4, 2.1)	1.9 (1.5, 2.4)	1.9 (1.5, 2.4)	2.1 (1.6, 2.7)
Creatinine, mg/dL	0.8 (0.7, 1.0)	0.8 (0.6, 0.9)	0.7 (0.6, 0.9)	0.7 (0.6, 1.0)	0.8 (0.6, 1.0)	0.8 (0.6, 1.0)	0.9 (0.7, 1.2)
Albumin, g/L	31.9 (27.6, 36.0)	29.2 (25.2, 33.1)	28.3 (24.5, 34.0)	29.7 (26.3, 33.4)	29.8 (26.6, 33.0)	29.6 (26.2, 33.0)	31.0 (27.9, 34.0)
Alanine transaminase, IU/L	28.0 (17.7, 51.5)	45.3 (27.0, 89.6)	58.1 (31.0, 151.4)	100.6 (33.2, 391.9)	102.4 (45.5, 372.6)	206.3 (85.3, 566.6)	159.3 (61.5, 385.3)
Aspartate transaminase, IU/L	37.0 (24.6, 62.9)	65.0 (42.0, 116.6)	92.3 (51.0, 179.0)	126.3 (62.9, 330.0)	145.4 (77.3, 291.5)	191.8 (110.1, 429.3)	177.9 (100.8, 332.8)
Sodium, mmol/L	139.7 (137.0, 141.8)	138.6 (135.8, 141.0)	137.4 (134.9, 140.0)	137.3 (133.4, 139.5)	137.3 (133.3, 139.8)	136.9 (133.9, 138.9)	135.0 (131.0, 138.0)
Score, mean (SD)							
MELD	9.7 (4.0)	14.4 (3.9)	18.2 (5.0)	20.8 (4.6)	23.0 (4.8)	25.1 (5.1)	28.6 (5.7)
MELD-Na	10.5 (5.1)	15.7 (5.1)	19.6 (6.6)	22.6 (4.8)	24.5 (5.5)	26.6 (4.9)	30.0 (5.3)
CTP	7.3 (1.4)	9.4 (1.5)	10.2 (1.6)	10.3 (1.7)	10.5 (1.8)	10.6 (1.7)	10.8 (1.7)
Transplantation-free mortality							
28-day, No. (%)	17 (2.0)	26 (4.3)	14 (6.5)	19 (8.6)	22 (12.8)	26 (18.7)	117 (28.8)
90-day, No. (%)	35 (4.2)	51 (8.5)	25 (11.6)	36 (16.4)	38 (22.1)	44 (31.7)	201 (49.5)

SD, standard deviation; HBV, hepatitis B virus; IQR, interquartile range; MELD, the model of end-stage liver disease; MELD-Sodium, the model of end-stage liver disease with sodium; CTP, Child-Turcotte-Pugh.

19.2 and 44.8% of adjusted 28- and 90-day transplantation-free mortality, respectively.

We furtherly plotted the TB-mortality correlation curves of patients with compensated cirrhosis (**Figures 5A,B**) and decompensated cirrhosis (**Figures 5C,D**). The trends of the two curves were similar, and both have two inflection points. Compared with the curve of compensated cirrhosis, the curve of decompensated cirrhosis is closer to linearity, and the TB value corresponding to the two inflection points were smaller (7.5 vs. 16.0 mg/dL, and 18.6 vs. 26.0 mg/dL).

Liver Failure Is Insufficient to Be Defined by TB Alone in Advanced Fibrosis

The independent effects of TB on mortality in patients with advanced fibrosis (**Figure 6A**) are significantly different from that in cirrhotic patients, either compensated or decompensated (**Figure 7**). Compared with the TB-mortality correlation curve of cirrhosis, besides the lower overall mortality, there is only one inflection point (the peak in **Figure 6B**, 12.1 mg/dL) in the TB-mortality correlation curve of advanced fibrosis, dividing the curve into two parts. The TB-mortality correlation curve is

TABLE 2 | Comparison of baseline characteristics of patients with advanced fibrosis based on different level of total bilirubin (mg/dL) at admission.

Variable	0<TB≤2 *N* = 239	2<TB≤5 *N* = 188	5< TB≤8 *N* = 96	8<TB≤12 *N* = 104	12<TB≤16 *N* = 103	16< TB≤20 *N* = 74	20<TB *N* = 136
Demographics							
Age, mean (SD)	44.8 (12.7)	42.1 (11.3)	41.4 (12.5)	43.8 (12.0)	42.5 (11.2)	43.8 (11.6)	43.3 (12.5)
Gender, No. (%)	159 (66.5)	146 (77.7)	76 (79.2)	72 (69.2)	79 (76.7)	60 (81.1)	114 (83.8)
Etiology, No. (%)							
HBV	160 (66.9)	133 (70.7)	71 (74.0)	77 (74.0)	86 (83.5)	47 (63.5)	113 (83.1)
Alcoholic	9 (3.8)	5 (2.7)	3 (3.1)	2 (1.9)	2 (1.9)	2 (2.7)	2 (1.5)
Others	70 (29.3)	50 (26.6)	22 (22.9)	25 (24.0)	15 (14.6)	25 (33.8)	21 (15.4)
Complications, No. (%)							
Ascites	6 (2.5)	6 (3.2)	9 (9.4)	15 (14.4)	19 (18.4)	14 (18.9)	51 (37.5)
Bacterial infection	10 (4.2)	8 (4.3)	7 (7.3)	9 (8.7)	16 (15.5)	16 (21.6)	41 (30.1)
Hepatic encephalopathy							
Not overt	239 (100.0)	188 (100.0)	96 (100.0)	104 (100.0)	99 (96.1)	72 (97.3)	122 (89.7)
Grade 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.9)	0 (0.0)	8 (5.9)
Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	2 (2.7)	4 (2.9)
Grade 4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	2 (1.5)
Laboratory results, median (IQR)							
Hemoglobin, g/L	141.0 (127.0, 152.0)	138.0 (128.8, 153.0)	136.0 (121.5, 145.2)	136.0 (117.2, 147.0)	136.0 (124.5, 151.0)	130.0 (114.8, 142.0)	128.0 (115.0, 144.0)
White blood cell, 10^9/L	4.6 (4.0, 5.4)	4.7 (3.9, 5.9)	5.2 (4.1, 6.3)	5.2 (4.2, 6.6)	6.2 (5.0, 7.6)	6.6 (5.2, 7.9)	7.2 (5.7, 9.8)
Platelet, 10^9/L	147.0 (113.0, 187.2)	139.5 (103.2, 177.5)	128.0 (102.0, 168.5)	136.5 (94.8, 171.2)	132.0 (98.0, 160.0)	129.0 (98.0, 173.5)	122.0 (90.2, 166.0)
International normalized ratio	1.1 (1.0, 1.2)	1.2 (1.0, 1.3)	1.3 (1.1, 1.6)	1.4 (1.1, 1.7)	1.6 (1.3, 2.1)	1.5 (1.2, 2.0)	1.8 (1.5, 2.5)
Creatinine, mg/dL	0.7 (0.6, 0.9)	0.8 (0.7, 0.9)	0.7 (0.6, 0.8)	0.7 (0.6, 0.9)	0.7 (0.6, 0.8)	0.8 (0.7, 0.9)	0.8 (0.6, 1.0)
Albumin, g/L	40.1 (36.8, 43.5)	38.3 (34.2, 42.0)	35.9 (33.0, 39.5)	34.5 (31.8, 37.9)	33.2 (30.0, 37.4)	32.7 (29.3, 36.2)	32.2 (29.9, 35.6)
Alanine transaminase, IU/L	408.0 (184.0, 751.5)	790.0 (383.5, 1137.0)	832.0 (310.5, 1360.0)	653.0 (301.1, 1331.2)	639.4 (273.2, 1275.5)	509.9 (147.2, 952.0)	302.4 (145.0, 818.1)
Aspartate transaminase, IU/L	248.0 (144.0, 431.2)	428.9 (220.0, 750.2)	535.5 (223.1, 1007.2)	508.4 (267.7, 908.2)	433.0 (197.0, 949.7)	284.6 (153.7, 680.0)	207.1 (144.8, 571.2)
Sodium, mmol/L	140.4 (138.0, 142.0)	140.0 (138.0, 141.4)	139.0 (136.5, 140.8)	139.0 (136.2, 140.8)	138.0 (136.0, 140.0)	137.0 (135.0, 139.0)	137.2 (135.0, 140.0)
Score, mean (SD)							
MELD	8.2 (2.8)	12.4 (3.3)	16.6 (3.0)	19.1 (3.9)	21.5 (4.2)	23.1 (4.8)	26.8 (5.7)
MELD-Na	8.1 (4.0)	12.7 (3.9)	17.5 (3.5)	20.1 (4.0)	22.4 (4.3)	24.3 (4.8)	27.7 (5.6)
CTP	5.3 (0.8)	6.9 (1.0)	7.8 (1.0)	8.3 (1.3)	8.7 (1.6)	8.9 (1.7)	9.6 (1.9)
Transplantation-free mortality							
28-day, No. (%)	0 (0.0)	1 (0.5)	0 (0.0)	1 (1.0)	7 (6.8)	4 (5.4)	27 (19.9)
90-day, No. (%)	3 (1.3)	2 (1.1)	2 (2.1)	5 (4.8)	10 (9.7)	11 (14.9)	37 (27.2)

SD, standard deviation; HBV, hepatitis B virus; IQR, interquartile range; MELD, the model of end-stage liver disease; MELD-Sodium, the model of end-stage liver disease with sodium; CTP, Child-Turcotte-Pugh.

almost horizontal when TB is below 12.1 mg/dL. While with TB exceeding 12.1 mg/dL, mortality rate is positively and linearly related to TB. It was consistent with the findings from the COXPH model presented in **Table 4**.

The mathematical TB cutoff was 12.1 mg/dL, with 33.1% (311/940) patients with advanced fibrosis exceeding the cutoff, corresponding to 2.9 and 8.0% adjusted 28- and 90-day transplantation-free mortality, respectively. The clinical TB cutoff was calculated to be 36.0 mg/dL (**Figure 4B**). With this cutoff, only 1.3% (12/940) patients could be stratified with a higher TB, among which 22.1% died during 90 days (**Table 5**).

DISCUSSION

The present prospective study with large sample size first time established the respective TB cutoffs for liver failure in cirrhosis and advanced fibrosis *via* calculation. Specifically, any increase of TB independently increases the risk of 90-day transplantation free mortality in patients with cirrhosis. While, for patients with

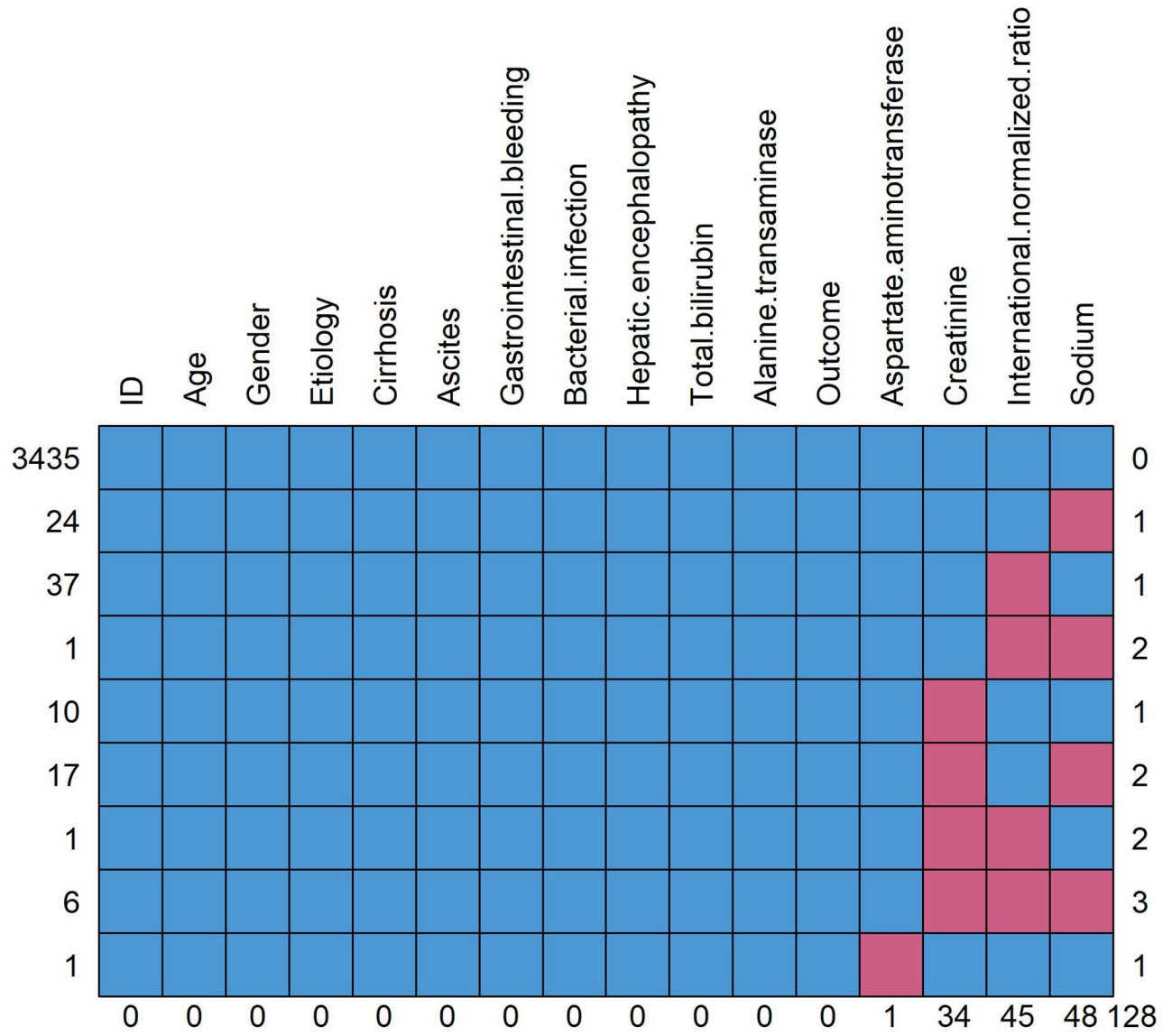


FIGURE 2 | Pattern of missing data. The red square means data missing and the blue square means data complete. The figure shows the overall number of missing data for each variable and the distribution of different missing types. Taking the "sodium" in the rightmost column as an example, a total of 48 patients have missing data of sodium, of which 24 patients (line 2) have missing data of sodium alone, 1 patient (line 4) has missing data of international standardized ratio and sodium, 17 patients (line 6) have missing data of creatinine and sodium and 6 patients (line 8) have missing data of creatinine, international normalized ratio and sodium.

advanced fibrosis, only TB >12 mg/dL increased the risk. More importantly, based on the sufficient data of large prospective cohorts and rigorous analysis, we intuitively demonstrated the different effects of TB on the mortality in patients with cirrhosis and advanced fibrosis, and calculated their mathematical and clinical cutoffs for liver failure, respectively. The findings from this study offer an essential component of evidence-based ACLF diagnostic criteria in HBV high-endemic areas.

Western researchers represented by EASL-CLIF believe that the three major conditions of ACLF are cirrhosis, ADs and organ failure (4). They did not think that ACLF will present in non-cirrhotic patients. In contrast, the Eastern researchers represented by APASL emphasize the "reversibility of ACLF" and exclude decompensated cirrhosis out of ACLF. To achieve a universally acceptable definition, the type of ACLF were suggested to be divided into three categories depending on underlying chronic liver diseases: type A (non-cirrhotic ACLF), type B (cirrhotic ACLF), and type C (decompensated cirrhotic ACLF) (13, 33, 34). However, these subjective concepts are not appropriate to serve as solid evidence.

A clear definition of liver failure has fundamental significance for the diagnosis of ACLF. From this perspective view, our

TABLE 3 | The unadjusted and adjusted hazard ratios of 90-day transplantation-free mortality due to total bilirubin in patients with cirrhosis.

Total bilirubin (mg/dL)		Number of patients	Number of death (%)	Hazard ratio of transplantation-free mortality (95% CI), *p*-value	
				Unadjusted	Adjusted*
As a continuous variable		2,592	430 (15.3)	1.077 (1.070–1.084), <0.001	1.060 (1.051–1.069), <0.001
As a categorical variable	0–2	839	35 (3.9)	1 (Reference)	1 (Reference)
	2–5	601	51 (8)	2.081 (1.354–3.200), 0.001	2.120 (1.364–3.295), 0.001
	5–8	215	25 (10.9)	2.915 (1.745–4.870), <0.001	2.520 (1.467–4.331), 0.001
	8–12	220	36 (15.7)	4.170 (2.619–6.640), <0.001	3.527 (2.154–5.776), <0.001
	12–16	172	38 (20.5)	5.858 (3.701–9.273), <0.001	4.808 (2.934–7.878), <0.001
	16–20	139	44 (28.8)	8.988 (5.765–14.012), <0.001	7.927 (4.861–12.928), <0.001
	Over 20	406	201 (42.8)	16.171 (11.289–23.166), <0.001	10.422 (6.874–15.802), <0.001
	P-value for trend			0.004	<0.001

**Adjusted for age, gender, etiology, hepatic encephalopathy grade, infection, ascites, gastrointestinal bleeding, international normalized ratio, creatinine, serum sodium and alanine transaminase.*

TABLE 4 | The unadjusted and adjusted hazard ratios of 90-day transplantation-free mortality due to total bilirubin in patients with advanced fibrosis.

Total bilirubin (mg/dL)		Number of patients	Number of death (%)	Hazard ratio of transplantation-free mortality (95% CI), *p*-value	
				Unadjusted	Adjusted*
As a continuous variable		940	70 (7.3)	1.109 (1.087–1.131), <0.001	1.080 (1.049–1.112), <0.001
As a categorical variable	0–2	239	3 (1.3)	1 (Reference)	1 (Reference)
	2–5	188	2 (1.1)	0.845 (0.141–5.056), 0.854	0.802 (0.130–4.956), 0.812
	5–8	96	2 (2.1)	1.653 (0.276–9.895), 0.582	1.443 (0.237–8.803), 0.691
	8–12	104	5 (4.7)	3.887 (0.929–16.267), 0.063	1.866 (0.401–8.681), 0.426
	12–16	103	10 (9.5)	8.131 (2.238–29.545), 0.001	4.186 (1.070–16.371), 0.040
	16–20	74	11 (14.5)	12.606 (3.517–45.188), <0.001	5.829 (1.476–23.026), 0.012
	Over 20	136	37 (25.9)	25.624 (7.899–83.118), <0.001	8.455 (2.358–30.312), 0.001
	P-value for trend			0.008	0.002

**Adjusted for age, gender, etiology, hepatic encephalopathy grade, infection, ascites, international normalized ratio, creatinine, serum sodium, and alanine transaminase.*

findings were obtained from quantitative analysis of the data from large cohort studies, which provides the possibility to stop this controversy. At first, we revealed the different effects of TB on mortality in patients with cirrhosis and advanced fibrosis, respectively, and proved that liver failure can be diagnosed by TB alone in cirrhosis but not in advanced fibrosis. Therefore, we demonstrated our hypothesis that the controversy between the East and the West on how to use TB to diagnose liver failure was caused by differences of the study population. Then, in the comparison of the TB-mortality correlation curves of different underlying chronic liver diseases, we found that the trend of the curve of compensated cirrhosis was similar to that of decompensated cirrhosis and quite different from that of advanced fibrosis. We observed the saturation effects in both the two curves of cirrhosis, which didn't present in the curve of advanced fibrosis. On the other hand, when TB increases from 0 to 12 mg/dL, the curves of cirrhosis rises simultaneously while the curve of advanced fibrosis keeps horizontal. These findings suggest that the mechanism of liver failure in cirrhosis, whether compensated and decompensated, could be similar and the same criteria can be used for diagnosis. However, it does not support the view of APASL to exclude decompensated cirrhosis but use the same criteria to diagnose ACLF in compensated cirrhosis and non-cirrhotic chronic liver diseases.

The definition of TB cutoff for liver failure is controversial as well. Though various TB cutoffs have been proposed in relevant studies, they were all initialized from expert consensus or existing models. Actually, few studies illuminated the independent and quantitative correlation between TB level and the short-term mortality of patients. A clinically meaningful cutoff would be one particular value that correlates with a marked change in physiological response and patient outcome (35). Thus, we calculated the TB cutoffs based on the mathematical definition (maximum of acceleration) and the clinical definition (15% 28-day transplantation-free mortality), respectively. In patients with cirrhosis, the two TB cutoffs are 14.2 mg/dL (mathematical) and 18.1 mg/dL (clinical), respectively, both the diagnosis rate (about

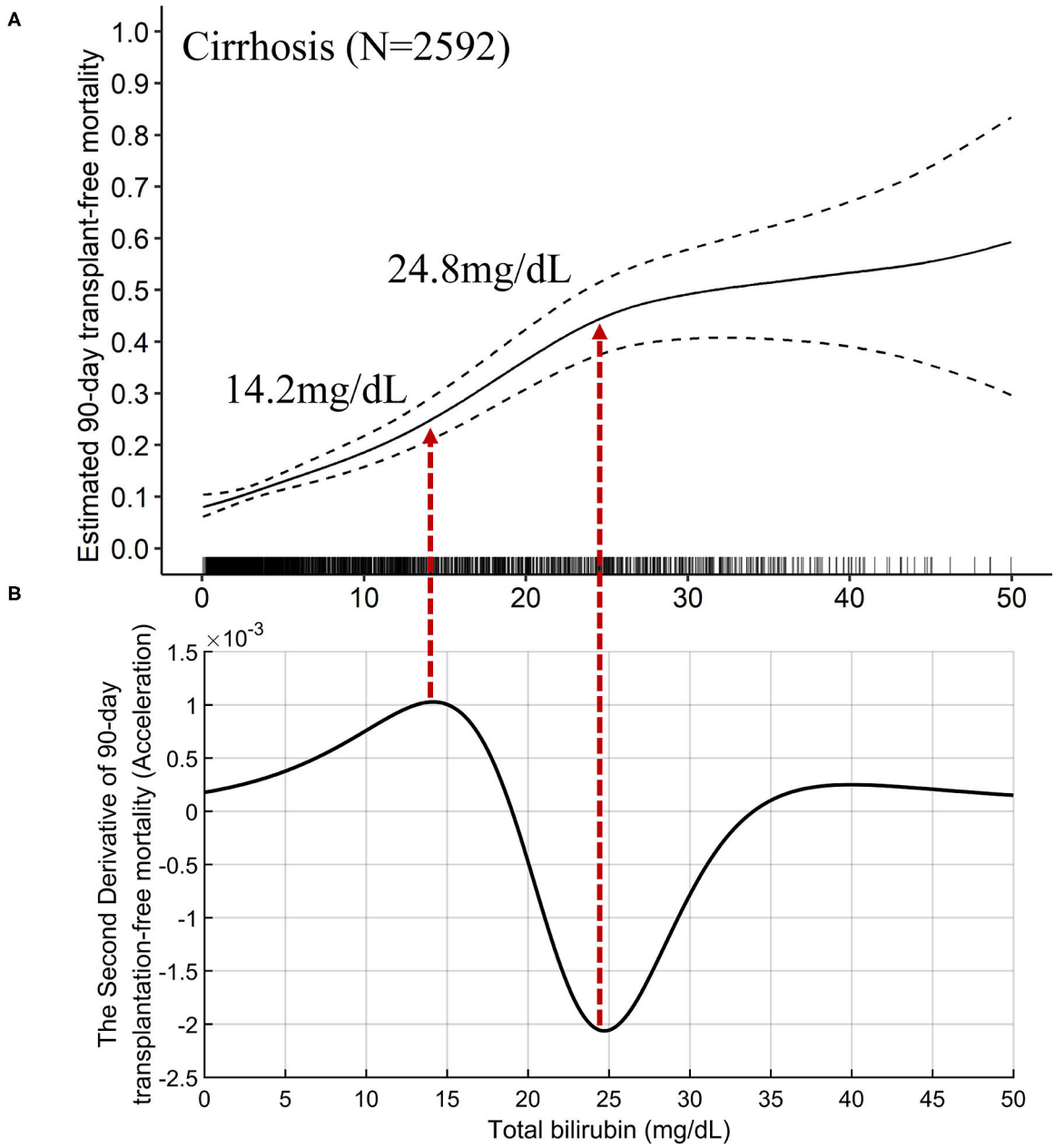


FIGURE 3 | The TB-mortality (90-day) correlation curve of cirrhosis and the corresponding second derivative (acceleration) curve. **(A)** the TB-mortality correlation curve of cirrhosis; **(B)** the second derivative (acceleration) of TB to mortality. TB, total bilirubin.

one fifth) and the corresponding adjusted 28-day mortality (about 15%) were acceptable. In patients with advanced fibrosis, the mathematical TB cutoff is 12.1 mg/dL, whose corresponding mortality (2.9%) was much lower than the clinical definition (15%). In case the TB cutoff of clinical definition (36.0 mg/dL) was applied, only 1.3% patients can be diagnosed as liver failure. Therefore, from the perspective of high short-term mortality, liver failure in advanced fibrosis cannot be diagnosed by TB alone.

This study has several strengths. First, it is the unique study in demonstrating the independent and quantitative correlation between TB and short-term mortality in hospitalized patients with cirrhosis and advanced fibrosis, respectively. Based on the findings of the study, we proved that it is unfeasible to use the same criteria to diagnose liver failure in patients with cirrhosis and advanced fibrosis, settling this protracted controversy between the ACLF consortiums from East and West. Second, the TB cutoffs for liver failure were calculated completely

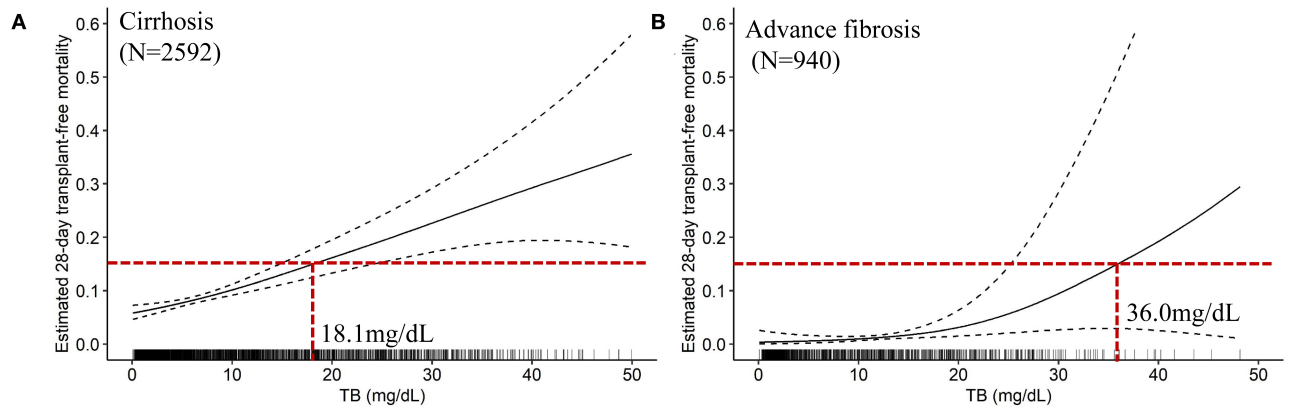


FIGURE 4 | The TB-mortality (28-day) correlation curves and the clinical cutoffs of cirrhosis and advanced fibrosis. **(A)** the TB-mortality correlation curve (28-day) of cirrhosis; **(B)** the TB-mortality correlation curve (28-day) of advanced fibrosis. TB, total bilirubin.

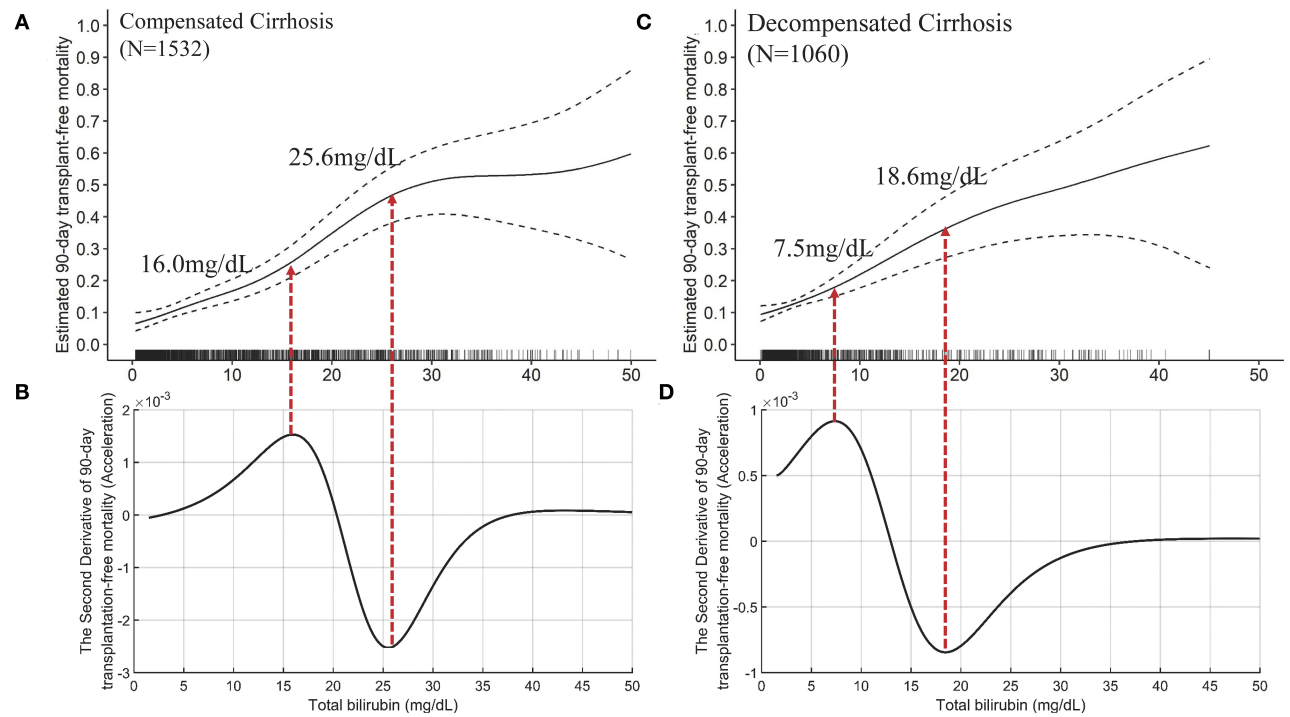


FIGURE 5 | The TB-mortality (90-day) correlation curves of compensated cirrhosis and decompensated cirrhosis and their corresponding second derivative (acceleration) curves. **(A)** the TB-mortality correlation curve of compensated cirrhosis; **(B)** the second derivative (acceleration) of TB to mortality *(compensated cirrhosis); **(C)** the TB-mortality correlation curve of decompensated cirrhosis; **(D)** the second derivative (acceleration) of TB to mortality (decompensated cirrhosis). TB, total bilirubin.

based on mathematical methods, provide evidence-based reference for the establishment of ACLF diagnosis criteria. Third, the study based on the data of hospitalized patients with representative characteristics of cirrhosis and advanced in China, which implied that our findings could be applicable to the 2-billion-population HBV high-epidemic area (15). Finally, the perspective multicenter design will result in low rates of loss to follow-up and missing variables, ensuring the reliability of our conclusions.

Several limitations should be acknowledged. First, we excluded the non-cirrhotic patients with a FIB-4 score < 1.45. However, this strategy is implemented to rule-out the patients with merely mild or without fibrosis. The liver conditions of these patients are close to normal, whose features of liver failure (if presented) are similar to that of acute liver failure. Moreover, the 90-day mortality of these patients was only 1.1% (2/178), not the potential victims of ACLF. Second, we excluded the patients received LT within 90 days, which may

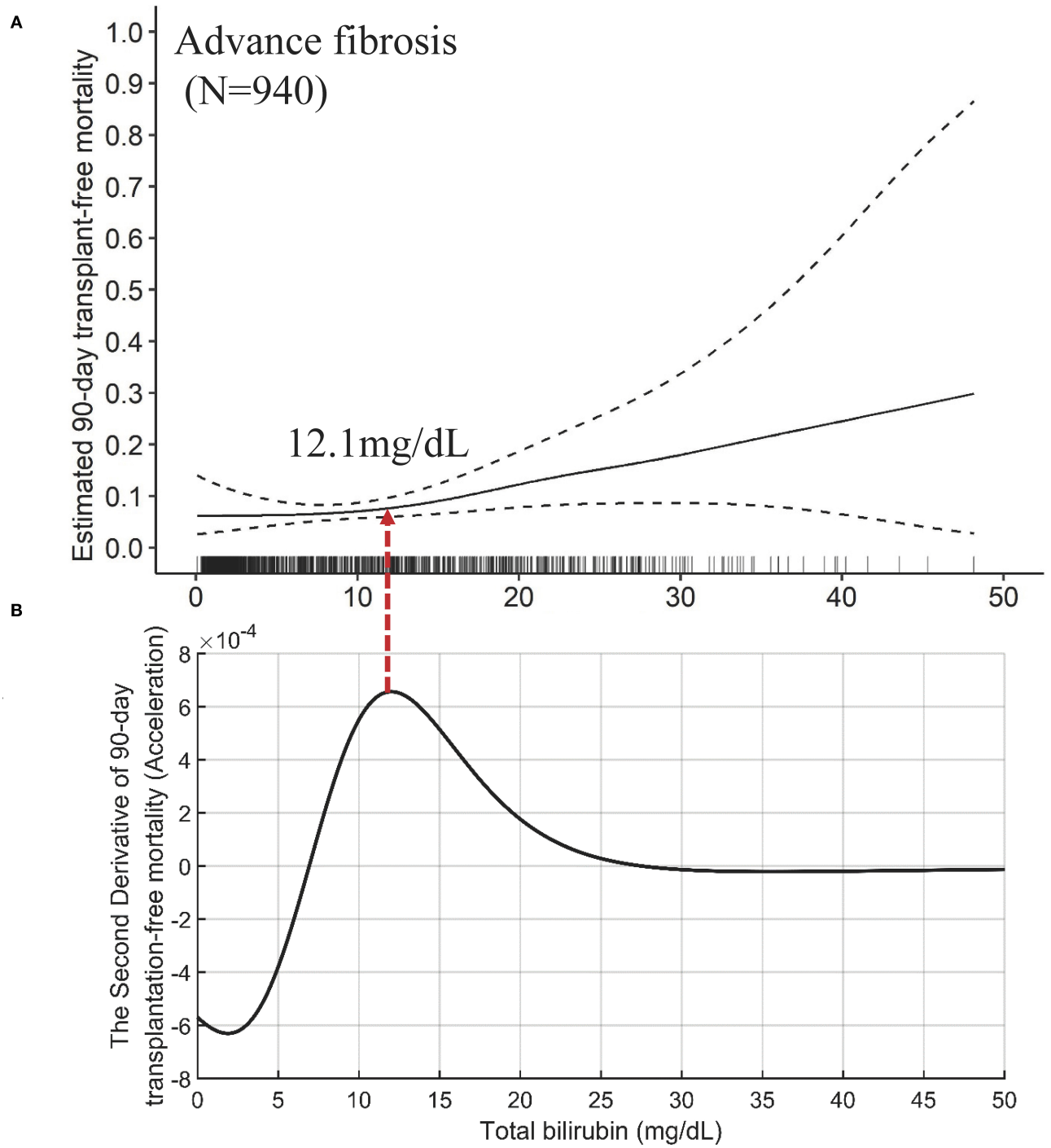


FIGURE 6 | The TB-mortality (90-day) correlation curve of advanced fibrosis and the corresponding second derivative (acceleration) curve. **(A)** the TB-mortality correlation curve of advanced fibrosis; **(B)** the second derivative (acceleration) curve of TB to mortality. TB, total bilirubin.

have some potential inference on the results. However, taking transplant-free mortality as the primary outcome is a common practice in ACLF research to obtain the natural prognosis of patients.

CONCLUSION

This study is the first one to clearly demonstrate the different effects of TB on 90-day mortality in patients with cirrhosis

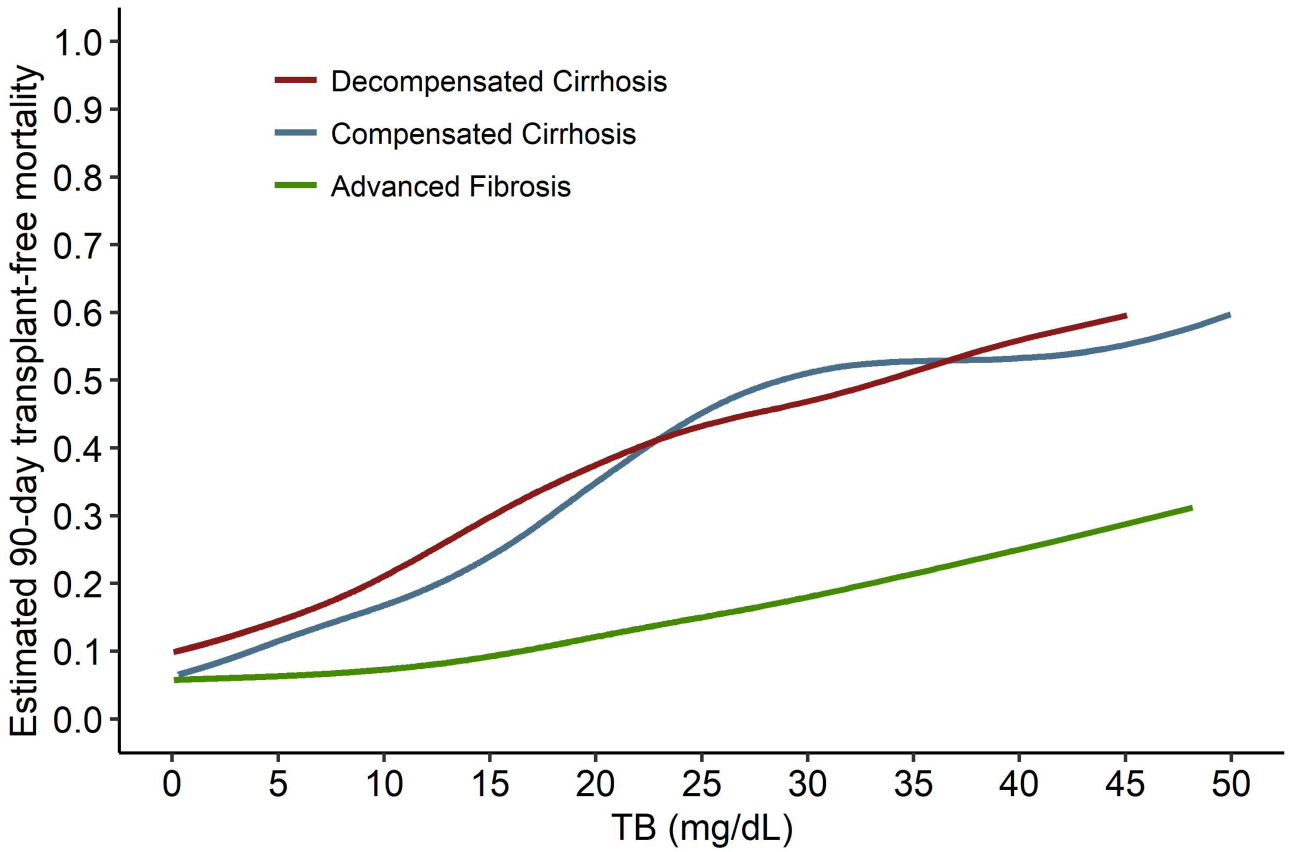


FIGURE 7 | The comparison of the TB-mortality curves between decompensated cirrhosis, compensated cirrhosis, and advanced fibrosis. TB, total bilirubin.

TABLE 5 | The multivariable adjusted 28-day and 90-day mortality based on the calculated the mathematical and clinical TB cutoffs for liver failure.

	Meaning of the TB cutoff	Value of the cutoff	Percentage of patients with TB exceeding the cutoff	Corresponding adjusted transplantation-free mortality	
				28 days	90 days
Cirrhosis	Peak of acceleration curve (mathematical cutoff)	14.2 mg/dL	23.3% (605/2592)	13.3%	25.0%
	Valley of acceleration curve	24.8 mg/dL	10.3% (268/2592)	19.1%	44.8%
	Reaching 15% 28-day transplantation-free mortality (clinical cutoff)	18.1 mg/dL	18.2% (471/2592)	15.0%	32.7%
Advanced fibrosis	Peak of acceleration curve (mathematical cutoff)	12.1 mg/dL	33.1% (311/940)	2.9%	8.0%
	Reaching 15% 28-day transplantation-free mortality (clinical cutoff)	36.0 mg/dL	1.3% (12/940)	15.0%	22.1%

TB, total bilirubin.

and advanced fibrosis, proving that liver failure can be diagnosed by TB alone in cirrhosis but not in advanced fibrosis. We also proposed the first mathematical and clinical TB cutoffs (14.2 and 18.1 mg/dL in cirrhosis, and 12.1 and 36.0 mg/dL in advanced fibrosis) for liver failure via calculation. Our innovative work will become the foundation of the establishing evidence-based ACLF diagnostic criteria in HBV high-endemic areas.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s at: aclf_group@163.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethics Board of Shanghai Renji Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HL had full access to all the data used in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. HL: concept, design, and supervision. LQ and HL: drafting of the manuscript. WZ and HL: critical revision of the manuscript for important intellectual content. LQ and WZ: statistical analysis. HL, GD, XW, XZhe, YHu, JiC, ZM, YG, FL, and XL: obtained funding. All authors: acquisition of data, administrative, technical, or material support.

FUNDING

This work was supported by Grants from the National Science and Technology Major Project (2018ZX10302206, 2018ZX10723203, and 2017ZX10202202). It was partially supported by the National Key Research and Development Program of China (No. 2017YFC0908100 and 2017YFC0908103), Shanghai Hospital Development Commission (16CR1024B and SHDC2020CR1037B), Shanghai Municipal Education Commission–Guofeng Clinical Medicine and Shanghai Municipal Government Funding, the National Natural Science Foundation of China (82070650, 81271884, 81930061, 81401665, 81461130019, 81470038, 81470869, 81473641, 81900579, 81660333, 81670576, 81700561, 81870425, and GZ1263), the Foundation for Innovative Research groups of the Natural Science Foundation of Hubei Province of China (2018CFA031), and the Shandong Province Natural Science Foundation (No. ZR2019PH052).

ACKNOWLEDGMENTS

We thank all the patients who participated in this study. We thank the following Chinese (Acute on) Chronic Liver Failure Consortium (Ch-CLIF.C) members and participants for their contributions to this study: Department of Gastroenterology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University— Bo Zeng, Liuying Chen, Shijin Wang, Tongyu Wang, Fuchen Dong and Dandan Wu; Centre of Integrative Medicine, Beijing Ditan Hospital, Capital Medical University— Yuxin Li, Yunyi Huang; Department of Infectious Diseases, Southwest Hospital, Third Military Medical University (Army Medical University)— Jie Xia; Yi Zhou; Baoyan Xu; Shuning Sun, Yunjie Dan; Department of Infectious Disease, Hunan Key Laboratory of Viral Hepatitis, Xiangya Hospital, Central South University— Chengjin Liao, Xiaoxiao Liu; Department of Infectious Diseases, Institute of Infection and Immunology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology— Jing Liu, Ling Xu, Shue Xiong; Hepatology Unit, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University— Guotao Zhong, Congyan Zhu; Department of Hepatology, First Hospital of Jilin University— Chang Jiang, Xiaoyu Wen; Department of Infectious Disease, Taihe Hospital, Hubei University of Medicine—Qing Lei; Department of Liver Intensive Care Unit, Shanghai Public Health Clinical Centre, Fudan University —Liujuan Ji, Jiefei Wang; Department of Infectious Diseases and Hepatology, Second Hospital of Shandong University—Xuanqiong Fang, Ziyu Wang; Liver Disease Centre, First Affiliated Hospital of Xinjiang Medical University—Fang, rong Jie, Nan Li; Department of Infectious Disease, Henan Provincial People's Hospital—Huiming Jin; Department of Infectious Diseases, Henan Provincial People's Hospital— Hai Li; Department of Infectious Diseases, Affiliated Hospital of Logistics University of People's Armed Police Force— Shaoyang Wang.

REFERENCES

1. Mezzano G, Juanola A, Cardenas A, Mezey E, Hamilton JP, Pose E, et al. Global burden of disease: acute-on-chronic liver failure, a systematic review and meta-analysis. *Gut.* (2021). doi: 10.1136/gutjnl-2020-322161
2. Bajaj JS, O'Leary JG, Reddy KR, Wong F, Biggins SW, Patton H, et al. Survival in infection-related acute-on-chronic liver failure is defined by extrahepatic organ failures. *Hepatology.* (2014) 60:250–6. doi: 10.1002/hep.27077
3. Kim TY, Song DS, Kim HY, Sinn DH, Yoon EL, Kim CW, et al. Characteristics and discrepancies in acute-on-chronic liver failure: need for a unified definition. *PLoS ONE.* (2016) 11:e0146745. doi: 10.1371/journal.pone.0146745
4. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology.* (2013) 144:1426–37; 1437.e1421–9. doi: 10.1053/j.gastro.2013.02.042
5. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. *Hepatol Int.* (2019) 13:353–90. doi: 10.1007/s12072-019-09946-3
6. Wu T, Li J, Shao L, Xin J, Jiang L, Zhou Q, et al. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure. *Gut.* (2018) 67:2181–91. doi: 10.1136/gutjnl-2017-314641
7. Choudhury A, Jindal A, Maiwall R, Sharma MK, Sharma BC, Pamecha V, et al. Liver failure determines the outcome in patients of acute-on-chronic liver failure (ACLF): comparison of APASL ACLF research consortium (AARC) and CLIF-SOFA models. *Hepatol Int.* (2017) 11:461–71. doi: 10.1007/s12072-017-9816-z
8. Arroyo V, Moreau R, Jalan R, Gines P, EASL-CLIF Consortium CANONIC Study. Acute-on-chronic liver failure: a new syndrome that will re-classify cirrhosis. *J Hepatol.* (2015) 62:S131–43. doi: 10.1016/j.jhep.2014.11.045

9. Gustot T, Fernandez J, Garcia E, Morando F, Caraceni P, Alessandria C, et al. Clinical course of acute-on-chronic liver failure syndrome and effects on prognosis. *Hepatology.* (2015) 62:243–52. doi: 10.1002/hep.27849
10. Li H, Chen LY, Zhang NN, Li ST, Zeng B, Pavesi M, et al. Characteristics, diagnosis and prognosis of acute-on-chronic liver failure in cirrhosis associated to hepatitis B. *Sci Rep.* (2016) 6:25487. doi: 10.1038/srep25487
11. Shawcross DL, Austin MJ, Abeles RD, McPhail MJW, Yeoman AD, Taylor NJ, et al. The impact of organ dysfunction in cirrhosis: survival at a cost? *J Hepatol.* (2012) 56:1054–62. doi: 10.1016/j.jhep.2011.12.014
12. Olson JC, Wendon JA, Kramer DJ, Arroyo V, Jalan R, Garcia-Tsao G, et al. Intensive care of the patient with cirrhosis. *Hepatology.* (2011) 54:1864–72. doi: 10.1002/hep.24622
13. Hernaez R, Sola E, Moreau R, Gines P. Acute-on-chronic liver failure: an update. *Gut.* (2017) 66:541–53. doi: 10.1136/gutjnl-2016-312670
14. Trebicka J, Fernandez J, Papp M, Caraceni P, Laleman W, Gambino C, et al. The PREDICT study uncovers three clinical courses in acutely decompensated cirrhosis with distinct pathophysiology. *J Hepatol.* (2020) doi: 10.1016/j.jhep.2020.11.012
15. Polaris Observatory Collaborators. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *Lancet Gastroenterol Hepatol.* (2018) 3:383–403. doi: 10.1016/S2468-1253(18)30056-6
16. Sarin SK, Kumar M, Eslam M, George J, Al Mahtab M, Akbar SMF, et al. Liver diseases in the Asia-Pacific region: a Lancet Gastroenterology & Hepatology Commission. *Lancet Gastroenterol Hepatol.* (2019) 5:167–228. doi: 10.1016/S2468-1253(19)30342-5
17. Shi Y, Yang Y, Hu Y, Wu W, Yang Q, Zheng M, et al. Acute-on-chronic liver failure precipitated by hepatic injury is distinct from that precipitated by extrahepatic insults. *Hepatology.* (2015) 62:232–42. doi: 10.1002/hep.27795
18. Malinchoc M, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology.* (2000) 31:864–71. doi: 10.1053/he.2000.5852
19. Jalan R, Pavesi M, Saliba F, Amoros A, Fernandez J, Holland-Fischer P, et al. The CLIF Consortium Acute Decompensation score (CLIF-C ADs) for prognosis of hospitalised cirrhotic patients without acute-on-chronic liver failure. *J Hepatol.* (2015) 62:831–40. doi: 10.1016/j.jhep.2014.11.012
20. Bernal W, Jalan R, Quaglia A, Simpson K, Wendon J, Burroughs A. Acute-on-chronic liver failure. *Lancet.* (2015) 386:1576–87. doi: 10.1016/S0140-6736(15)00309-8
21. Sarin SK, Kumar A, Almeida JA, Chawla YK, Fan ST, Garg H, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL). *Hepatol Int.* (2009) 3:269–82. doi: 10.1007/s12072-008-9106-x
22. Chen T, Yang Z, Choudhury AK, Al Mahtab M, Li J, Chen Y, et al. Complications constitute a major risk factor for mortality in hepatitis B virus-related acute-on-chronic liver failure patients: a multi-national study from the Asia-Pacific region. *Hepatol Int.* (2019) 13:695–705. doi: 10.1007/s12072-019-09992-x
23. Gu WY, Xu BY, Zheng X, Chen J, Wang XB, Huang Y, et al. Acute-on-chronic liver failure in China: rationale for developing a patient registry and baseline characteristics. *Am J Epidemiol.* (2018) 187:1829–39. doi: 10.1093/aje/kwy083
24. Qiao L, Wang X, Deng G, Huang Y, Chen J, Meng Z, et al. Cohort profile: a multicentre prospective validation cohort of the Chinese Acute-on-Chronic Liver Failure (CATCH-LIFE) study. *BMJ open.* (2021) 11:e037793. doi: 10.1136/bmjopen-2020-037793
25. Moore KP, Wong F, Gines P, Bernardi M, Ochs A, Salerno F, et al. The management of ascites in cirrhosis: report on the consensus conference of the International Ascites Club. *Hepatology.* (2003) 38:258–66. doi: 10.1053/jhep.2003.50315
26. Blei AT, Cordoba J, Practice Parameters Committee of the American College of Gastroenterology. Hepatic encephalopathy. *Am J Gastroenterol.* (2001) 96:1968–76. doi: 10.1111/j.1572-0241.2001.03964.x
27. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection comparison with liver biopsy and fibrotest. *Hepatology.* (2007) 46:32–6. doi: 10.1002/hep.21669
28. Kim WR, Biggins SW, Kremers WK, Wiesner RH, Kamath PS, Benson JT, et al. Hyponatremia and mortality among patients on the liver-transplant waiting list. *N Engl J Med.* (2008) 359:1018–26. doi: 10.1056/NEJMoa0801209
29. Child C, Turcotte J. Surgery and portal hypertension. *Major Probl Clin Surg.* (1964) 1964:1–85.
30. Wood S. *Generalized Additive Models: An Introduction with R*. Boca Raton, FL: Chapman & Hall (2006). doi: 10.1201/9781420010404
31. Wood SN, Augustin NH. GAMs with integrated model selection using penalized regression splines and applications to environmental modelling. *Ecol Modell.* (2002) 157:157–77. doi: 10.1016/S0304-3800(02)00193-X
32. Deboeck PR, Nicholson J, Kouros C, Little TD, Garber J. Integrating developmental theory and methodology: using derivatives to articulate change theories, models, and inferences. *Appl Dev Sci.* (2016) 19:217–31. doi: 10.1080/10888691.2015.1021924
33. Jalan R, Yurdaydin C, Bajaj JS, Acharya SK, Arroyo V, Lin HC, et al. Toward an improved definition of acute-on-chronic liver failure. *Gastroenterology.* (2014) 147:4–10. doi: 10.1053/j.gastro.2014.05.005
34. Bajaj JS, Moreau R, Kamath PS, Vargas HE, Arroyo V, Reddy KR, et al. Acute-on-chronic liver failure: getting ready for prime time? *Hepatology.* (2018) 68:1621–32. doi: 10.1002/hep.30056
35. Spaite DW, Hu C, Bobrow BJ, Chikani V, Sherrill D, Barnhart B, et al. Mortality and prehospital blood pressure in patients with major traumatic brain injury: implications for the hypotension threshold. *JAMA Surg.* (2017) 152:360–8. doi: 10.1001/jamasurg.2016.4686

Disclaimer: This study is based in part on data provided by 15 hospitals located in 14 different cities in China (Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University [Shanghai]; Southwest Hospital, Third Military Medical University (Army Medical University) [Chongqing]; Beijing Ditan Hospital, Capital Medical University [Beijing]; Union Hospital, Tongji Medical College, Huazhong University of Science and Technology [Wuhan]; Xiangya Hospital, Central South University [Changsha]; Nanfang Hospital, Southern Medical University [Guangzhou]; Taihe Hospital, Hubei University of Medicine [Shiyan]; The First Hospital of Jilin University [Changchun]; Shanghai Public Health Clinical Centre, Fudan University [Shanghai]; The Second Hospital of Shandong University [Jinan]; The First Affiliated Hospital of Xinjiang Medical University [Urumqi]; The First Affiliated Hospital of School of Medicine, Zhejiang University [Hangzhou]; Henan Provincial People's Hospital [Zhengzhou]; Affiliated Hospital of Logistics University of People's Armed Police Force [Tianjin]; Fuzhou General Hospital of Nanjing Military Command [Fujian]. The interpretations and conclusions contained herein are those of the researchers and do not necessarily represent the views of the Health Services of the involved cities or of the national government. None of the involved cities' or national Health Services express any opinion in relation to this study. The opinions, results, and conclusions reported herein are those of the authors and are independent of the funding sources.

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.

frontiers in Medicine

ORIGINAL RESEARCH
published: 06 July 2021
doi: 10.3389/fmed.2021.655863

Low Growth Hormone Levels Predict Poor Outcome of Hepatitis B Virus-Related Acute-on-Chronic Liver Failure

Daxian Wu[1], Lingjian Zhang[2,3], Shanshan Ma[2,3], Yalei Zhao[2,3], Ronggao Chen[4], Fen Zhang[2,3], Qiuhong Liu[2,3], Xiaowei Xu[2,3,5] and Zhongyang Xie[2,3,5]**

[1] Department of Infectious Diseases, The First Affiliated Hospital of Nanchang University, Nanchang, China, [2] Collaborative Innovation Centre for Diagnosis and Treatment of Infectious Diseases, College of Medicine, First Affiliated Hospital, Zhejiang University, Hangzhou, China, [3] State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, School of Medicine, First Affiliated Hospital, Zhejiang University, Hangzhou, China, [4] Department of Hepatobiliary and Pancreatic Surgery, School of Medicine, First Affiliated Hospital, Zhejiang University, Hangzhou, China, [5] Department of Infectious Diseases, School of Medicine, First Affiliated Hospital, Zhejiang University, Hangzhou, China

OPEN ACCESS

Edited by:
Yu-Chen Fan,
Shandong University, China

Reviewed by:
Enqiang Chen,
Sichuan University, China
Faouzi Saliba,
Hôpital Paul Brousse, France

***Correspondence:**
Xiaowei Xu
xxw69@zju.edu.cn
Zhongyang Xie
zyxie@zju.edu.cn

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 19 January 2021
Accepted: 02 June 2021
Published: 06 July 2021

Citation:
Wu D, Zhang L, Ma S, Zhao Y, Chen R, Zhang F, Liu Q, Xu X and Xie Z (2021) Low Growth Hormone Levels Predict Poor Outcome of Hepatitis B Virus-Related Acute-on-Chronic Liver Failure. Front. Med. 8:655863. doi: 10.3389/fmed.2021.655863

Background and Aims: Hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF) remains a serious entity with high mortality. Growth hormone (GH) is related to the liver metabolism and regeneration. The present study aimed to explore the changes and prognostic efficacy of GH on the outcome of HBV-ACLF.

Methods: A prospective cohort of 124 patients and a cross-sectional cohort of 142 subjects were enrolled. GH and insulin-like growth factor-1(IGF-1) were detected by ELISA. Thirty-day survival was collected and the association between GH and the 30-day mortality of HBV-ACLF was analyzed.

Results: The mean age of the whole prospective cohort was 46.61 ± 12.71 years, and 19 (15.3%) patients were female. The median (IQR) of GH levels in non-survivors were 1106.55 (674.25, 1922.4) pg/ml, which were significantly lower than in survivors ($p < 0.001$). In the cross-sectional cohort, GH level was significantly higher in liver cirrhosis - acute decompensation (LC-AD) group than liver cirrhosis (LC) group ($p < 0.001$) while IGF-1 decreased significantly in LC, LC-AD, ACLF groups than health control (HC) and chronic Hepatitis B (CHB) groups ($p < 0.001$). The area under the receiver operating characteristic curve (AUROC) of GH for predicting 30-day mortality was 0.793. We built a new prognostic model, namely MELD-GH, which showed better predictive efficacy than Child-Pugh, MELD, CLIF-SOFA, and CLIF-C ACLF scores.

Conclusions: Low GH predicted the poor outcome of HBV-ACLF patients. GH and IGF-1 levels were differently distributed among HC, CHB, LC, LC-AD, and ACLF patients. MELD-GH had better predictive accuracy when compared to Child-Pugh, MELD, CLIF-SOFA, and CLIF-C ACLF scores.

Keywords: growth hormone, hepatitis B, acute-on-chronic liver failure, mortality, prognostic model

INTRODUCTION

Currently in China, there are around 35 million patients with hepatitis B virus (HBV) infection, and the social burden caused by chronic hepatitis B (CHB) is still heavy (1). Moreover, the reactivation of HBV is the leading cause of acute-on-chronic liver failure (ACLF) (2, 3). ACLF is characterized as acute deterioration of liver function, organ failure and high short-term mortality in the presence of preexisting chronic liver diseases (CLDs) (4, 5), which are associated with organ failures and high short-term mortality.

So far, the pathophysiological mechanism of ACLF hasn't been elucidated clearly. Disordered immune function and liver regeneration are considered two factors to play key roles on the prognosis of ACLF patients (2). After the injury of a precipitating event, massive and sub-massive hepatic necrosis occurred (6), which caused excessive immune responses and pulled the trigger of liver regeneration. Recent studies suggested that, there might be two distinct regeneration patterns in livers with massive hepatic necrosis in patients with acute liver failure (7). Unfortunately, our knowledge on the mechanism of the regeneration is quite limited and need further study.

Growth hormone (GH), as one of the most important endocrine hormones in human body, plays an important role in promoting growth and regulating carbohydrate, lipid, protein, and mineral metabolism. It also plays a vital role on liver regeneration (8–10). Some studies have reported that there might be a correlation between GH and liver disease (11, 12). For example, in alcoholic liver disease and non-alcoholic liver disease, GH may be involved in the development of diseases and affect lipid metabolism in hepatocytes (13, 14). Some reports have also reported a reduction in GH concentrations in patients with reciprocal cirrhosis, which was associated with prognosis (15). Insulin-like-growth factor-1 (IGF-1), which is synthesized mainly in the liver, mediates most of the biological functions of GH (16) and is reported to participated in the process of liver regeneration (17). However, whether GH and IGF-1 regulate the liver regeneration and affect the prognosis of ACLF patients remains unknown.

In this study, we built a prospective cohort of HBV-ACLF patients, and identified differences in serum levels of GH and IGF-1 between survival and non-survival group and then explored their variability in a cross-sectional cohort. Furthermore, we built a prognostic model containing GH and evaluated its efficacy of for predicting short-term outcomes of HBV-ACLF patients.

METHODS

Patients

Two cohorts were enrolled in this study, and the flow chart was in **Figure 1**. In the first cohort, we enrolled HBV-ACLF patients who presented with a new episode of acute hepatic insult manifesting as acute jaundice, coagulopathy, ascites, upper gastrointestinal bleeding, and hepatic encephalopathy (HE) between 1 January 2018 and 1 October 2018. Patients were divided into survivor and non-survivor groups according to their survival status of 30 days after admission. Patients undergoing liver transplantation were excluded. In the second cohort, a cross-sectional investigation was performed using a cohort of 142 subjects including healthy controls (HCs), patients with CHB, liver cirrhosis (LC), liver cirrhosis-acute decompensation (LC-AD) and ACLF. All HCs, CHB and LC subjects were recruited from the outpatient clinic. Among the 38 enrolled ACLF patients, 21 survived patients were placed into ACLF-S group while the remaining 17 were placed into ACLF-D group. Patients with ACLF received a standard treatment, including nucleoside analogs for HBV DNA-positive patients; diammonium glycyrrhizinate and Ademetionine for protection of liver function; sodium restriction, diuretics and paracentesis combined with albumin infusion for ascites; lactulose and L-ornithine aspartate for HE; prophylactic antibiotics for bacterial infections and renal replacement therapy for hepato-renal syndrome. Eleven non-survivors (11/38) and 35 survivors (35/124) received plasma exchange (PE) combined with hemofiltration (HF) treatment. All subjects were enrolled or recruited in the First Affiliated Hospital of Zhejiang University. This study was conducted in compliance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University. Written informed consent was obtained from all patients or their legal representatives.

Inclusion and Exclusion Criteria

ACLF was diagnosed according to the Asian Pacific Association for the Study of the Liver (APASL) criteria published in 2014 (3). According to the APASL criteria, ACLF was defined as an acute hepatic insult manifesting as jaundice (serum levels of bilirubin $\geq$ 5 mg/dl) and coagulopathy (international normalized ratio [INR] $\geq$ 1.5 or prothrombin activity $<$ 40%) within 4 weeks in a patient with previously diagnosed or undiagnosed CLD/cirrhosis. Cirrhosis patients with a history of AD who met the above criteria were also diagnosed as ACLF. The Model for End-stage Liver Disease (MELD), the chronic liver failure-sequential organ failure assessment (CLIF-SOFA), CLIF-C ACLF (18) scores were calculated to evaluate the severity of patients. The diagnosis of LC was based on previous liver biopsy, endoscopic signs, radiologic imaging, clinical symptoms and laboratory parameters. HBV reactivation was defined as a $\geq$ 2 log increase in the HBV DNA level from previously stable baseline level or a level $\geq$ 100 IU/mL in patients in whom HBV DNA had been undetectable, or $\geq$ 20,000 IU/mL in those negative for HBV DNA at baseline (19). The exclusion criteria included: age $<$ 18 or $>$80 years, liver malignancy or any other type of cancer, history of liver transplantation or bone marrow transplantation, and any severe disease in other systems.

Data Collection

Data were collected at enrollment, and included vital signs, physical examination results, histories, complications and precipitating events. The laboratory measurements collected included white blood cell (WBC) count, neutrophils, red blood cell (RBC) count, platelet (PLT) count, C-reactive protein (CRP), alpha-fetoprotein (AFP), ferritin, total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST),

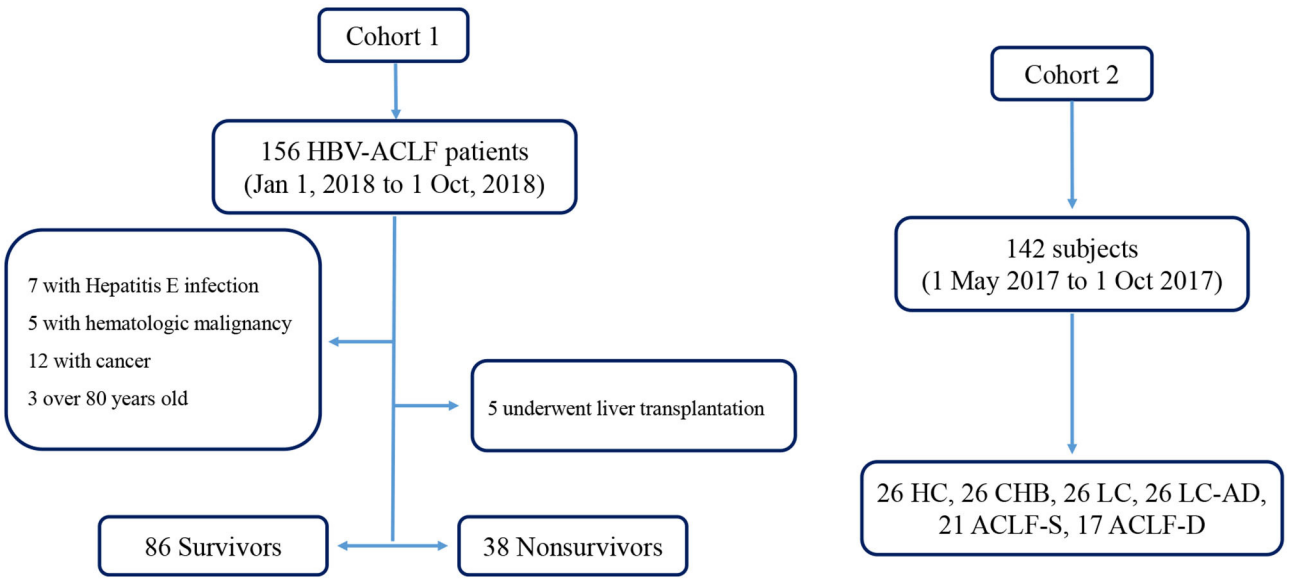


FIGURE 1 | Flow chart of enrolled cohorts. ACLF-D, acute-on-chronic liver failure-death; ACLF-S, acute-on-chronic liver failure-survival; CHB, chronic Hepatitis B; HBV-ACLF, Hepatitis B related acute-on-chronic liver failure; HC, health control; LC, liver cirrhosis; LC-AD, liver cirrhosis-acute decompensation.

serum levels of albumin (ALB), cholinesterase, gamma-glutamyl transpeptidase (GGT), triglycerides, cholesterol, total bilirubin (TB) level, serum levels of sodium, potassium (K), creatinine (Cr), blood urea nitrogen (BUN), international normalized ratio (INR), pulse oximetry, HBV infection biomarker levels, and HBV-DNA levels. Image measurements, including ultrasound, CT, and MRI, were also collected. Follow-up information was collected from medical records and follow-up phone calls at 30 days.

Sample Collection and Testing

Blood samples were obtained on the day following hospitalization and were centrifuged at 3,500 rpm for 10 min to separate the serum, which were stored at −80°C. GH was measured using an enzyme-linked immunosorbent assay (ELISA) with an ELISA detection kit (ab190811, Abcam). IGF-1, IGF-2, IGF receptor-1, and IGF receptor-2 levels in prospective cohort were detected using an immunoassay (QAH-IGF-1, RayBiotech). IGF-1 level in cross-sectional cohort was detected using an ELISA detection kit (ab211651, Abcam).

Statistical Analyses

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, v.22.0; SPSS, Inc., Chicago, IL, USA) and MEDCALC (MedCalc Software, Belgium). Continuous data are expressed as means ± standard deviations or medians with interquartile ranges (p25, p75), while categorical data are expressed as numbers (percentages). All tests were two-tailed, and $P < 0.05$ were considered indicative of statistical significance. Student's *t*-tests or non-parametric Mann–Whitney *U*-tests, as appropriate, were used to compare continuous data. Categorical data and ordered categorical data were compared using chi-square tests and Spearman rank correlation tests, respectively. The area under the receiver operating characteristic curve (AUROC) of the various prognostic scoring systems was compared by Z-tests using Delong's method.

RESULTS

Characteristics of the Prospective HBV-ACLF Cohort

A total of 124 HBV-ACLF patients were enrolled in the prospective cohort. As shown in **Table 1**, the mean patient age was 46.61 ± 12.71 years, and 19 (15.3%) patients were female. Among the whole cohort, 69 (55.6%) patients were cirrhotic, and 25 (20.2%) had acute decompensation (AD) history. The MELD, CLIF-SOFA, CLIF-C ACLF scores were 23.09 ± 5.61, 8.15 ± 1.95, and 42.01 ± 9.79, respectively.

Based on 30-day survival, the patients were divided into non-survivor ($n = 38$) and survivor groups ($n = 86$). The mean age of survivor and non-survivor groups was 44.58 ± 12.15 and 51.21 ± 12.9, while 12.8% in survivor group were female and 21.1% in non-survivor group. Compared to the survivor group, non-survivors were older ($P = 0.009$) and had lower levels of cholesterol ($P = 0.003$) and albumin ($P = 0.035$). Conversely, neutrophil counts ($P = 0.005$), INR ($P < 0.0001$), TB ($P = 0.001$) and creatine ($P = 0.005$) levels, and MELD ($P < 0.0001$), CLIF-SOFA ($P < 0.0001$), and CLIF-C ACLF ($P < 0.0001$) scores were higher in the non-survivor group.

Distribution of GH in Prospective Cohort Patients

In the prospective cohort of HBV-ACLF patients, the median GH levels in non-survivors were 1106.55 pg/ml, which were significantly lower ($P < 0.0001$) than in survivors (2930.55 pg/ml)

TABLE 1 | Characteristics of non-survivors and survivors in the HBV-ACLF cohort.

	Total ($n = 124$)	Non-survivor ($n = 38$)	Survivor ($n = 86$)	p-value (non-survivor vs. survivor)
Female, n(%)	19 (15.3)	8 (21.1)	11 (12.8)	0.239
Age (y)	46.61 ± 12.71	51.21 ± 12.9	44.58 ± 12.15	0.009
MAP (mm Hg)	89.86 ± 12.77	90.11 ± 17.16	89.75 ± 10.38	0.903
Alcoholism	13 (10.5)	7(18.4)	6 (7.0)	0.055
Cirrhosis	69 (55.6)	17 (44.7)	52 (60.5)	0.104
Previous decompensation	25 (20.2)	4 (10.5)	21 (24.4)	0.075
GIB	13 (10.5)	4 (10.5)	9 (10.5)	0.608
Infection	38 (30.6)	14 (36.8)	24 (27.9)	0.32
Ascites	74 (59.7)	24 (63.2)	50 (58.2)	0.599
HE	19 (15.3)	16 (42.1)	3 (3.5)	<0.0001
HBeAg-positive	50 (40.3)	14 (36.8)	36 (41.9)	0.599
LgDNA	5.37 ± 2.02	5.27 ± 2.25	5.41 ± 1.93	0.724
WBC ($\times 10^9$/L)	7.05 (5.4, 9.88)	8.45 (5.75, 11.55)	6.7 (4.98, 8.9)	0.011
NEU ($\times 10^9$/L)	5.1 (3.43, 6.98)	6.4 (4.23, 10.2)	4.65 (3.28, 6.15)	0.005
RBC ($\times 10^9$/L)	4.15 (3.68, 4.63)	4.19 (3.69, 4.65)	4.13 (3.68, 4.6)	0.856
PLT ($\times 10^9$/L)	113.4 ± 49.92	110.29 ± 48.22	114.77 ± 50.87	0.647
GH (pg/ml)	2,232 (1176.9, 4300.88)	1106.55 (674.25, 1922.4)	2930.55 (1778.85, 4799.33)	<0.0001
Ferritin (ng/ml)	2567.95 (1170.2, 4166.35)	2459.45 (1021.25, 5021.18)	2567.95 (1262.83, 3766.18)	0.704
Alpha-fetoprotein (ng/ml)	117.9 (40.48, 294.48)	111.85 (39.63, 277.6)	124.1 (40.48, 320.28)	0.615
CRP (mg/L)	11.15 (8.28, 15.45)	11.75 (9.3,15.78)	10.65 (8.1, 15.5)	0.191
TP (g/L)	58.15 (53.53, 62.38)	58.2 (54.03, 61.35)	58.1 (53.3, 62.55)	0.901
ALT (U/L)	268 (107.5, 596.25)	281 (140.75, 430.5)	257.5 (84, 700.75)	0.67
AST (U/L)	162.5 (86.5, 367)	178.5 (111, 401.75)	162 (85, 336.25)	0.265
ALB (g/L)	31.27 ± 4.26	30.06 ± 4.55	31.81 ± 4.03	0.035
ALP (U/L)	146.02 ± 39.74	148.05 ± 40.01	145.12 ± 39.82	0.706
Cholinesterase (U/L)	3721.79 ± 1438.38	3453.11 ± 1469.41	3840.51 ± 1416.85	0.168
GGT (U/L)	102.25 ± 58.9	93.03 ± 56.19	106.33 ± 59.93	0.248
Total Bilirubin (mmol/L)	299.65 (232.03, 405.3)	354.5 (288.73, 454.1)	278.15 (213.75, 381.78)	0.001
Creatine (mmol/L)	64 (57, 73.75)	72 (57, 115.25)	63 (57, 69.25)	0.005
BUN (mmol/L)	4.06 (3.11, 6.16)	5.28 (3.64, 7.74)	3.75 (3.05, 5.52)	0.003
Triglycerides (mmol/L)	1.28 (1.02, 1.66)	1.17 (0.98, 1.41)	1.35 (1.03, 1.79)	0.039
Cholesterol (mmol/L)	2.22 (1.69, 2.76)	1.7 (1.48, 2.62)	2.38 (1.84, 2.79)	0.003
Potassium (mmol/L)	4.22 (3.9, 4.78)	4.39 (3.94, 4.95)	4.16 (3.87, 4.6)	0.08
Sodium (mmol/L)	138 (135.25, 139)	137.5 (134.75, 139.25)	138 (136, 139)	0.703
Blood glucose (mmol/L)	4.05 (3.21, 5.1)	3.85 (2.96, 5.67)	4.12 (3.25, 4.92)	0.612
INR	1.99 (1.64, 2.53)	2.69 (2.32, 3.28)	1.86 (1.59, 2.19)	<0.0001
MELD	23.09 ± 5.61	28.44 ± 5.74	20.73 ± 3.57	<0.0001
CLIF-SOFA	8.15 ± 1.95	10 ± 2.41	7.33 ± 1.14	<0.0001
CLIF-C ACLF	42.01 ± 9.79	50.49 ± 9.69	38.27 ± 7.18	<0.0001

Continuous data are expressed as means ± SD or medians with interquartile ranges (p. 25, p. 75), and categorical data are expressed as numbers (percentages). ALP, phosphatase alkaline; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CLIF-C ACLF, European Association for the Study of Chronic Liver Failure; COSSH-ACLF, Chinese Group on the Study of Severe Hepatitis B; CRP, C-reactive protein; GGT, gamma-glutamyl transpeptidase; HBV, hepatitis B virus; HE, hepatic encephalopathy; INR, international normalized ratio; MAP, mean arterial pressure; MELD, Model for End-stage Liver Disease; RBC, red blood count; GIB, gastrointestinal hemorrhage; WBC, white blood count.

(**Figure 2A**). All patients were divided into four groups according the number of organ failures; patients with ≥3 organ failures had significantly lower GH levels compared to 1 ($P < 0.001$), and 2 ($P = 0.045$) organ failure groups (**Figure 2B**). Furthermore, patients with a MELD score > 25 had lower GH levels than those with a score between 20 and 25 ($P = 0.009$) (**Figure 2C**). However, there were no significant difference in GH levels between the MELD > 25 group and the MELD < 20 group. Comparisons of GH levels between patients with and without certain kinds of organ failure are shown in **Supplementary Figure 1**. Interestingly, patients

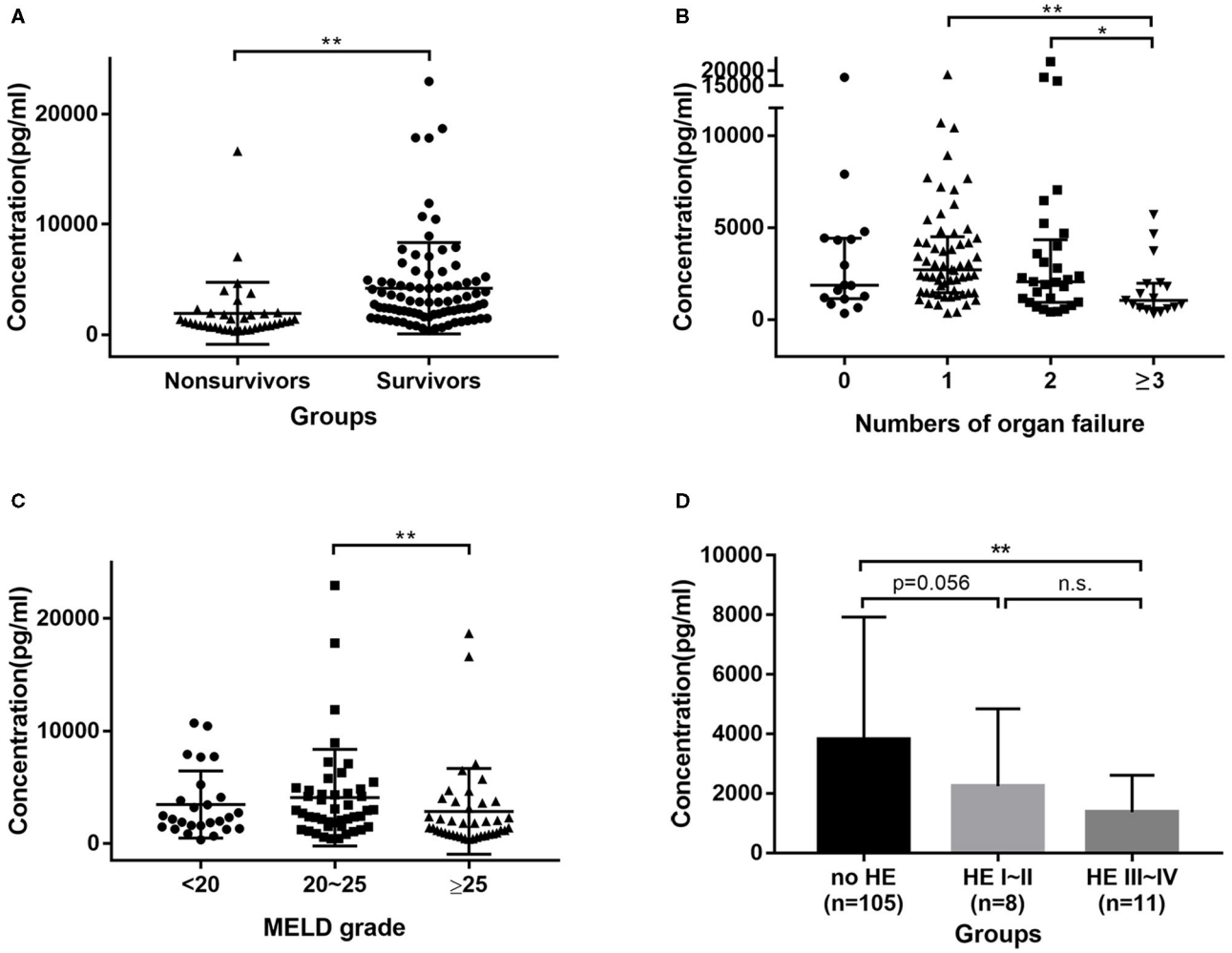


FIGURE 2 | Distribution of GH in HBV-ACLF cohort. **(A)** GH levels were higher in survivors group than in non-survivors group. **(B)** Patients with 3 or more organs failure had lower GH levels than patients with 2, 1, or no organ failure. **(C)** Patients with MELD > 25 had lower GH levels than patients with MELD between 20 and 25. **(D)** patients with Grade III∼IV encephalopathy had lower GH levels than no HE group. * < 0.05, ** < 0.01.

with encephalopathy had lower GH levels than those without. We divided patients into no HE, HE I∼II, HE III∼IV groups (**Figure 2D**), and then found that GH level in no HE group was significantly higher than HE III∼IV group ($P = 0.002$), and showed a higher trend than HE I∼II group ($P = 0.056$).

The relationships between GH levels and other indicators are shown in **Supplementary Table 1**. GH levels were negatively related to neutrophil counts, CRP, and BUN, but positively related to ALB, triglycerides, cholesterol, and alkaline phosphatase. The serum levels of IGF-1, IGF-2, IGF-1R, and IGF-2R were measured, as shown in **Supplementary Table 2**, but there were no significant differences between survivors and non-survivors.

GH Levels in the Cross-Sectional Cohort

The characteristics of HCs and patients in the cross-sectional cohort are listed in **Table 2**. There were no differences in mean age or sex among groups. The median GH levels in each group were 427.66 pg/ml (HCs), 125.91 pg/ml (CHB), 362.94 pg/ml (LC), 1246.05 pg/ml (LC-AD), and 1299.8 pg/ml (ACLF). Comparisons of GH levels among the different groups are shown in **Figure 3A**. Compared to HC ($P < 0.001$), CHB ($P < 0.001$), and LC ($P < 0.001$), the ACLF group had significantly higher GH levels. Similarly, patients in the LC-AD group had higher GH levels than those in the HC ($P < 0.001$), CHB ($P < 0.001$), and LC ($P < 0.001$) groups. In ACLF group, ACLF-S group had higher GH level than ACLF-D group, which was shown in **Supplementary Figure 2**. A detailed comparison of GH levels among groups is listed in **Supplementary Table 3**. Correlation analysis revealed a positive correlation between GH levels and disease severity ($r = 0.462$, $P < 0.001$).

Distribution of IGF-1 among these groups was different from GH. As shown in **Figure 3B**, IGF-1 levels in HC group were higher than LC ($P < 0.001$), LC-AD ($P < 0.001$) and ACLF (P

TABLE 2 | Characteristics of the cross-sectional cohort.

	HC ($n = 26$)	CHB ($n = 26$)	LC ($n = 26$)	LC-AD ($n = 26$)	ACLF group		
					Overall ($n = 38$)	ACLF-S ($n = 21$)	ACLF-D ($n = 17$)
Age	42.42 ± 12.88	43.08 ± 12.94	48.81 ± 8.07	52.62 ± 13.12	49.1 ± 13.3	51.81 ± 13.47	45.65 ± 12.57
Sex (M/F)	11/15	16/10	20/6	20/6	32/6	17/4	15/2
WBC ($\times 10^9$/L)	5.85 (4.98, 7.03)	5.25 (3.73, 6.23)	4 (3.1, 5)	3.5 (2.13, 4.9)	7.2 (5.8, 8.6)	6.1 (4.9, 7.9)	7.8 (6.8, 9.9)
RBC ($\times 10^9$/L)	4.65 (4.35, 4.83)	4.33 (4.14, 4.64)	4.64 (4.12, 5.22)	3.43 (2.66, 3.79)	4.2 (3.6, 4.5)	4.06 (3.53, 4.33)	4.41 (4.01, 4.68)
Platelets ($\times 10^9$/L)	243.5 (203.25, 300)	190.5 (132.25, 245.5)	108 (65, 160)	52.5 (40, 92.75)	100.5 (73.5, 145.5)	93 (72, 138.5)	123 (69, 146.5)
TP (g/L)	73.7 (70.25, 76.13)	68.5 (62.8, 76.2)	70.4 (64, 76.35)	57.3 (53.08, 61.88)	57.8 (54, 62.7)	58.5 (54.5, 65.75)	55.8 (53.15, 61.65)
Albumin (g/L)	47.35 (45.28, 49.93)	41.6 (39.4, 44.5)	41.3 (36.4, 46)	28.9 (24.1, 34.1)	29.9 (27.8, 33.5)	29.8 (27.15, 33.55)	30.9 (27.9, 33.35)
ALT (U/L)	13.5 (10, 23)	47.5 (28.25, 89)	28.5 (22.5, 53.75)	27.5 (16.5, 90.5)	294.5 (158.3, 598.5)	248 (144, 404.5)	375 (213.5, 766)
AST (U/L)	17 (14, 20.5)	36 (21.5, 59)	29.5 (23.75, 42.5)	49.5 (24.75, 96)	294.5 (164.8, 409.8)	287 (127, 486)	306 (209.5, 376.5)
Total bilirubin (mmol/L)	11.5 (8, 14)	12.5 (8.75, 18.5)	12 (11,17.25)	40.5 (31.5, 76)	355.5 (226, 407)	292 (188.5, 384)	391 (301, 452.5)
Creatinine (mmol/L)	67.5 (56.75, 80)	73 (65, 85.5)	74.5 (64.5, 82.75)	79 (65.75, 91.25)	66 (55.5, 76.3)	68 (62, 75)	58 (52.5, 83)
BUN (mmol/L)	5.15 (4.25, 6.2)	5 (3.85, 6.45)	5.67 (4.55, 6.36)	5.6 (4.02, 7.78)	3.8 (2.8, 5.2)	3.8 (3.15, 4.25)	3.5 (2.15, 5.7)
INR	ND	ND	1.14 (1.07, 1.22)	1.45 (1.34, 1.58)	2.4 (1.9, 2.8)	2.1 (1.86, 2.37)	2.73 (2.47, 4)
GH (pg/ml)	427.66 (64.27, 1162.01)	125.91 (38.46, 373.35)	362.94 (69.27, 709.34)	1246.05 (723.66, 3397.5)	1299.8 (528.2, 2302.3)	1616.62 (843.18, 3101.43)	680.71 (398.71, 1451.91)

Continuous data are expressed as means ± SD or medians with interquartile ranges (p. 25, p. 75), and categorical data are expressed as numbers (percentages). ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CHB, chronic Hepatitis B; GH, growth hormone; INR, international normalized ratio; LC, liver cirrhosis; LC-AD, liver cirrhosis-acute decompensation; ND, not detected; RBC, red blood count; TP, total protein; WBC, white blood count.

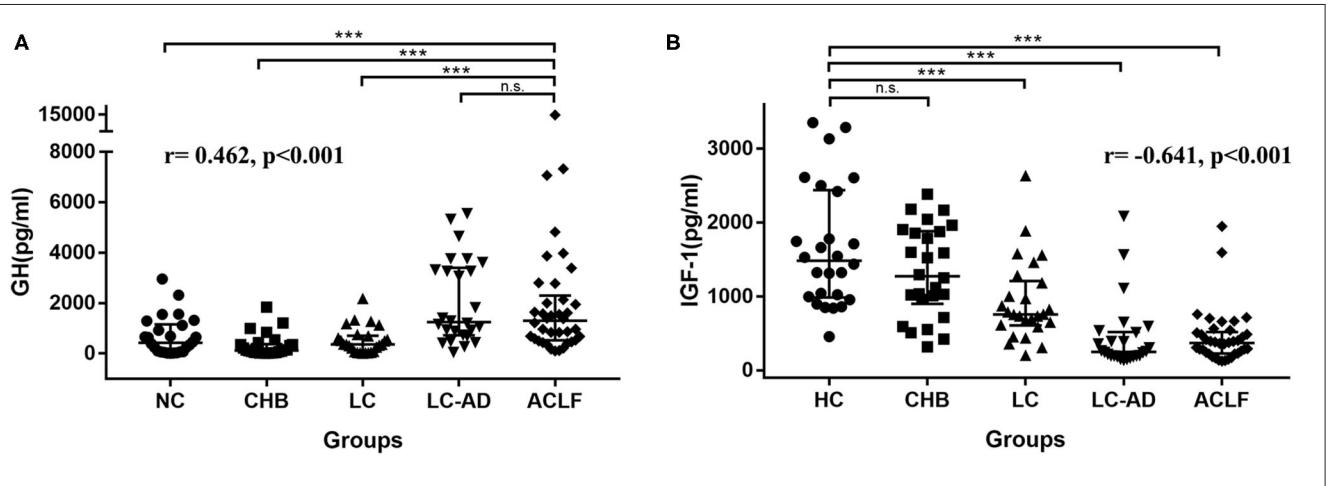


FIGURE 3 | Distribution of GH in cross-sectional cohort. **(A)** ACLF group showed higher GH levels than HC, CHB, and LC group, but no difference from LC-AD group, and the *r*-value of the correlation between GH levels and severity was 0.462, $p < 0.001$. **(B)** HC group showed higher IGF-1 levels than LC, LC-AD, and ACLF group, but no difference from CHB group, and the r value of the correlation between IGF-1 levels and severity was −0.641, $p < 0.001$. ACLF, acute-on-chronic liver failure; CHB, chronic hepatitis B; GH, growth hormone; HC, health control; IGF-1, insulin-like growth factor-1; LC, liver cirrhosis; LC-AD, liver cirrhosis-acute decompensation. *** <0.001.

< 0.001) group. Interestingly, IGF-1 levels in LC-AD group were significantly lower than in LC group ($P < 0.001$), but GH levels revealed much higher ($P < 0.001$). Consistent with the result in prospective cohort, there was no difference of IGF-1 levels between ACLF-S and ACLF-D groups. A detailed comparison of IGF-1 levels among groups is listed in **Supplementary Table 4**. Correlation analysis revealed a negative correlation between IGF-1 levels and disease severity ($r = -0.641$, $P < 0.001$).

TABLE 3 | Univariate and multivariate logistic regression analyses of 30-day survival in the study cohort.

Variables	Univariate logistic regression			Multivariate logistic regression		
	HR	95%CI	*p*-value	HR	95%CI	*p*-value
Age	0.958	[0.928, 0.989]	**0.009**			
Sex	1.818	[0.666, 4.964]	0.243			
MAP	0.998	[0.968, 1.028]	0.883			
Cirrhosis	1.889	[0.873, 4.088]	0.106			
Previous decompensation	2.746	[0.872, 8.646]	0.084			
Alcoholism	0.064	[0.103, 1.067]	0.064			
Bacterial infection	0.664	[0.295, 1.492]	0.321			
Acites	0.810	[0.369, 1.778]	0.600			
UGIB	0.994	[0.286, 3.450]	0.992			
HE	0.050	[0.013, 0.186]	**<0.0001**	0.03	[0.003, 0.275]	**0.002**
Growth hormone (mg/dL)	1.436	[1.122, 1.838]	**0.004**	1.708	[1.113, 2.619]	**0.014**
WBC(×109/L)	0.885	[0.800, 0.979]	**0.018**	1.345	[1.053, 1.718]	**0.017**
Neutrophil ($\times 10^9$/L)	0.861	[0.769, 0.964]	**0.010**			
RBC ($\times 10^9$/L)	1.030	[0.897, 1.181]	0.678			
Platelets ($\times 10^9$/L)	1.002	[0.994, 1.010]	0.644			
Ferritin (ng/ml)	0.942	[0.825, 1.076]	0.378			
Alpha-fetoprotein (ng/ml)	1.001	[0.999, 1.002]	0.333			
CRP (mg/L)	0.983	[0.949, 1.018]	0.337			
Total protein (g/L)	0.996	[0.951, 1.043]	0.878			
Albumin (g/L)	1.108	[1.006, 1.220]	**0.038**			
ALT (U/L)	1.000	[0.999, 1.001]	0.619			
AST (U/L)	0.999	[0.998, 1.000]	0.163			
ALP (U/L)	0.998	[0.989, 1.008]	0.703			
Cholinesterase (U/L)	0.169	[0.919, 1.620]	0.169			
Total bilirubin (mmol/L)	0.995	[0.991, 0.998]	**0.001**	0.987	[0.979, 0.996]	**0.002**
Cr (mmol/L)	0.968	[0.949, 0.987]	**0.001**			
BUN (mmol/L)	0.761	[0.647, 0.897]	**0.001**	0.566	[0.415, 0.772]	**0.0002**
Triglycerides (mmol/L)	2.385	[0.996, 5.709]	0.051			
Cholesterol (mmol/L)	2.163	[1.182, 3.957]	**0.012**			
Potassium (mmol/L)	0.543	[0.276, 1.069]	0.077			
Sodium (mmol/L)	1.024	[0.924, 1.136]	0.648			
Blood glucose (mmol/L)	0.208	[0.823, 1.043]	0.927			
INR	0.136	[0.060, 0.306]	**<0.0001**	0.037	[0.007, 0.214]	**<0.0001**

ALP, phosphatase alkaline; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRP, C-reactive protein; GGT, gamma-glutamyl transpeptidase; HE, hepatic encephalopathy; INR, international normalized ratio; MAP, mean arterial pressure; RBC, red blood count; UGIB, upper gastrointestinal hemorrhage; WBC, white blood count. The bold value mean the $p < 0.05$.

Roles of GH in Predicting the Outcome of HBV-ACLF Patients

Univariate analyses showed that age, HE, GH, WBC, neutrophil count, ALB, TB, Cr, BUN, cholesterol, and INR were significantly associated with 30-day outcomes of HBV-ACLF patients in the prospective cohort. Then, multivariate analyses revealed that HE, GH, WBC, TB, BUN, and INR were independently associated with prognosis at day 30 (**Table 3**).

Next, we analyzed the prognostic value of GH. The AUC of GH for predicting 30-day outcomes was 0.793 (**Figure 4A**). Then we divided patients into low-GH and high-GH groups according to the cut-off value (2,001 pg/mL). The 30-day survival rate in low-GH group was significantly lower than in the high-GH group (45.5 vs. 88.4%, $P < 0.001$) (**Figure 4B**).

Then we analyzed the prognostic efficacy among different prognostic models. The AUC of Child-Pugh, MELD, CLIF-SOFA and CLIF-C ACLF was 0.758, 0.882, 0.862, and 0.838. A new prognostic model was built based on MELD score by logistic regression analyses, namely MELD-GH, combining GH with MELD to predict 30-day outcome. The formula used to calculate MELD-GH is: 0.413*MELD-1.606*Ln (GH). The sensitivity and specificity of MELD-GH was 83.72% and 92.11%. The AUC of

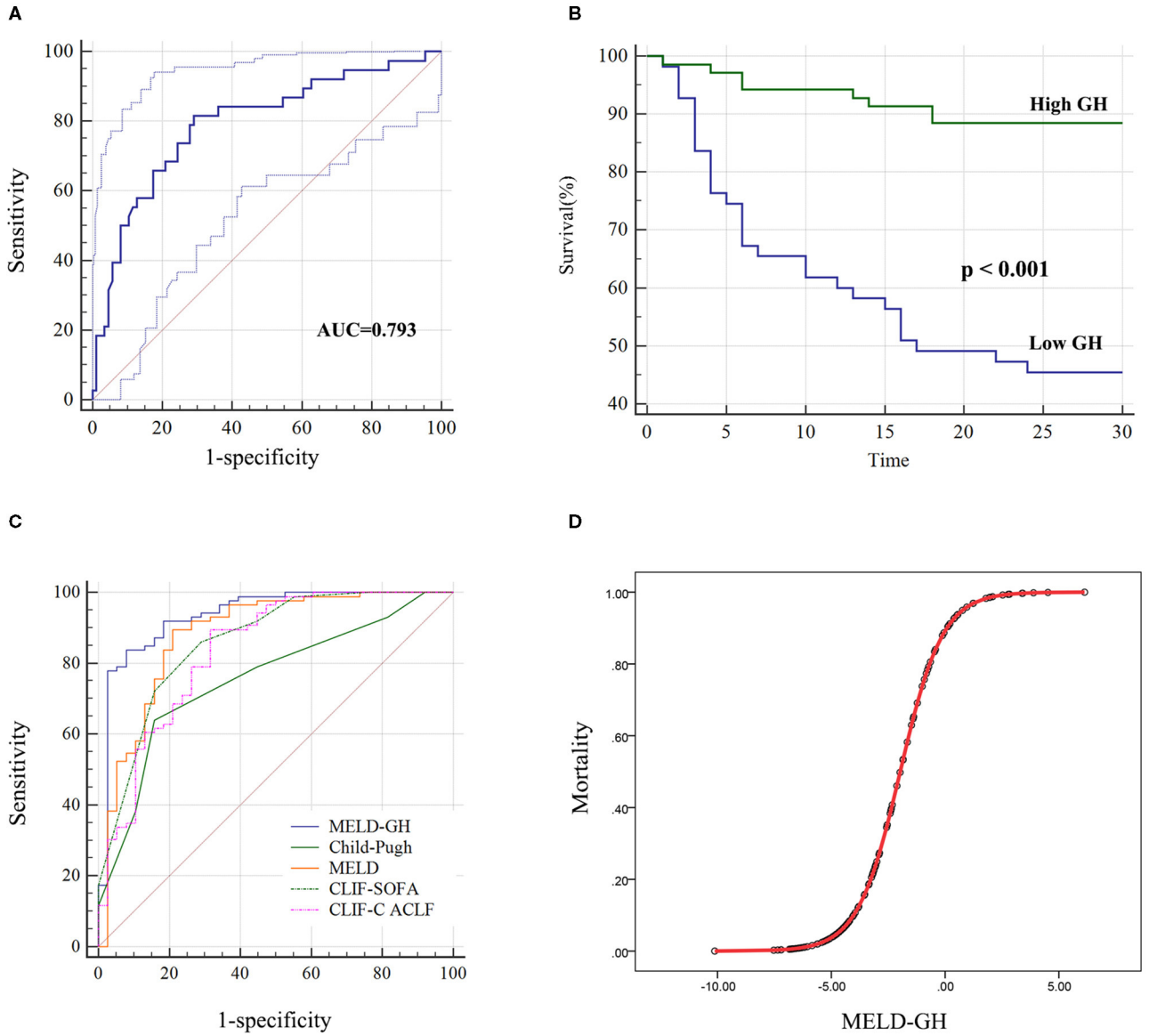


FIGURE 4 | Performance of GH on predicting 30-day survival of HBV-ACLF patients. **(A)** The AUC of GH predicting the outcome was 0.793, and low GH (<2,001 pg/ml) group **(B)** showed significantly worse 30-day outcome than high GH group. **(C)** MELD-GH score showed highest AUC than Child-Pugh, MELD, CLIF-SOFA, and CLIF-C ACLF scores. **(D)** The estimation of HBV-ACLF mortality based on MELD-GH score.

MELD-GH was 0.937, which was significantly superior to the Child-Pugh ($P < 0.0001$), MELD ($P = 0.0185$), CLIF-SOFA ($P = 0.034$), and CLIF-C ACLF ($P = 0.0227$) scores for predicting 30-day outcomes (**Figure 4C** and **Table 4**). When MELD-GH < -5, the mortality rate only reached 3.5%, and when MELD-GH > 0, the mortality rate increased above 90% (**Figure 4D**). The detailed comparison of each two prognostic models were listed in **Supplementary Table 5**.

DISCUSSION

We analyzed serum levels of GH between non-surviving and surviving HBV-ACLF patients. Low GH levels predicted poor outcomes in HBV-ACLF patients. GH is bound by the GH receptor on the cell membrane of hepatocytes, where it activates the Janus kinase 2 (JAK2)-signal transducer and activator of transcription 5 (STAT5) signaling pathway to upregulate genes such as IGF-1 and peroxisome proliferator-activated receptor γ (PPAR-γ) and thereby modulate lipid metabolism (13). IGF-1 is mainly synthesized in the liver under the regulation of GH, which further affects energy metabolism in hepatocytes. In addition, GH can promote the regeneration of hepatocytes (8, 20). In the current study, GH levels were significantly higher in the ACLF-S group than in the ACLF-D group, possibly because high concentrations of GH maintained the metabolic function or promoted the regeneration of hepatocytes.

TABLE 4 | Performance of different prognostic models.

Variables	Sensitivity (%)	Specificity (%)	AUC	SE	95% CI	*p*-value (*vs*. MELD-GH)
MELD-GH	83.72	92.11	0.937	0.026	0.878–0.973	
Child-pugh	63.95	84.21	0.758	0.045	0.673–0.830	<0.0001
MELD	89.53	78.95	0.882	0.037	0.812–0.933	0.0185
CLIF-SOFA	86.09	71.05	0.862	0.035	0.788–0.917	0.0344
CLIF-C ACLF	89.53	68.42	0.838	0.042	0.761–0.898	0.0227

CLIF-C ACLF, European Association for the Study of Chronic Liver Failure; CLIF-SOFA, chronic liver failure-sequential organ failure assessment; COSSH-ACLF, Chinese Group on the Study of Severe Hepatitis B; MELD, Model for End-stage Liver Disease.

The liver is the major source of circulating IGF-1, and its bioavailability is modified by insulin-like growth factor binding proteins (IGFBPs) (11). We measured the concentrations of IGF-1 and IGF-2, but there were no differences between the survivor and non-survivor groups. A possible explanation for this is that HBV-ACLF patients suffered GH resistance, which was defined by high levels of circulating GH and low levels of IGF (21). GH resistance is often observed in cirrhotic patients, who develop nutritional and metabolic complications such as insulin resistance, malnutrition, osteopenia, and hypogonadism, which is in part related to IGF-1 deficiency (17). In the current study, GH levels were increased in the survival group, but IGF-1 levels were not increased significantly due to GH resistance. Whether GH can upregulate the IGF-1 level remains uncertain, and how GH exerts its effects in hepatocytes remains unclear.

In the cross-sectional cohort, GH concentrations were higher in the LC-AD group than in the LC group, suggesting that elevated GH levels may be associated with acute insult of decompensation. In children with bacterial sepsis and septic shock, GH levels were elevated significantly, which is in contrast to the changes observed in IGF-1 levels (22). In addition, Wang et al. (23) demonstrated that inhibition of the GH pathway aggravates acetaminophen-induced acute mice liver injury. Therefore, when an acute insult occurs, GH secretion is increased in response to the stress. In our cross-sectional study, we observed that IGF-1 decreased as the severity of the HBV disease progressed. LC group had lower IGF-1 levels than HC and CHB groups, which is consistent with other studies (24, 25). Interestingly, in the LC-AD and ACLF groups, IGF-1 levels were much lower than LC group, which revealed that the synthesis function of hepatocytes was seriously damaged. However, IGF-1 level didn't decreased further in ACLF group than LC-AD group and was not affected by the increase of GH level.

The number of organ failures determines the mortality rate of patients with ACLF (26). Our study found that when patients had two or more organ failures, the concentration of GH dropped dramatically. This suggests that GH secretion may be closely related to the general condition of the patient. In the comparison of different organ failures, GH levels were significantly lower in the brain failure group compared to the non-brain failure group, which suggests that GH secretion is affected by encephalopathy. Liu et al. (27) found that IGF-1 and GH levels decreased as the severity of encephalopathy worsened, which is consistent with the current results. However, due to the small number of patients with encephalopathy, this result needs to be confirmed further.

In this study, we developed a new prognostic model, namely MELD-GH, which was based on the MELD score. Further analyses demonstrated a better prognostic efficacy of MELD-GH than Child-Pugh, MELD, CLIF-SOFA, and CLIF-C ACLF scores. This result suggested GH may be applied as one of the indicators for predicting the short-term outcome of HBV-ACLF patients. MELD-GH had better prognostic efficacy was significantly more convenient to calculate and apply in the clinical setting, because it only contained 4 indicators, which was less than CLIF-ACLF and CLIF-C ACLF. In our study, we used APASL criteria to diagnose ACLF and used CLIF-SOFA and CLIF-C ACLF to evaluated the severity and make a comparison with the new model. We used APASL consensus criteria to diagnose ACLF because our cohort characteristics were more similar to the Asian ACLF, which was mainly caused by HBV (3). Though CLIF-SOFA and CLIF-C-ACLF score were built according to the ACLF patients with decompensated cirrhosis, they were widely recognized and usually used for comparison with new models (28–30).

In a previous study, thyroid-related hormones also differed in patients with ACLF (31). Interestingly, thyroid-stimulating hormone can predict the prognosis of patients with ACLF. In addition, some studies have found that sex hormones (32) and adrenal hormones (33) have prognostic value in cirrhosis or liver failure. It could be speculated that endocrine organs, particularly the pituitary gland, may play an important role in the pathogenesis of ACLF. Some other studies have shown that pituitary function changes before and after liver transplantation (20, 34). and may be related to disease severity and liver regeneration.

There were some limitations to this study. First, the mechanism by which GH participates in the pathophysiology of the ACLF remains elusive. Without any change in IGF-1, the mechanism of the physiological role of GH and downstream regulatory molecules in the GH pathway remain elusive, which limits our observations regarding the GH-mediated regulation of liver regeneration and metabolism. Second, we only enrolled HBV-ACLF patients, whether these results can be applied to other causes of ACLF remains to be confirmed. Third, the prognostic

efficacy of GH and MELD-GH needs to be validated in a large multicenter study.

CONCLUSIONS

In our HBV-ACLF cohort, serum levels of GH were higher in the survival group and the high-GH patients also had longer survival than who with low-GH, which suggested that low levels predicted poor outcomes in HBV-ACLF patients. Low levels predicted poor outcomes in HBV-ACLF patients. MELD-GH scores had better predictive accuracy than Child-Pugh, MELD, CLIF-SOFA, and CLIF-C ACLF scores. Pituitary function might play a role in ACLF, which needs further research.

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 Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

XX, ZX, and DW: study concept and design. LZ, SM, YZ, and FZ: data acquisition. ZX, DW, and RC: data analysis and interpretation. XX and ZX: critical revision of the manuscript for important intellectual content. ZX and QL: statistical analysis. XX and DW: obtained funding. All authors reviewed and approved the final manuscript.

FUNDING

This study was supported by Key research and development project of Department of Science and Technology of Zhejiang Province (2017C03051), the Independent Task of State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, and the National Natural Science Foundation of China (82000601).

ACKNOWLEDGMENTS

We thank for the financial support by Key research and development project of Department of Science and Technology of Zhejiang Province (2017C03051), and Science Fund for Creative Research Groups of the National Natural Science Foundation of China (No. 81721091).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.655863/full#supplementary-material

REFERENCES

1. Liu J, Fan D. Hepatitis B in China. *Lancet.* (2007) 369:1582–3. doi: 10.1016/S0140-6736(07)60723-5
2. Sarin SK, Choudhury A. Acute-on-chronic liver failure: terminology, mechanisms and management. *Nat Rev Gastroenterol Hepatol.* (2016) 13:131–49. doi: 10.1038/nrgastro.2015.219
3. Sarin SK, Kedarisetty CK, Abbas Z, Amarapurkar D, Bihari C, Chan AC, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) 2014. *Hepatol Int.* (2014) 8:453–71. doi: 10.1007/s12072-014-9580-2
4. Bernal W, Jalan R, Quaglia A, Simpson K, Wendon J, Burroughs A. Acute-on-chronic liver failure. *Lancet.* (2015) 386:1576–87. doi: 10.1016/S0140-6736(15)00309-8
5. Hernaez R, Solà E, Moreau R, Ginès P. Acute-on-chronic liver failure: an update. *Gut.* (2017) 66:541–53. doi: 10.1136/gutjnl-2016-312670
6. Li H, Xia Q, Zeng B, Li ST, Liu H, Li Q, et al. Submassive hepatic necrosis distinguishes HBV-associated acute on chronic liver failure from cirrhotic patients with acute decompensation. *J Hepatol.* (2015) 63:50–9. doi: 10.1016/j.jhep.2015.01.029
7. Dezso K, Nagy P, Paku S. Human liver regeneration following massive hepatic necrosis - two distinct patterns. *J Gastroenterol Hepatol.* (2019) 35:124–34. doi: 10.1111/jgh.14721
8. Pennisi PA, Kopchick JJ, Thorgeirsson S, LeRoith D, Yakar S. Role of growth hormone (GH) in liver regeneration. *Endocrinology.* (2004) 145:4748–55. doi: 10.1210/en.2004-0655
9. Zerrad-Saadi A, Lambert-Blot M, Mitchell C, Bretes H, Collin de l'Hortet A, et al. GH receptor plays a major role in liver regeneration through the control of EGFR and ERK1/2 activation. *Endocrinology.* (2011) 152:2731–41. doi: 10.1210/en.2010-1193
10. Ishikawa M, Brooks AJ, Fernandez-Rojo MA, Medina J, Chhabra Y, Minami S, et al. Growth hormone stops excessive inflammation after partial hepatectomy, allowing liver regeneration and survival through induction of H2-Bl/HLA-G. *Hepatology.* (2020) 73:759–75. doi: 10.1002/hep.31297
11. Donaghy A, Ross R, Gimson A, Hughes SC, Holly J, Williams R. Growth hormone, insulinlike growth factor-1, and insulinlike growth factor binding proteins 1 and 3 in chronic liver disease. *Hepatology.* (1995) 21:680–8. doi: 10.1002/hep.1840210313
12. Kratzsch J, Blum WF, Schenker E, Keller E. Regulation of growth hormone (GH), insulin-like growth factor (IGF)I, IGF binding proteins−1,−2,−3 and GH binding protein during progression of liver cirrhosis. *Exp Clin Endocrinol Diabetes.* (1995) 103:285–91. doi: 10.1055/s-0029-1211366
13. Takahashi Y. The role of growth hormone and insulin-like growth factor-i in the liver. *Int J Mol Sci.* (2017) 18:1447. doi: 10.3390/ijms18071447
14. Rufinatscha K, Ress C, Folie S, Haas S, Salzmann K, Moser P, et al. Metabolic effects of reduced growth hormone action in fatty liver disease. *Hepatol Int.* (2018) 12:474–81. doi: 10.1007/s12072-018-9893-7
15. Greenhill C. Liver: Growth hormone in liver fibrosis. *Nat Rev Gastroenterol Hepatol.* (2014) 11:579. doi: 10.1038/nrgastro.2014.165
16. Takahashi Y. Essential roles of growth hormone (GH) and insulin-like growth factor-I (IGF-I) in the liver. *Endocr J.* (2012) 59:955–62. doi: 10.1507/endocrj.EJ12-0322
17. Bonefeld K, Moller S. Insulin-like growth factor-I and the liver. *Liver Int.* (2011) 31:911–9. doi: 10.1111/j.1478-3231.2010.02428.x
18. Jalan R, Saliba F, Pavesi M, Amoros A, Moreau R, Ginès P, et al. Development and validation of a prognostic score to predict mortality

in patients with acute-on-chronic liver failure. *J Hepatol.* (2014) 61:1038–47. doi: 10.1016/j.jhep.2014.06.012
19. Kumar M, Jain S, Sharma BC, Sarin SK. Differentiating acute hepatitis b from the first episode of symptomatic exacerbation of chronic hepatitis B. *Digestive Dis Sci.* (2006) 51:594–9. doi: 10.1007/s10620-006-3175-2
20. Jara M, Schulz A, Malinowski M, Puhl G, Lock JF, Seehofer D, et al. Growth hormone/insulin-like growth factor 1 dynamics in adult living donor liver transplantation. *Liver Transpl.* (2014) 20:1118–26. doi: 10.1002/lt.23922
21. Stiedl P, McMahon R, Blaas L, Stanek V, Svinka J, Grabner B, et al. Growth hormone resistance exacerbates cholestasis-induced murine liver fibrosis. *Hepatology.* (2015) 61:613–26. doi: 10.1002/hep.27408
22. Onenli-Mungan N, Yildizdas D, Yapicioglu H, Topaloglu AK, Yüksel B, Ozer G. Growth hormone and insulin-like growth factor 1 levels and their relation to survival in children with bacterial sepsis and septic shock. *J Paediatr Child H.* (2004) 40:221–6. doi: 10.1111/j.1440-1754.2004.00342.x
23. Wang T, Hai J, Chen X, Peng H, Zhang H, Li L, et al. Inhibition of GHRH aggravated acetaminophen-induced acute mice liver injury through GH/IGF-I axis. *Endocrine J.* (2012) 59:579–87. doi: 10.1507/endocrj.EJ11-0356
24. Khoshnood A, Toosi MN, Faravash MJ, Esteghamati A, Froutan H, Ghofrani H, et al. A Survey of correlation between insulin-like growth factor-I (IGF-I) levels and severity of liver cirrhosis. *Hepatitis Monthly.* (2013) 13:e6181. doi: 10.5812/hepatmon.6181
25. Shmueli E, Miell JP, Stewart M, Alberti KG, Record CO. High insulin-like growth factor binding protein 1 levels in cirrhosis: link with insulin resistance. *Hepatology.* (1996) 24:127–33. doi: 10.1002/hep.510240122
26. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology.* (2013) 144:1426–37:1437 e1421–9. doi: 10.1053/j.gastro.2013.02.042
27. Liu G, Wu HW, Li ZG. Study on the correlation of changes of IGF-1, GH, and NGB levels and NBNA score in neonates with hypoxic ischemic encephalopathy. *Eur Rev Med Pharmacol Sci.* (2018) 22:3173–81. doi: 10.26355/eurrev_201805_15078
28. Wu D, Zhang S, Xie Z, Chen E, Rao Q, Liu X, et al. Plasminogen as a prognostic biomarker for HBV-related acute-on-chronic liver failure. *J Clin Invest.* (2020) 130:2069–80. doi: 10.1172/JCI130197
29. Wu T, Li J, Shao L, Xin J, Jiang L, Zhou Q, et al. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure. *Gut.* (2017) 67:2181–91. doi: 10.1136/gutjnl-2017-314641
30. Song DS, Kim TY, Kim DJ, Kim HY, Sinn DH, Yoon EL, et al. Validation of prognostic scores to predict short-term mortality in patients with acute-on-chronic liver failure. *J Gastroenterol Hepatol.* (2018) 33:900–9. doi: 10.1111/jgh.13991
31. Wu D, Sun Z, Liu X, Rao Q, Chen W, Wang J, et al. HINT: a novel prognostic model for patients with hepatitis B virus-related acute-on-chronic liver failure. *Aliment Pharmacol Ther.* (2018) 48:750–60. doi: 10.1111/apt.14927
32. Balakrishnan CH, Rajeev H. Correlation of serum prolactin level to child pugh scoring system in cirrhosis of liver. *J Clin Diagn Res.* (2017) 11:OC30–3. doi: 10.7860/JCDR/2017/24730.10273
33. Wiest R, Moleda L, Zietz B, Hellerbrand C, Schölmerich J, Straub R. Uncoupling of sympathetic nervous system and hypothalamic-pituitary-adrenal axis in cirrhosis. *J Gastroenterol Hepatol.* (2008) 23:1901–8. doi: 10.1111/j.1440-1746.2008.05456.x
34. Seehofer D, Steinmueller T, Graef KJ, Rayes N, Wiegand W, Tullius SG, et al. Pituitary function test and endocrine status in patient with cirrhosis of the liver before and after hepatic transplantation. *Ann Transplant.* (2002) 7:32–7.

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.

frontiers in Medicine

ORIGINAL RESEARCH
published: 27 July 2021
doi: 10.3389/fmed.2021.689865

Characteristics of Peripheral Lymphocyte Subsets in Patients With Acute-On-Chronic Liver Failure Associated With Hepatitis B

*Juan Li[1,2], Chun-Hua Hu[1,2], Yi Chen[2], Mi-Mi Zhou[2], Zhi-Jie Gao[1,2], Meng-Jun Fu[1,2], Jing Wang[2], Jian-Zhou Li[1,2], Tian-Yan Chen[1,3], Ying-Ren Zhao[1,3] and Ying-Li He[2,3]**

[1] Department of Infectious Diseases, School of Medicine, First Affiliated Teaching Hospital, Xi'an Jiaotong University, Xi'an, China, [2] School of Medicine, Institution of Hepatology, First Affiliated Teaching Hospital, Xi'an Jiaotong University, Xi'an, China, [3] Shaanxi Clinical Research Center of Infectious Diseases, Xi'an, China

OPEN ACCESS

Edited by:
Yu-Chen Fan,
Shandong University, China

Reviewed by:
Dongbo Wu,
Sichuan University, China
Ran Xue,
Beijing Cancer Hospital, China

***Correspondence:**
Ying-Li He
heyingli2000@xjtu.edu.cn

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 01 April 2021
Accepted: 22 June 2021
Published: 27 July 2021

Citation:
Li J, Hu C-H, Chen Y, Zhou M-M, Gao Z-J, Fu M-J, Wang J, Li J-Z, Chen T-Y, Zhao Y-R and He Y-L (2021) Characteristics of Peripheral Lymphocyte Subsets in Patients With Acute-On-Chronic Liver Failure Associated With Hepatitis B. Front. Med. 8:689865. doi: 10.3389/fmed.2021.689865

Background and Aims: Acute-on-chronic liver failure (ACLF) is a rare, but dramatic clinical syndrome. There is substantial evidence suggesting that immunity-mediated inflammation plays an important role in HBV-ACLF. Our aim was to characterize the proportion and cell counts of peripheral blood lymphocyte subsets in acute-on-chronic liver failure patients caused by HBV infection.

Methods: One hundred and seventeen patients were enrolled in this study, including those with HBV-related ACLF (HBV-ACLF; $n = 70$), and HBV related non-ACLF patients (HBV non-ACLF; $n = 47$). Demographics, clinical and laboratory data at hospital admission were retrospectively analyzed. The percentage and cell count of peripheral lymphocyte subsets were evaluated by flow cytometry. Comparison analysis was performed by *t*-test or non-parametric Mann–Whitney *U*-test. Actuarial probabilities of death were calculated by the Kaplan-Meier method.

Results: Both circulating lymphocyte count and lymphocyte percentage were significantly reduced in patients with HBV-ACLF ($P < 0.001$). The $CD8^+$ T cell, $CD4^+$ T cell, and $CD16^+CD56^+$ NK cell counts were significantly decreased in HBV-ACLF. Consistently, flow cytometric analysis showed that $CD8^+$ T cell counts were significantly decreased in non-survivors, while no significant differences were found in $CD4^+$ T cell, $CD19^+$ B cell, or $CD56^+CD16^+$ NK cell counts. Furthermore, the group with the lower $CD8^+$ T cell count displayed a significantly higher mortality rate compared with the group with the higher $CD8^+$ T cell count.

Conclusions: The abnormal prevalence of lymphocyte subsets may be important in the pathogenesis of HBV-ACLF. The decrease in $CD8^+$ T cell counts may be related to poor survival in HBV-ACLF patients.

Keywords: lymphocyte subsets, hepatitis B virus, acute-on-chronic liver failure, immune response, flow cytometry

INTRODUCTION

Acute-on-chronic liver failure (ACLF) is a rare, but dramatic clinical syndrome characterized by massive hepatocyte death leading to multiorgan failure in patients with pathological damage caused by chronic liver disease (1). The most common cause of ACLF in China is chronic hepatitis B virus (HBV) infection (2). ACLF results in an extremely high mortality rate due to its unclear pathogenesis and the lack of effective treatment approaches (3). There is substantial evidence to suggest that immunity-mediated inflammation plays an important role in HBV-ACLF. The neutrophil–lymphocyte ratio (NLR) and lymphocyte–monocyte ratio (LMR), which reflect systemic inflammation, are valuable prognostic markers in ACLF patients (4–8).

Several reports on the immune pathogenesis of chronic HBV infection have suggested that $CD8^+$ T cells, $CD4^+$ T cells, and NK cells as well as cytokines participate in the development of liver injury (9–13). A recent study (13) revealed that interleukin 21 (IL-21) enhanced the antiviral responses of $CD8^+$ T cells in chronic HBV infection. Zou et al. (14) characterized lymphocyte subsets in peripheral blood and liver tissue, and reported that the abnormal distribution of circulating and liver-infiltrating immune competent cells may be an important factor for the development of HBV-related ACLF. Dong et al. (15) demonstrated that HBV-ACLF patients displayed immune disorders from the perspective of adaptive immunity, which were characterized by a reduction in the number of $CD4^+$ T lymphocytes. Similarly, another study on peripheral lymphocytes reported the exhaustion of differentiated $CD4^+$ T cells from the circulation in HBV-ACLF patients compared to patients without ACLF (7). Our previous study found that Natural Killer Group 2A (NKG2A) expressed on peripheral $CD3^-CD56^+$ NK cells and $CD3^+CD8^+$ T cells played a key negative regulatory role in the progress of HBV-related ACLF (16). However, there are still insufficient data to show the overall characteristics of immune cell status in HBV-related ACLF patients and their association with prognosis. Therefore, this study focused on the proportion and number of peripheral blood lymphocyte subsets in HBV-related ACLF patients to characterize changes in immune status and clarify the correlation between liver injury and its immunological characteristics.

MATERIALS AND METHODS

Study Population

The data from patients with chronic HBV infection who were admitted from July 2013 to November 2019 in the First Affiliated Hospital of Xi'an Jiaotong University, the largest general hospital in northwest China under the direct administration of the Chinese Ministry of Health, were retrospectively collected, including 70 patients with HBV-ACLF and 47 patients with HBV-non-ACLF. HBV-non-ACLF referred to patients with chronic hepatitis B, or HBV-related compensated cirrhosis, who had abnormal liver function due to chronic HBV infection, but do not meet the diagnostic criteria of ACLF. ACLF was diagnosed according to the recommendation by the Asian Pacific Association for the Study of the Liver (APASL) (17): serum bilirubin $\geq$5 mg/dL, an international normalized ratio (INR) $\geq$1.5 or prothrombin activity <40%, recent development of complications such as hepatic encephalopathy, or an abrupt and obvious increase in ascites, spontaneous bacterial peritonitis, or hepatorenal syndrome. Briefly, CHB patients were HBsAg positive for more than 6 months and may have exhibited signs or symptoms of hepatitis and abnormal liver function. Cirrhosis was diagnosed based on liver biopsy (if available), or the combination of clinical symptoms, laboratory tests, and CT/MRI scan. HBV-non-ACLF refers to patients who had alanine aminotransferase or aspartate aminotransferase levels >2 times the upper limit of normal (ULN) (40 U/L) and a total bilirubin level <5 ULN (1 mg/dL).

Patients were excluded if they had any of the following conditions: (1) malignancies, such as hepatocellular carcinoma; (2) concurrent hepatitis A, hepatitis C, hepatitis D, hepatitis E, Epstein–Barr virus, or other virus infections; (3) with one or more additional known primary or secondary causes of liver disease, other than hepatitis B. All patients received standard care and treatments as recommended by the guidelines.

TABLE 1 | Demographic and clinical parameters of the study population.

Parameters	HBV ACLF (n = 70)	HBV non-ACLF (n = 47)	P-value
Age (years)	38 (28–49)	39 (29–51)	0.885
Gender (m/f)	57/13	34/13	0.246
ALT (U/L)	193.00 (88.66–689.80)	123.15 (41.28–670.73)	0.018
AST (U/L)	189.00 (90.08–782.50)	58.75 (44.70–413.83)	<0.001
TBIL (μmol/L)	229.60 (154.47–372.10)	16.80 (10.20–37.96)	<0.001
Albumin (g/L)	32.86 ± 1.05	36.70 ± 1.62	<0.001
Creatinine (μmol/L)	56.40 (49.70–64.60)	60.35 (45.55–67.75)	0.748
PT (s)	22.10 (19.45–27.45)	13.65 (13.18–14.90)	<0.001
INR	1.83 (1.54–2.34)	1.12 (1.04–1.23)	<0.001
HBeAg positive	34 (48.57)	18 (38.30)	0.273
lgHBsAg (lg IU/mL)	2.68 ± 0.17	3.13 ± 0.25	0.108
lgHBV DNA (lg IU/mL)	5.10 (3.87–6.03)	4.21 (3.06–6.18)	0.237
WBC ($\times10^9$/L)	5.37 (4.48–7.29)	5.15 (3.83–5.85)	0.027
PLT ($\times10^9$/L)	92.98 ± 8.05	145.94 ± 17.66	<0.001
Lymphocyte count ($\times10^9$/L)	1.23 ± 0.08	1.70 ± 0.16	<0.001
Lymphocytes percentage (%)	22.56 ± 1.87	33.50 ± 2.21	<0.001
Mortality, *n* (%)	21 (30.0)	0 (0)	<0.001

Data are expressed as mean ± standard deviation (SD), number (percentage), or median (interquartile range). ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; AST, aspartate Aminotransferase; TBIL, total bilirubin; INR, international normalized ratio; HBV, hepatitis B virus, HBsAg, HBV surface antigen WBC, white blood cell count; PLT, platelet count.

This retrospective study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University.

Laboratory Examinations

Patients and controls underwent routine laboratory evaluations for liver diseases, including clinical assessments, complete blood count, liver function tests (alanine aminotransferase, total bilirubin, serum albumin), renal function (serum creatinine), electrolytes (serum sodium, potassium) and coagulation (international normalized ratio), HBV DNA load (COBAS TaqMan, lower detection limit 20 IU/mL). The tests were performed at the central laboratory in the hospital. Model for end-stage liver disease (MELD) score and MELD-Na score were used to assess disease severity and calculated as previously described (18).

Flow Cytometry for Detection of Lymphocytes Subsets

One hundred microliters of blood sample were stained with 10 μL of fluoroisothiocyanate (FITC)-conjugated CD4, PE-conjugated CD8, Per-CP-conjugated CD3, APC-conjugated CD19, and Multitest $CD16^+CD56$ reagent (Beckman Coulter). Then incubation for 15 min in the dark and red blood cells lysis were done. After washing, the cells were resuspended in Cytomics FC 500 (Beckman Coulter) flow cytometric analysis was done with Cell Quest software. Lymphocytes were defined with their forward and side scatter characteristic. T lymphocytes were identified ($CD3^+$), and then subdivided into $CD4^+$ or $CD8^+$ populations. B lymphocyte ($CD3^-CD19^+$) and natural killer cell ($CD3^-CD56^+CD16^+$) numbers and percentages were also determined.

Statistical Analysis

Statistical analysis was performed using SPSS 23.0 for Windows (SPSS, Chicago, IL, USA), with graphs drawn using GraphPad Prism 8.0 (GraphPad, La Jolla, CA, USA). Quantitative data were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR), and the categorical data were expressed as the number (percentage). *T*-test or the non-parametric Mann–Whitney *U*-test was used where appropriate. A Pearson's Chi-square or Fisher's exact test was performed to compare qualitative data. Actuarial probabilities of death during follow-up were calculated by the Kaplan-Meier method and compared by log-rank test. Results with a two-tailed *P*-value of <0.05 were considered statistically significant.

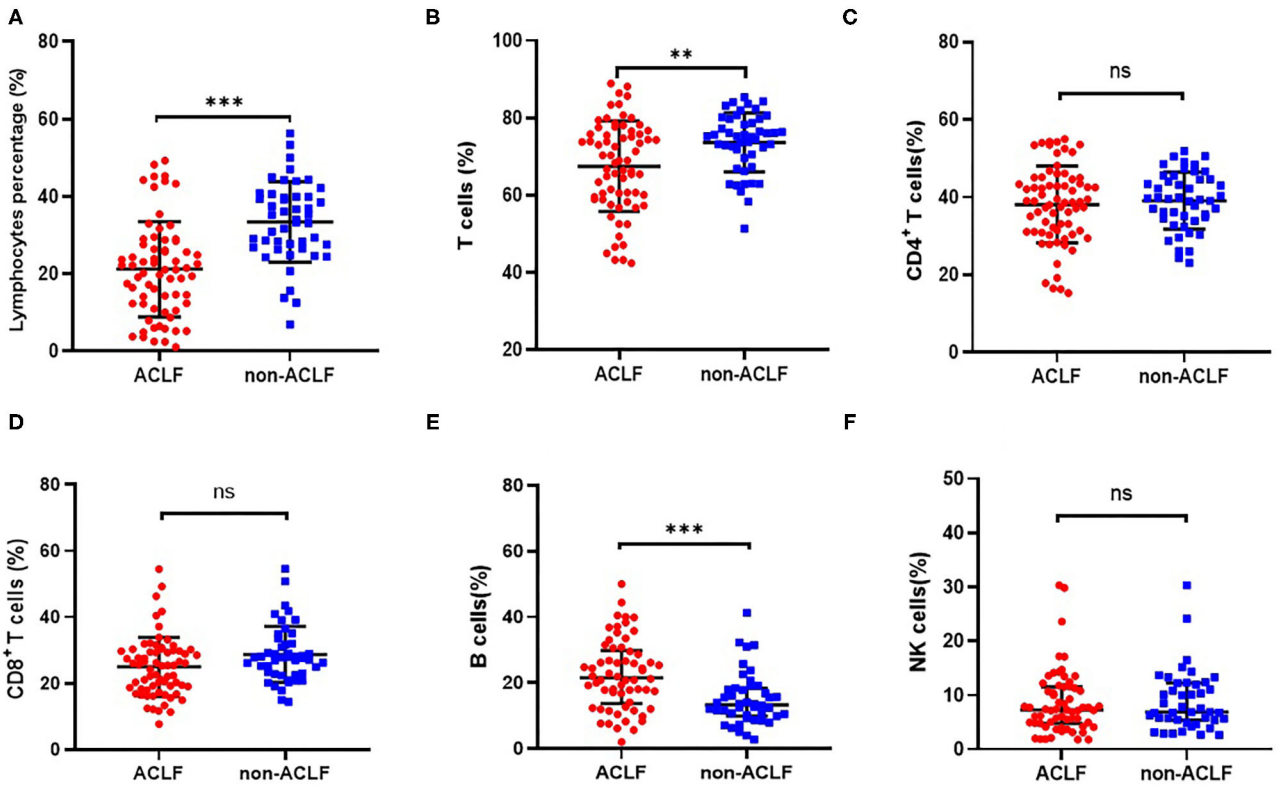


FIGURE 1 | Comparison of the proportion of lymphocyte **(A)**, $CD3^+$ T cells **(B)**, $CD4^+$ T cells **(C)**, $CD8^+$ T cells **(D)**, $CD19^+$ B cells **(E)** and $CD16^+CD56^+$ NK cells **(F)** in patients with hepatitis B-related acute-on-chronic liver failure (ACLF) ($n = 70$) and non-ACLF ($n = 47$), where the lines indicated the mean or median. $^{**}P < 0.01$, $^{***}P < 0.001$.

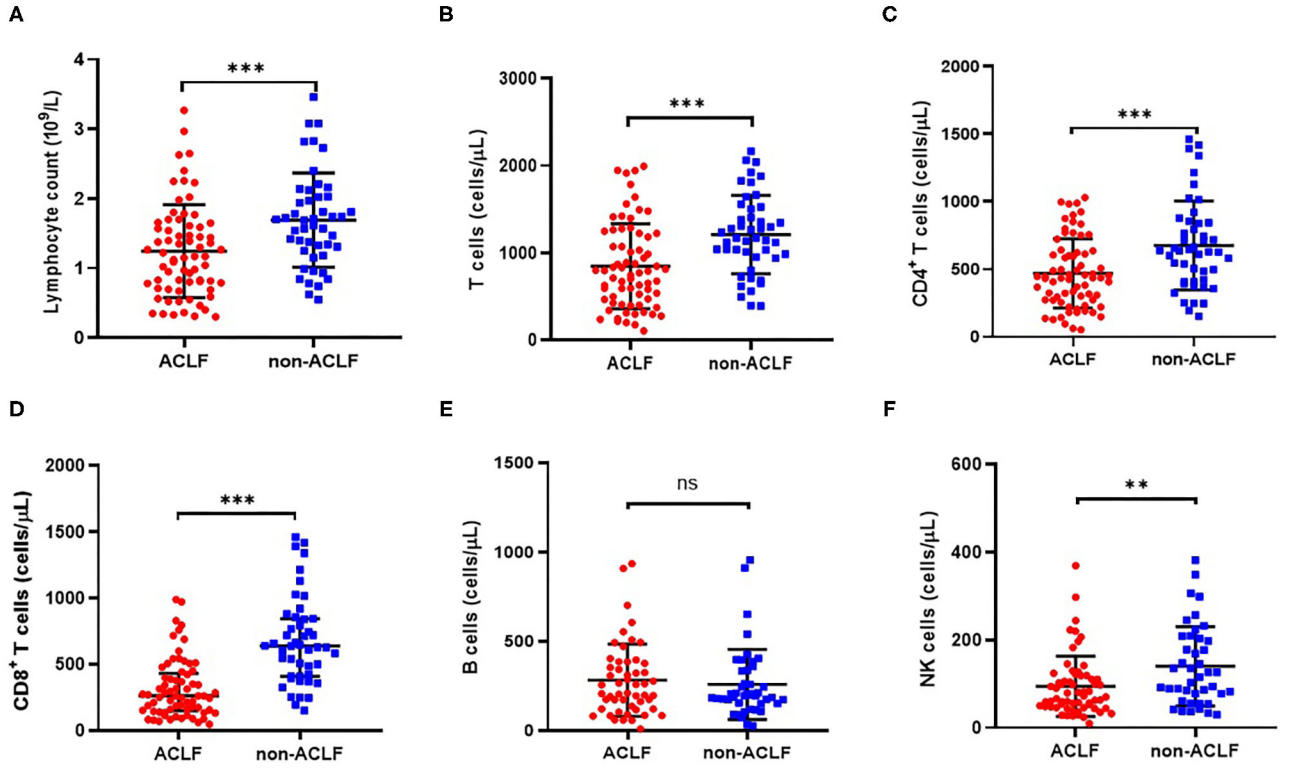


FIGURE 2 | Comparison of cell counts of lymphocyte **(A)**, $CD3^+$ T cells **(B)**, $CD4^+$ T cells **(C)**, $CD8^+$ T cells **(D)**, $CD19^+$ B cells **(E)** and $CD16^+CD56^+$ NK cells **(F)** in patients with hepatitis B-related acute-on-chronic liver failure (ACLF) ($n = 70$) and non-ACLF ($n = 47$), where the lines indicated the mean or median. $^{**}P < 0.01$, $^{***}P < 0.001$.

RESULTS

Demographic and Clinical Characteristics of the Study Population

The clinical and biochemical characteristics of the enrolled groups were summarized in **Table 1**. There were no significant differences in age, gender, creatinine, HBeAg positive ratio and HBV DNA load between the two groups. As expected, HBV-related ACLF patients displayed higher levels of ALT, AST, total bilirubin and INR, but lower levels of albumin and decreased platelet count compared with non-ACLF group. The 28-day mortality rate in the ACLF group was 30.0% (21/70), which was significantly higher than that in the non-ACLF group.

Peripheral Lymphocyte Subsets in Patients With HBV-Related ACLF and Non-ACLF

Compared with patients in the HBV non-ACLF group, both circulating lymphocyte count and lymphocyte percentage were significantly reduced in patients with liver failure ($P < 0.001$, **Figures 1A**, **2A**). With regard to lymphocyte subset proportion, no significant differences were observed in $CD4^+$ T cells, $CD8^+$ T cells and $CD16^+CD56^+$ NK cells (**Figures 1C,D,F**). The proportion of $CD3^+$ T cells in HBV-ACLF patients was significantly lower than that in the non-ACLF group ($P = 0.003$, **Figure 1B**), while the percentage of $CD19^+$ B cells was significantly higher compared with the non-ACLF group ($P < 0.001$, **Figure 1E**).

Additionally, lymphocyte subset counts between the two groups were analyzed. Compared with the HBV non-ACLF group, the decrease in total lymphocyte count in ACLF patients (**Figure 2A**) was possibly due to a relative decrease in $CD8^+$ T cell (**Figure 2D**), $CD4^+$ T cell (**Figure 2C**), and $CD16^+CD56^+$ NK cell counts (**Figure 2F**). However, the lower number of $CD19^+$ B cells in ACLF patients was not significant (**Figure 2E**).

Comparison of Peripheral Lymphocyte Subsets Between Non-Surviving and Surviving ACLF Patients

To investigate whether peripheral lymphocyte subsets are correlated with short-term prognosis in HBV-related ACLF, a comparative analysis was performed between the surviving and non-surviving patients during a 28-day follow-up period. Of 70 ACLF patients, 21 patients died (mortality rate: 30.0%). Non-surviving patients showed higher total bilirubin, creatinine and INR at hospital admission ($P = 0.007$, $P = 0.017$ and $P < 0.001$, respectively, **Table 2**). In addition, the presence of encephalopathy (42.85 vs. 18.36%) and bacterial infection (85.71 vs. 57.14%) were both significantly higher in non-survivors compared with survivors ($P = 0.032$, $P = 0.042$, respectively, **Table 2**). Non-survivors showed a significantly higher MELD and

TABLE 2 | Demographics, clinical data, and laboratory parameters in survivors and non-survivors of patients with HBV-related ACLF.

Parameters	Survivors ($n = 49$)	Non-survivors ($n = 21$)	*P*-value
Age (years)	35.60 ± 1.83	52.78 ± 6.11	0.009
Gender (m/f)	42/7	15/6	0.159
ALT (U/L)	297.00 (105.83–746.33)	107.00 (82.50–450.90)	0.878
AST (U/L)	201.50 (88.25–809.00)	189.00 (101.75–755.05)	0.590
Cholesterol (mmol/L)	2.31 ± 0.10	1.76 ± 0.26	<0.001
TBIL (μmol/L)	244.88 ± 26.15	379.75 ± 62.51	0.007
Albumin (g/L)	31.95 (29.20–38.93)	29.24 (27.85–33.05)	0.039
Creatinine (μmol/L)	55.25 (48.05–60.15)	69.30 (55.20–83.36)	0.017
Sodium (mmol/L)	137.55 (135.00–139.00)	135.00 (129.50–137.75)	0.119
PT (s)	20.60 (17.85–23.85)	27.60 (24.90–39.25)	<0.001
INR	1.74 (1.46–1.95)	2.36 (2.06–3.50)	<0.001
HBeAg positive	23 (46.94)	11 (52.38)	0.676
lgHBsAg (lg IU/mL)	2.50 ± 0.20	3.20 ± 0.32	0.090
lgHBV DNA (lg IU/mL)	4.65 ± 0.31	6.13 ± 0.65	0.014
WBC ($\times 10^9$/L)	5.22 (4.12–7.13)	7.37 (5.47–10.98)	0.006
PLT ($\times 10^9$/L)	99.90 ± 10.02	82.11 ± 11.90	0.018
Lymphocyte count ($\times 10^9$/L)	1.35 ± 0.10	1.01 ± 1.13	0.050
Lymphocytes percentage (%)	23.70 (16.95–32.72)	8.72 (5.05–21.85)	<0.001
Ascites, *n* (%)	20 (40.82)	13 (61.90)	0.105
Encephalopathy, *n* (%)	9 (18.36)	9 (42.85)	0.032
Bacterial infection, *n* (%)	28 (57.14)	18 (85.71)	0.042
PE/DPMAS, *n* (%)	18 (36.73)	9 (42.85)	0.630
Use of antibiotic, *n* (%)	28 (57.14)	18 (85.71)	0.042
MELD score	23.40 ± 0.99	29.44 ± 2.65	<0.001
MELD-Na score	22.90 ± 1.04	25.67 ± 2.04	0.001

Data are expressed as mean ± standard deviation (SD), number (percentage) or median (interquartile range). ALT, alanine aminotransferase; AST, aspartate Aminotransferase; TBIL, total bilirubin; INR, international normalized ratio; WBC, white blood cell count; PLT, platelet count; PE, plasma exchange; DPMAS, double plasma molecular absorption system; MELD, model for end-stage liver disease.

MELD-Na score and lower lymphocyte percentage compared with survivors ($P < 0.001$, **Table 2**).

As shown in **Figure 3A**, total lymphocyte count was lower in non-survivors compared with survivors (1.01 ± 1.13 vs. 1.35 ± 0.10) although the difference was not statistically significant ($P = 0.050$, **Table 2**). Since the absolute number of lymphocytes was already small in ACLF, it is of little sense to explore the proportion of lymphocyte subgroups between survivors and non-survivors. Consequently, we analyzed the absolute counts of lymphocyte subsets. Flow cytometric analysis showed that the $CD8^+$ T cell count was significantly decreased in non-survivors ($P = 0.003$, **Figure 3D**), while no significant differences were found in $CD4^+$ T cell, $CD19^+$ B cell, or $CD56^+CD16^+$ NK cell counts (**Figures 3C,E,F**).

Decreased $CD8^+$ T Cell Count at Admission Was Correlated With Poor Prognosis in Patients With HBV-ACLF

We further divided the patients into the lowest and highest groups according to the median of absolute numbers of peripheral blood lymphocytes to compare their short-term prognosis. The group with the lower $CD8^+$ T cell count displayed a significantly higher mortality rate compared to the group with the higher $CD8^+$ T cell count (42.9 vs. 17.1%, $P = 0.019$, **Table 3**). Similar results were found for $CD3^+$ T cell counts, which may have been the relative result of reduced $CD8^+$ T cell counts. With the median of the $CD8^+$ T cell counts as the cut-off value (277.95 cells/μl), survival probability in patients with HBV-ACLF was shown in **Figure 4**. These results indicated that reduced $CD8^+$ T cell counts might be related to poor prognosis in HBV-ACLF patients.

DISCUSSION

The results of the current study showed that the $CD8^+$ T cell, $CD4^+$ T cell, and $CD16^+CD56^+$ NK cell counts were significantly decreased in HBV-ACLF. Consistently, decreased $CD8^+$ T cell counts was observed in non-survivors in comparison with survivors. Furthermore, the group with the lower $CD8^+$ T cell count displayed a significantly higher mortality rate compared with the group with the higher $CD8^+$ T cell count. The present results indicated that decreased $CD8^+$ T cell counts might relate to the poor outcome in patients of HBV-ACLF.

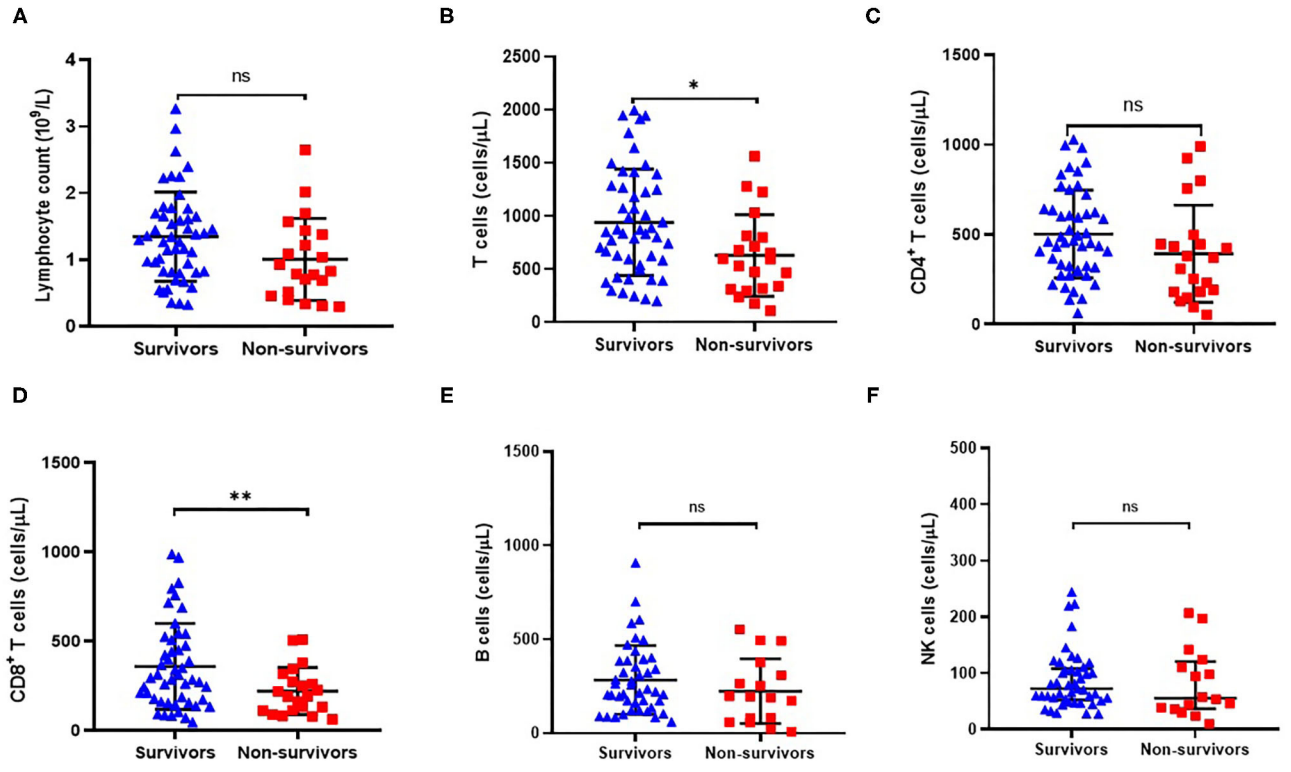


FIGURE 3 | Comparison of peripheral absolute counts of lymphocyte **(A)**, $CD3^+$ T cells **(B)**, $CD4^+$ T cells **(C)**, $CD8^+$ T cells **(D)**, $CD19^+$ B cells **(E)** and $CD16^+CD56^+$ NK cells **(F)** in surviving ($n = 49$) and non-surviving ($n = 21$) patients with HBV-related ACLF. $^*P < 0.05$, $^{**}P < 0.01$.

TABLE 3 | Comparison of mortality rate between the higher and lower group divided by median values of lymphocyte count in HBV-related ACLF patients.

Mortality (*n*, %)	Lower group	Higher group	*P*-value
$CD3^+$ T cells (cells/μl)	15 (42.9)	6 (17.1)	0.019
$CD4^+$ T cells (cells/μl)	14 (40.0)	7 (20.0)	0.068
$CD8^+$ T cells (cells/μl)	15 (42.9)	6 (17.1)	0.019
$CD19^+$ B cells (cells/μl)	13 (37.1)	8 (22.9)	0.192
$CD56^+CD16^+$ NK cells (cells/μl)	12 (34.3)	9 (25.7)	0.434

It is known that chronic HBV infection lead to T cell exhaustion or dysfunction, resulting in the immunotolerance and viral persistence (19). The pathogenesis of HBV-ACLF is also a dysfunctional immune response caused by increased systemic inflammation and immune cell paralysis, which has been manifested in pre-existing liver diseases. Moreover, it was reported that immune disorders in ACLF were similar to sepsis, with abnormalities in the immune response, ranging from excessive inflammation to immune depression (20, 21). This study investigated the distribution characteristics of dominant peripheral lymphocyte subsets in patients with HBV-related ACLF. Consistent with previous findings (6, 8, 14), significant reductions were observed both in peripheral lymphocyte percentage and absolute number in ACLF patients compared with non-ACLF patients. We noted that the decrease in total lymphocyte counts in ACLF patients was possibly the result of a relative decrease in $CD8^+$ T cell as well as $CD4^+$ T cell and $CD16^+CD56^+$ NK cell counts (**Figure 2**, respectively). However, no significant difference was observed in the proportion of $CD4^+$ T cells, $CD8^+$ T cells and $CD16^+CD56^+$ NK cells between HBV-ACLF and non-ACLF patients. This observation could be explained by exhausted lymphocytes during liver failure. It seems that under the immunosuppressive condition of ACLF, the change in lymphocyte subset counts was more obvious than the proportion of these subsets. Notably, we found that the frequency of circulating $CD19^+$ B cells in ACLF patients was significantly increased. A recent study demonstrated that HBV-ACLF patients had higher serum IgG, IgA, and IgM levels compared to CHB patients (22). Moreover, an overwhelming B cell response apparently centered in liver tissue was observed in patients with HBV-associated acute liver failure (23). These studies indicated that the B-cell immune response might play a role in the pathogenesis of HBV-related ACLF.

Importantly, we found that $CD8^+$ T cell counts were significantly decreased in non-survivors compared with survivors (**Figure 3D**). Furthermore, the group with the lowest $CD8^+$ T cell count displayed a significantly higher mortality rate compared to the group with the highest $CD8^+$ T cell count, indicating that a reduction in $CD8^+$ T cell count might be related to the poor prognosis of HBV-ACLF patients (**Figure 4**). Thus, circulating lymphocyte numbers could be a potential parameter for monitoring disease progression, especially $CD8^+$ T cells.

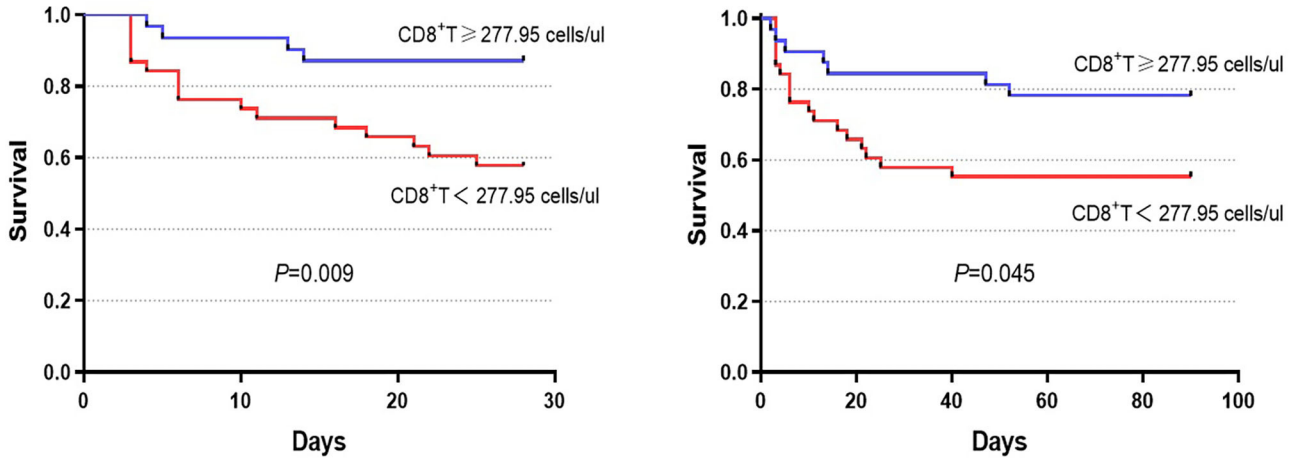


FIGURE 4 | Survival was evaluated using Kaplan–Meier curves, and the statistics were compared by log-rank tests. Significant differences were found between the higher group ($CD8^+$ T cell count ≥ 277.95 cells/μl) and the lower group ($CD8^+$ T cell count < 277.95 cells/μl) in 28-day (chi-square = 6.803, $P = 0.009$) and 90-day (chi-square = 4.015, $P = 0.045$) survival.

It is reasonable to believe that lower circulating lymphocytes in peripheral blood, like $CD8^+$ T cells, increase the risk of infection and result in endotoxemia, which in turn, exacerbates inflammatory damage of liver tissue (13, 24–26). A recent study of ACLF patients reported that peripheral mononuclear myeloid-derived suppressor cell expansion suppressed T-cell proliferation and increased sensitivity to bacterial infections (27). In addition, previous studies reported that $CD8^+$ T cells were more prone to undergoing apoptosis or clonal deletion, especially in the absence of activated $CD4^+$ T cells (28, 29). Therefore, monitoring these circulating lymphocytes, especially $CD8^+$ T cells, might help to predict the extent of liver damage.

There are however some limitations in our study. First, this is a retrospective review of a single-center experience. The current findings need to be confirmed in large, multicenter prospective studies. Second, we only investigated the prevalence of dominant peripheral lymphocyte subsets, and the cellular immune responses in liver tissue are unclear. Studying the histology of the liver may be interesting as changes in T cells in peripheral blood do not necessarily reflect the T cell population in liver tissue. However, for a life-threatening disease like ACLF, barriers existed to get liver biopsy for such severe diseases. Nevertheless, the overall features of peripheral lymphocyte subsets in ACLF shown in this study are valuable and could help to narrow the ranges in further studies.

CONCLUSION

The abnormal distribution of circulating lymphocytes probably associated with the progressive development of HBV-related ACLF. The decrease in $CD8^+$ T cell counts may be related to poor prognosis in HBV-ACLF patients. Our findings will contribute to a further understanding of the immune pathogenesis of HBV-related ACLF.

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 First Affiliated Hospital of Xi'an Jiaotong University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JL and Y-LH planned and designed the study and wrote the protocol. Y-LH, T-YC, and Y-RZ were responsible for the treatment of patients. C-HH, YC, M-MZ, Z-JG, and M-JF participated in the study monitoring and management. JL, J-ZL, and JW were biostatisticians and participated in the data analysis and writing of the report. All authors read and approved the final version of the work.

FUNDING

This research was supported by the National Natural Science Foundation of China Grant Nos. 81770622 and 81700559, Shaanxi Provincial Natural Science Foundation (2019JM-021), and the Clinical Research Award of the First Affiliated Hospital of Xi'an Jiaotong University, China (No. XJTU1AF-CRF-2018-002). The funding sources had no involvement in the study design, writing the manuscript, or decision to submit for publication.

ACKNOWLEDGMENTS

We all express our gratitude to the patients, who kindly gave consent for the study to be presented in this paper.

REFERENCES

1. Bernal W, Jalan R, Quaglia A, Simpson K, Wendon J, Burroughs A. Acute-on-chronic liver failure. *Lancet.* (2015) 386:1576–87. doi: 10.1016/S0140-6736(15)00309-8
2. Tang LSY, Covert E, Wilson E, Kottilil S. Chronic hepatitis B infection: a review. *JAMA.* (2018) 319:1802–13. doi: 10.1001/jama.2018.3795
3. Zhao RH, Shi Y, Zhao H, Wu W, Sheng JF. Acute-on-chronic liver failure in chronic hepatitis B: an update. *Expert Rev Gastroenterol Hepatol.* (2018) 12:341–50. doi: 10.1080/17474124.2018.1426459
4. Fan Z, EnQiang C, Yao DL, LiBo Y, Hong L, Lang B, et al. Neutrophil-lymphocyte ratio predicts short term mortality in patients with hepatitis B virus-related acute-on-chronic liver failure treated with an artificial liver support system. *PLoS ONE.* (2017) 12:e0175332. doi: 10.1371/journal.pone.0175332
5. Moreau N, Wittebole X, Fleury Y, Forget P, Laterre PF, Castanares-Zapatero D. Neutrophil-to-lymphocyte ratio predicts death in acute-on-chronic liver failure patients admitted to the intensive care unit: a retrospective cohort study. *Shock.* (2018) 49:385–92. doi: 10.1097/SHK.0000000000000993
6. Zhu S, Waili Y, Qi X, Chen Y, Lou Y. Lymphocyte-monocyte ratio at admission predicts possible outcomes in patients with acute-on-chronic liver failure. *Eur J Gastroenterol Hepatol.* (2017) 29:31–5. doi: 10.1097/MEG.0000000000000767
7. Wu W, Yan H, Zhao H, Sun W, Yang Q, Sheng J, et al. Characteristics of systemic inflammation in hepatitis B-precipitated ACLF: differentiate it from no-ACLF. *Liver Int.* (2018) 38:248–57. doi: 10.1111/liv.13504
8. Cai J, Wang K, Han T, Jiang H. Evaluation of prognostic values of inflammation-based makers in patients with HBV-related acute-on-chronic liver failure. *Medicine.* (2018) 97:e13324. doi: 10.1097/MD.0000000000013324
9. Guidotti LG, Isogawa M, Chisari FV. Host-virus interactions in hepatitis B virus infection. *Curr Opin Immunol.* (2015) 36:61–6. doi: 10.1016/j.coi.2015.06.016
10. Tan G, Zhao W, Liu X, Wang J, Wu Y. Immunophenotypic profile of intrahepatic and circulating lymphocytes in chronic hepatitis B patients. *Hepatogastroenterology.* (2012) 59:1516–21. doi: 10.5754/hge11710
11. Asabe S, Wieland SF, Chattopadhyay PK, Roederer M, Engle RE, Purcell RH, et al. The size of the viral inoculum contributes to the outcome of hepatitis B virus infection. *J. Virol.* (2009) 83:9652–62. doi: 10.1128/JVI.00867-09
12. Nakamoto Y, Suda T, Momoi T, Kaneko S. Different procarcinogenic potentials of lymphocyte subsets in a transgenic mouse model of chronic hepatitis B. *Cancer Res.* (2004) 64:3326–33. doi: 10.1158/0008-5472.CAN-03-3817
13. Tang L, Chen C, Gao X, Zhang W, Yan X, Zhou Y, et al. Interleukin 21 reinvigorates the antiviral activity of hepatitis B virus (HBV)-specific CD8+ T cells in chronic HBV infection. *J Infect Dis.* (2019) 219:750–9. doi: 10.1093/infdis/jiy576
14. Zou Z, Xu D, Li B, Xin S, Zhang Z, Huang L, et al. Compartmentalization and its implication for peripheral immunologically-competent cells to the liver in patients with HBV-related acute-on-chronic liver failure. *Hepatol. Res.* (2009) 39:1198–207. doi: 10.1111/j.1872-034X.2009.00571.x
15. Dong X, Gong Y, Zeng H, Hao Y, Wang X, Hou J, et al. Imbalance between circulating CD4+ regulatory T and conventional T lymphocytes in patients with HBV-related acute-on-chronic liver failure. *Liver Int.* (2013) 33:1517–26. doi: 10.1111/liv.12248
16. Yi RT, Niu YH, Liu HL, Zhang TY, Yang YC, Zhang Y, et al. Natural killer group 2A expressed on both peripheral CD3(-)CD56(+)NK cells and CD3(+)CD8(+)T cells plays a pivotal negative regulatory role in the progression of hepatitis B virus-related acute-on-chronic liver failure. *J Interferon Cytokine Res.* (2016) 36:689–97. doi: 10.1089/jir.2015.0166
17. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. *Hepatol Int.* (2019) 13:353–90. doi: 10.1007/s12072-019-09946-3
18. Kamath PS, Kim WR. The model for end-stage liver disease (MELD). *Hepatology.* (2007) 45:797–805. doi: 10.1002/hep.21563
19. Shin E-C, Sung PS, Park S-H. Immune responses and immunopathology in acute and chronic viral hepatitis. *Nat Rev Immunol.* (2016) 16:509–23. doi: 10.1038/nri.2016.69
20. Hensley MK, Deng JC. Acute on chronic liver failure and immune dysfunction: a mimic of sepsis. *Semin Respir Crit Care Med.* (2018) 39:588–97. doi: 10.1055/s-0038-1672201
21. Albillos A, Lario M, Alvarez-Mon M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. *J Hepatol.* (2014) 61:1385–96. doi: 10.1016/j.jhep.2014.08.010
22. Zhang GL, Zhao QY, Xie C, Peng L, Zhang T, Gao ZL. Elevated serum IgG levels positively correlated with IL-27 may indicate poor outcome in patients with HBV-related acute-on-chronic liver failure. *J Immunol Res.* (2019) 2019:1538439. doi: 10.1155/2019/1538439
23. Farci P, Diaz G, Chen Z, Govindarajan S, Tice A, Agulto L, et al. B cell gene signature with massive intrahepatic production of antibodies to hepatitis B core antigen in hepatitis B virus-associated acute liver failure. *Proc Natl Acad Sci USA.* (2010) 107:8766–71. doi: 10.1073/pnas.1003854107
24. Zhuang Y, Xu HC, Shinde PV, Warfsmann J, Vasilevska J, Sundaram B, et al. Fragile X mental retardation protein protects against tumour necrosis factor-mediated cell death and liver injury. *Gut.* (2020) 69:133–45. doi: 10.1136/gutjnl-2019-318215
25. Malik G, Wilting J, Hess CF, Ramadori G, Malik IA. PECAM-1 modulates liver damage induced by synergistic effects of TNF-alpha and irradiation. *J Cell Mol Med.* (2019) 23:3336–44. doi: 10.1111/jcmm.14224
26. Liu T, Luo X, Li ZH, Wu JC, Luo SZ, Xu MY. Zinc-alpha2-glycoprotein 1 attenuates non-alcoholic fatty liver disease by negatively regulating tumour necrosis factor-alpha. *World J Gastroenterol.* (2019) 25:5451–68. doi: 10.3748/wjg.v25.i36.5451
27. Zeng Y, Li Y, Xu Z, Gan W, Lu L, Huang X, et al. Myeloid-derived suppressor cells expansion is closely associated with disease severity and progression in HBV-related acute-on-chronic liver failure. *J Med Virol.* (2019) 91:1510–8. doi: 10.1002/jmv.25466
28. Benseler V, Warren A, Vo M, Holz LE, Tay SS, Le Couteur DG, et al. Hepatocyte entry leads to degradation of autoreactive CD8 T cells. *Proc Natl Acad Sci USA.* (2011) 108:16735–40. doi: 10.1073/pnas.1112251108
29. Holz LE, Benseler V, Bowen DG, Bouillet P, Strasser A, O'Reilly L, et al. Intrahepatic murine CD8 T-cell activation associates with a distinct phenotype leading to Bim-dependent death. *Gastroenterology.* (2008) 135:989–7. doi: 10.1053/j.gastro.2008.05.078

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.

frontiers in Medicine

ORIGINAL RESEARCH
published: 02 August 2021
doi: 10.3389/fmed.2021.709884

Impact of Hepatic Encephalopathy on Clinical Characteristics and Adverse Outcomes in Prospective and Multicenter Cohorts of Patients With Acute-on-Chronic Liver Diseases

Liyuan Long[1†], Hai Li[2,3,4†], Guohong Deng[4,5†], Xianbo Wang[4,6†], Sihong Lu[7†], Beiling Li[8†], Zhongji Meng[4,9], Yanhang Gao[4,10], Zhiping Qian[4,11], Feng Liu[4,12,13], Xiaobo Lu[4,14], Haotang Ren[15], Jia Shang[16], Hai Li[17], Shaoyang Wang[18], Yubao Zheng[4,19], Huadong Yan[4,20], Shan Yin[2,3], Wenting Tan[5], Qun Zhang[6], Xin Zheng[4,7], Jinjun Chen[4,8], Sen Luo[9], Jinming Zhao[10], Wei Yuan[11], Tao Li[13], Rongjiong Zheng[14], Junping Liu[16], Xiaoxiao Liu[1], Wenyi Gu[2,3], Sumeng Li[7], Xue Mei[11], Ruochan Chen[1] and Yan Huang[1,4*]*

[1] Department of Infectious Diseases, Hunan Key Laboratory of Viral Hepatitis, Xiangya Hospital, Central South University, Changsha, China, [2] Department of Gastroenterology, School of Medicine, Ren Ji Hospital, Shanghai Jiao Tong University, Shanghai, China, [3] Key Laboratory of Gastroenterology and Hepatology, Shanghai Institute of Digestive Disease, Chinese Ministry of Health (Shanghai Jiao Tong University), Shanghai, China, [4] Chinese Chronic Liver Failure (CLIF) Consortium, Shanghai, China, [5] Department of Infectious Diseases, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China, [6] Center of Integrative Medicine, Beijing Ditan Hospital, Capital Medical University, Beijing, China, [7] Department of Infectious Diseases, Institute of Infection and Immunology, Tongji Medical College, Union Hospital, Huazhong University of Science and Technology, Wuhan, China, [8] Hepatology Unit, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China, [9] Department of Infectious Diseases, Taihe Hospital, Hubei University of Medicine, Shiyan, China, [10] Department of Hepatology, The First Hospital of Jilin University, Changchun, China, [11] Department of Liver Intensive Care Unit, Shanghai Public Health Clinical Centre, Fudan University, Shanghai, China, [12] Department of Hepatology, Tianjin Institute of Hepatology, Nankai University Second People's Hospital, Tianjin, China, [13] Department of Infectious Diseases and Hepatology, The Second Hospital of Shandong University, Jinan, China, [14] Infectious Disease Center, The First Affiliated Hospital of Xinjiang Medical University, Ürümqi, China, [15] State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Disease, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, [16] Department of Infectious Diseases, Henan Provincial People's Hospital, Zhengzhou, China, [17] Department of Infectious Diseases, Affiliated Hospital of Logistics University of People's Armed Police Force, Tianjin, China, [18] Department of Infectious Diseases, Fuzhou General Hospital of Nanjing Military Command, Fuzhou, China, [19] Department of Infectious Diseases, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, [20] Department of Hepatology, Ningbo No. 2 Hospital, Ningbo, China

Importance: Hepatic encephalopathy is a severe complication, and its contribution to clinical adverse outcomes in patients with acute-on-chronic liver diseases from the East is unclear.

Objective: We aimed to investigate the impact of hepatic encephalopathy on clinical characteristics and adverse outcomes in prospective and multicenter cohorts of patients with acute-on-chronic liver diseases.

Design: We conducted a cohort study of two multicenter prospective cohorts.

Setting: China.

Participants: Acute-on-chronic liver disease patients with various etiologies.

OPEN ACCESS

Edited by:
Tao Chen,
Huazhong University of Science and Technology, China

Reviewed by:
Yu-Chen Fan,
Shandong University, China
Daxian Wu,
First Affiliated Hospital of Nanchang University, China
Qiao Yang,
Sir Run Run Shaw Hospital, China

***Correspondence:**
Ruochan Chen
84172332@qq.com
Yan Huang
drhyan@163.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *14 May 2021*
Accepted: *07 July 2021*
Published: *02 August 2021*

Citation:
Long L, Li H, Deng G, Wang X, Lu S, Li B, Meng Z, Gao Y, Qian Z, Liu F, Lu X, Ren H, Shang J, Li H, Wang S, Zheng Y, Yan H, Yin S, Tan W, Zhang Q, Zheng X, Chen J, Luo S, Zhao J, Yuan W, Li T, Zheng R, Liu J, Liu X, Gu W, Li S, Mei X, Chen R and Huang Y (2021) Impact of Hepatic Encephalopathy on Clinical Characteristics and Adverse Outcomes in Prospective and Multicenter Cohorts of Patients With Acute-on-Chronic Liver Diseases. Front. Med. 8:709884. doi: 10.3389/fmed.2021.709884

Exposure: The diagnosis and severity of hepatic encephalopathy were assessed using the West Haven scale.

Main Outcome Measure: The correlation between clinical adverse outcomes and varying hepatic encephalopathy grades was analyzed in the target patients.

Results: A total of 3,949 patients were included, and 340 of them had hepatic encephalopathy. The incidence of hepatic encephalopathy was higher in patients with alcohol consumption (9.90%) than in those with hepatitis B virus infection (6.17%). The incidence of 28- and 90-day adverse outcomes increased progressively from hepatic encephalopathy grades 1–4. Logistic regression analysis revealed that hepatic encephalopathy grades 3 and 4 were independent risk factors for the 28- and 90-day adverse outcome in the fully adjusted model IV. Stratified analyses showed similar results in the different subgroups. Compared to grades 1–2 and patients without hepatic encephalopathy, those with grade 3 hepatic encephalopathy had a significant increase in clinical adverse outcomes, independent of other organ failures.

Conclusions and Relevance: Hepatic encephalopathy grades 3–4 were independent risk factors for 28- and 90-day adverse outcomes. Hepatic encephalopathy grade 3 could be used as an indicator of brain failure in patients with acute-on-chronic liver disease.

Keywords: hepatic encephalopathy, brain failure, acute on chronic liver disease, prospective, multicenter

KEY POINTS

- **Question:** What is the contribution of hepatic encephalopathy grades to clinical adverse outcomes in patients with acute-on-chronic liver diseases from the East.
- **Findings:** Logistic regression analysis revealed that hepatic encephalopathy grades 3 and 4 were independent risk factors for the 28- and 90-day adverse outcome of patients with acute-on-chronic liver diseases in the fully adjusted model IV.
- **Meaning:** Prevention of progression to higher grades of HE should be an important therapeutic goal. Hepatic encephalopathy grade 3 could be used as an indicator of brain failure in patients with acute-on-chronic liver disease.

INTRODUCTION

Chronic liver disease (CLD) is currently an increasing global problem and burden (1–3). Acute event is a common clinical condition in patients with CLDs, and subsequently progresses to severe liver injury or even liver failure if the condition continues to be aggravated (4). Patients with CLD and acute events are considered to have acute-on-chronic liver diseases (AoCLD) (5). Hepatic encephalopathy (HE) remains one of the most complex and worrisome complications due to severe hepatocellular dysfunction, the presence of large portal-systemic shunts, or both. It usually presents with a wide spectrum of neurological/psychiatric abnormalities, ranging from subclinical alterations, sleep disturbances, personality changes, abnormal behaviors, and coma (6, 7). A combined clinical practice guideline of the European Association for the Study of the Liver (EASL) and American Association for the Study of Liver Disease concluded that HE prevalence could increase as high as 80% in the course of follow-up, whereas overt HE will occur in 30–40% of patients with cirrhosis during their overall clinical courses (8). HE leads to considerable mortality and exerts a multidimensional burden on patients, their caregivers, and the national healthcare system (9, 10).

Considering the recent advances in treatment available for HE, the higher proportion of cognitively impaired older patients with AoCLD, and the increased significance of HE with the relative reduction in variceal bleeding, re-evaluation of HE-associated prognosis is necessary (9). The contribution of HE to clinical outcome as an independent risk factor is important for analysis in a multicenter eastern setting because the etiologies of CLDs, management strategies, and available therapeutic options for HE in the eastern region differ from those around the world (4). To define the impact of HE on characteristics and adverse outcomes, we evaluated two multicenter prospective cohorts including 3,970 patients with CLD (both cirrhotic and non-cirrhotic) with various etiologies and acute events in China.

METHODS

Study Design and Patients

Patients were recruited from two prospective multicenter cohorts with acute events of CLD, named CATCH-LIFE (NCT02457637 and NCT03641872), established by the Chinese Chronic Liver Failure Consortium composed of 15 tertiary hospitals in hepatitis B virus (HBV) endemic areas from January 2015 to December 2017 and July 2018 to January 2019, respectively (11, 12). The

study was approved by the Renji Hospital Ethics Committee of Shanghai Jiaotong University School of Medicine [ethics codes: (2014)148k and (2016)142k], and all written consent was obtained from the patients.

Together, the two independent cohort studies enrolled 3,970 patients with acute events of CLD, with 2,600 and 1,370 patients in the first and second cohorts, respectively. Acute events of CLD were defined as CLD with acute decompensation (AD) or acute liver injury (ALI). CLD was defined as cirrhotic or non-cirrhotic liver disease with a history of liver dysfunction lasting >6 months. Cirrhosis was diagnosed based on computed tomography/magnetic resonance imaging findings, laboratory test results, clinical symptoms, and history of liver disease. A diagnosis of CATCH-LIFE-defined AD required individuals to have acute development of gastrointestinal (GI) hemorrhage, HE, ascites, infection (e.g., spontaneous peritonitis and pneumonia), jaundice [total bilirubin (TB) level > 5 mg/dL], or any combination of these within 1 month before enrollment (13, 14). ALI was defined when total bilirubin was >2 mg/dl or alanine aminotransferase/aspartate aminotransferase was 3 times the range within 1 week (12, 15). The exclusion criteria were hepatocellular carcinoma or other liver malignancies before or during admission, extrahepatic malignancies or severe chronic extrahepatic disease, patients younger than 18 years of age or older than 80 years of age, and pregnancy. Among the 3,970 patients, we excluded one patient with an outlier of the international normalized ratio (INR) and 20 scheduled liver transplantations; thus, the final number of patients analyzed was 3,949. A flow chart of patient recruitment is shown in **Figure 1**.

Data Collection and Diagnosis of HE

We collected clinical data from all enrolled patients, including clinical manifestations and laboratory measurements. HE was diagnosed based on impaired cognition, consciousness, or motor function. The diagnosis and severity of HE were assessed according to the West Haven scale (16, 17) and grouped into four levels (from grades I to IV). The day of admission was day 1. Clinical adverse outcomes were defined as patient mortality or liver transplantation.

Statistical Analysis

Continuous variables were analyzed for normality using the Kolmogorov–Smirnov test. Normally distributed variables were compared using the Student *t*-test and are represented as the mean ± standard deviation. Non-normally distributed variables were compared using the Mann–Whitney U test and are presented as median with interquartile range (IQR). Categorical variables were compared using the χ^2 or Fisher exact test and are represented as count and percentage. Univariate logistic regression analysis was used to determine the potential correlation between HE and 28- and 90-day adverse outcomes. Stepwise regression was used to build logistic regression model I-IV. Model I was unadjusted. Model II was adjusted for age, sex, and etiology. Model III was adjusted on the basis of model II plus cirrhosis, ascites, infection, and gastrointestinal bleeding. Model IV is adjusted based on model III plus total bilirubin, international normalized ratio, and creatinine. Every Model is adjusted stepwise to further demonstrate the influence of these parameters on adverse outcome. Model IV is adjusted based

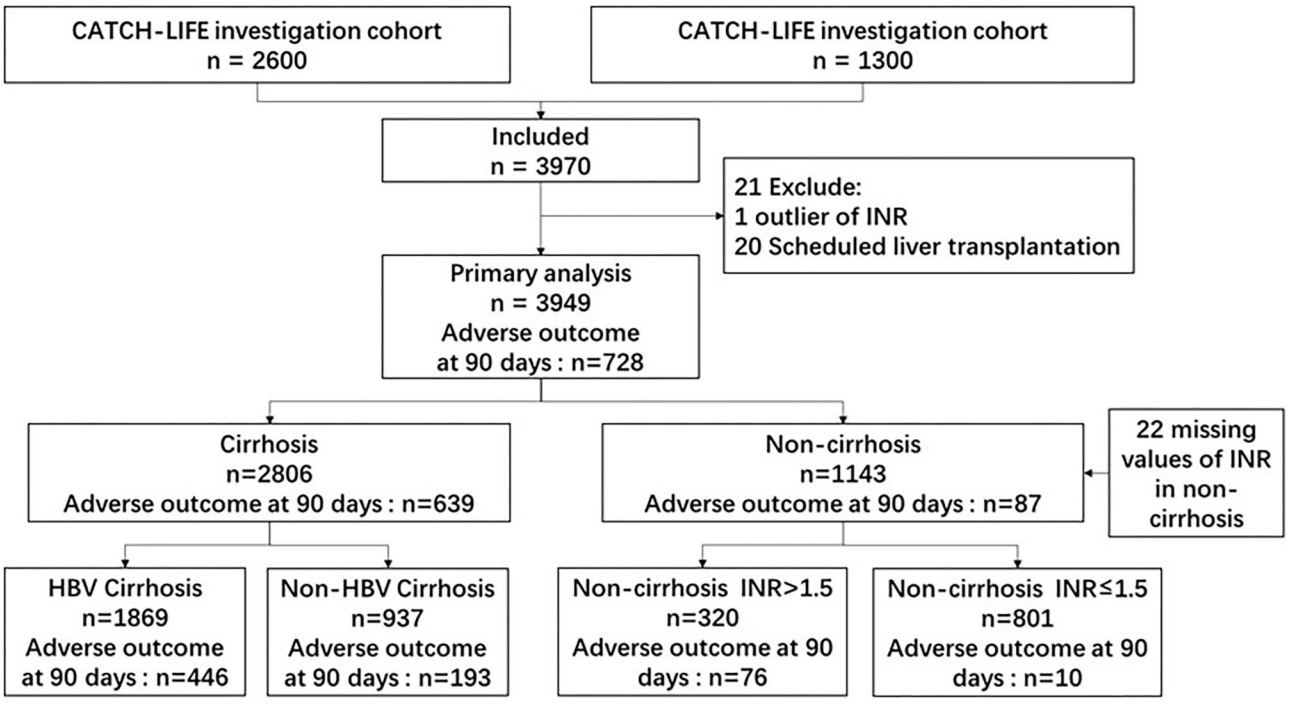


FIGURE 1 | Flow chart of patient recruitment. INR, international normalized ratio; HBV, hepatitis B virus.

TABLE 1 | Association of HE grade with characteristics of AoCLD patients.

Characteristics	NO HE (*N* = 3,610)	HE 1 (*N* = 127)	HE 2 (*N* = 137)	HE 3 (*N* = 55)	HE 4 (*N* = 20)
Demographic					
Age	48.00 (39.81, 57.25)	53.01 (45.18, 61.08)	52.51 (44.08, 60.00)	49.74 (40.45, 61.91)	51.16 (42.20, 55.66)
Gender (male)	2,638 (73.1)	101 (79.5)	112 (81.8)	44 (80.0)	15 (75.0)
Etiology					
HBV	2,600 (72.0)	74 (58.3)	87 (63.5)	26 (47.3)	11 (55.0)
Alcohol	628 (17.4)	35 (27.6)	38 (27.7)	19 (34.5)	4 (20.0)
Autoimmune	363 (10.1)	10 (7.9)	8 (5.8)	7 (12.7)	3 (15.0)
HCV	129 (3.6)	7 (5.5)	6 (4.4)	1 (1.8)	0 (0.0)
HEV	79 (2.2)	3 (2.4)	5 (3.6)	0 (0.0)	1 (5.0)
NAFLD	146 (4.0)	4 (3.1)	0 (0.0)	1 (1.8)	0 (0.0)
Schistosomiasis	48 (1.3)	4 (3.1)	2 (1.5)	1 (1.8)	0 (0.0)
Cryptogenic	171 (4.7)	9 (7.1)	9 (6.6)	5 (9.1)	2 (10.0)
Cirrhosis status					
Non_cirrhosis	1,101 (30.5)	17 (13.4)	13 (9.5)	8 (14.5)	4 (20.0)
Cirrhosis	2,509 (69.5)	110 (86.6)	124 (90.5)	47 (85.5)	16 (80.0)
Compensated	224 (6.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Decompensated	2,285 (63.3)	110 (86.6)	124 (90.5)	47 (85.5)	16 (80.0)
Acute decompensation					
Infection	740 (20.5)	29 (22.8)	49 (35.8)	17 (30.9)	6 (30.0)
Jaundice	1,662 (46.1)	57 (44.9)	85 (62.0)	39 (70.9)	13 (65.0)
Ascites	1,692 (46.9)	57 (44.9)	67 (48.9)	24 (43.6)	10 (50.0)
GI_Bleeding	530 (14.7)	16 (12.6)	20 (14.6)	9 (16.4)	3 (15.0)
Laboratory tests					
TB	4.13 (1.52, 13.38)	3.71 (2.18, 16.02)	10.36 (2.40, 26.21)	10.07 (3.86, 26.52)	13.11 (3.44, 23.41)
INR	1.40 (1.17, 1.76)	1.58 (1.41, 2.22)	1.84 (1.40, 2.79)	1.98 (1.48, 2.98)	2.13 (1.75, 3.01)
CR	0.77 (0.64, 0.93)	0.78 (0.62, 0.96)	0.84 (0.66, 1.11)	0.88 (0.64, 1.32)	0.78 (0.66, 1.08)
BUN	4.49 (3.40, 6.23)	5.05 (3.53, 8.02)	5.99 (4.25, 8.26)	7.11 (3.82, 15.09)	7.36 (4.55, 10.74)
ALB	32.30 (28.20, 37.00)	31.00 (27.00, 34.60)	30.20 (26.80, 34.30)	28.30 (26.70, 32.10)	31.70 (26.45, 35.39)
ALT	100.3 (34.4, 436.0)	45.2 (23.0, 147.0)	45.0 (25.7, 140.6)	57.0 (26.2, 152.2)	102.3 (39.0, 399.3)
AST	113.0 (49.0, 292.0)	75.9 (35.3, 181.1)	76.5 (38.4, 159.8)	89.0 (46.4, 181.2)	133.9 (70.0, 251.5)
WBC	4.95 (3.60, 6.86)	4.91 (3.60, 6.91)	6.36 (4.09, 9.99)	6.90 (5.19, 12.01)	9.44 (6.36, 13.07)
PLT	96.0 (59.0, 148.0)	78.0 (50.4, 118.5)	69.0 (48.0, 119.0)	79.0 (56.5, 128.5)	84.5 (43.6, 112.2)
FIO_2	476.2 (466.7, 476.2)	471.4 (461.9,476.2)	476.2 (466.7, 476.2)	471.4 (433.3, 476.2)	466.7 (410.3, 476.2)
MAP	89.00 (83.00, 94.67)	89.00 (80.66, 96.67)	87.00 (82.00, 94.00)	90.00 (76.84, 96.34)	94.50 (87.83, 101.75)
AKP	127.0 (91.0, 172.0)	116.0 (94.0, 163.4)	121.7 (83.7, 162.1)	117.2 (83.4, 161.2)	113.0 (80.0, 158.5)
γ-GT	82.00 (40.05, 155.88)	60.00 (25.50, 115.00)	48.00 (25.23, 93.40)	44.00 (30.60, 92.60)	54.90 (33.50, 69.50)
NL_ratio	2.46 (1.54, 4.24)	3.03 (1.81, 5.43)	4.33 (2.64, 7.74)	5.61 (3.23, 7.83)	9.03 (4.43, 11.72)
Hemoglobin	118.0 (97.0, 136.0)	113.0 (92.5, 128.5)	108.0 (84.0, 129.0)	98.0 (78.6, 120.5)	109.7 (97.2, 130.7)
Neutrophil	2.99 (1.99, 4.55)	3.10 (2.17, 5.12)	4.57 (2.46, 7.76)	5.39 (3.15, 9.03)	7.18 (4.25, 10.88)
Lymphocyte	1.22 (0.81, 1.75)	1.09 (0.76, 1.46)	1.00 (0.68, 1.50)	1.19 (0.70, 1.83)	0.96 (0.71, 1.12)
K	3.87 (3.52, 4.20)	3.81 (3.50, 4.12)	3.82 (3.41, 4.28)	4.00 (3.70, 4.39)	3.75 (3.14, 4.16)
Na	138.3 (135.7, 141.0)	137.9 (135.0,140.9)	136.0 (133.0, 141.0)	135.2 (131.3, 139.0)	138.7 (135.0, 141.5)
Scores					
MELD	15.50 (10.00, 22.00)	16.00 (12.00, 27.00)	21.50 (13.75, 30.25)	24.50 (18.00, 33.75)	23.00 (17.00, 31.00)
MELD_Na	17.00 (11.00, 24.00)	19.00 (13.00, 29.00)	24.00 (15.00, 32.00)	27.00 (16.00, 35.00)	22.00 (16.00, 33.00)
IMELD	34.00 (27.00, 41.00)	37.00 (30.00, 45.00)	41.00 (34.00, 50.50)	47.00 (35.00, 56.00)	42.00 (34.50, 47.50)
CLIF_SOFA	5.00 (3.00, 6.00)	6.00 (5.00, 8.00)	9.00 (6.75, 9.00)	10.00 (8.00, 11.75)	11.00 (9.00, 12.00)
SOFA	8.00 (7.00, 9.00)	9.00 (8.00, 10.00)	10.00 (9.00, 11.00)	10.00 (9.00, 11.00)	9.00 (8.00, 11.50)
CHILD_PUGH	8.00 (7.00, 10.00)	10.00 (8.00, 11.75)	11.00 (9.00, 12.00)	12.00 (11.00, 13.00)	11.50 (11.00, 13.00)

on model III plus the parameters with clinical significance as indicated in other study, like TB, INR and Cr (18). The survival rates in patients with different HE grades were estimated using the Kaplan–Meier method. All statistical analyses were performed with R (version 4.0.2, http://www.r-project.org).

RESULTS

Clinical Characteristics and Adverse Outcome of Patients With Acute-on-Chronic Liver Diseases According to the HE Grades

Among 3,949 patients with AoCLD, including those with and without cirrhosis, 340 developed HE. The incidences of HE grades 1, 2, 3, and 4 were 37.35, 40.29, 16.18, and 5.88%, respectively. People with HE grades 1–4 were older than those without HE ($p < 0.001$), and male patients were more common than female patients in HE grades 1–4. Regarding etiology, the top three were HBV infection, alcohol consumption, and autoimmune disease. Among patients with HE grades 1–4, there was a high incidence of AD, such as infection and jaundice (**Table 1**).

Patients with and without HE showed significant differences in several laboratory variables. Compared to the patients without HE, those with HE grades 1–4 showed a gradual increase in leukocyte counts, more deteriorated liver parameters (bilirubin, albumin, and INR), and progressively decreased hemoglobin and platelet (PLT) values. Renal function was also significantly worse in patients with HE grades 1–4 than in those without HE ($p < 0.001$). Other alterations in parameters indicated a statistically significant frequency of organ failure and unstable internal environment, such as respiratory failure, renal function, and sodium ion concentration, in patients with HE grades 1–4 compared to those without HE. At inclusion, liver function assessed with the Model of End-stage Liver Disease (MELD), Chronic Liver Failure (CLIF)-Sequential Organ Failure Assessment (SOFA), SOFA score, and Child-Pugh score were significantly worse among the HE groups than among the non-HE group, especially in HE grades 1–4 (**Table 1**).

Next, we sought to assess the association of HE grades with 28- and 90-day adverse outcomes in patients with AoCLD. The incidence of 28- and 90-day adverse outcomes increased progressively from HE grades 1 to 4 (**Table 2**). The Kaplan–Meier curve also revealed that the survival rate was negatively correlated with HE grades, indicating a poorer prognosis in the HE group than in the non-HE group (**Figure 2**).

The major etiologies contributing to HE are different in the Eastern and Western hemispheric regions. We further investigated the impact of HBV infection and alcohol consumption, the two major etiologies of CLD, on the clinical features of patients with HE. The incidence of HE was higher in patients with alcohol consumption (9.90%) than in patients with HBV infection (6.17%). Patients with alcohol-associated HE were older than those with HBV-associated HE ($p = 0.008$), and male patients were more common than female patients in the alcohol-associated HE group ($p = 0.004$). Jaundice was more common in patients with HBV-associated HE than in those with alcohol-associated HE ($p = 0.017$), while GI bleeding was more common in patients with alcohol-associated HE than in those with HBV-associated HE ($p = 0.001$). The values of TB, INR, alanine aminotransferase (ALT), aspartate aminotransferase (AST), MELD, MELD and sodium (MELD-Na), and integrated MELD (IMELD) were significantly higher in the HBV-associated HE group than in the alcohol-associated HE group ($p < 0.05$), suggesting worse clinical conditions with HBV-associated HE. The value of gamma-glutamyl transferase was higher, whereas that of hemoglobin was lower in patients with alcohol-associated HE than in those with HBV-associated HE. Moreover, the incidence of 28- and 90-day adverse outcomes was higher in patients with HBV-related HE than in those with alcohol-related HE ($p = 0.003$ and $p = 0.041$, respectively) (**Table 3**).

The incidence of HE was higher in cirrhotic patients than in non-cirrhotic patients (10.58 vs. 3.76%). Patients with cirrhosis-associated HE were older than patients with non-cirrhosis-associated HE ($p < 0.001$). Jaundice, ascites, and GI bleeding were more common in patients with cirrhosis-associated HE than in those with non-cirrhosis-associated ($p < 0.05$). The values of most laboratory tests, such as TB, INR, blood urea nitrogen, ALT, AST, white blood cell, and PLT, and clinical scores such as MELD, MELD-Na, IMELD, and CLIF-SOFA were worse in cirrhotic patients than in non-cirrhotic patients, as expected. There was no obvious difference in the incidence of 28- and 90-day adverse outcomes between patients with and without cirrhosis (**Table 3**).

Role of HE as an Independent Risk Factor of Adverse Outcome in Patients With Acute-on-Chronic Liver Diseases

We further assessed the role of HE as an independent risk factor for adverse outcomes in patients with AoCLD. We gradually controlled for other risk factors such as age, sex, etiology, cirrhosis, ascites, infection, GI bleeding, TB, INR, and creatinine (CR) in models I–IV. Univariate analysis using logistic regression revealed that HE grades 1–4 were all independent prognostic factors of 28-day adverse outcomes in models I–III (**Table 4**). Moreover, the odds ratio (OR) increased significantly as the HE grade progressed. In model IV, adjusted for model III plus values of TB, INR, and CR, HE grades 3 and 4 were significantly correlated with 90-day adverse outcomes, and the ORs were 4.03 ($p < 0.0001$) and 16.74 ($p < 0.0001$), respectively. HE grades 3 and 4 were also independent prognostic factors in the 90-day adverse outcome analysis in the fully adjusted model IV (**Table 5** and **Figure 3**).

To investigate whether the contribution of HE to clinical adverse outcomes is independent of non-HE organ failures, such as hepatic, renal, pulmonary, and coagulation failures, we excluded patients with a TB level > 12 mg/dL, INR > 1.5, CR level > 2 mg/dL, and need for mechanical ventilation as respiratory failure, according to the definition of organ failure in prior studies (14, 19). We found that there was a significant increase in the clinical adverse outcomes in HE grades 3 and 4, when compared with grades 1–2 and patients without HE, both at 28 and 90 days (**Figure 4**).

TABLE 2 | Association of HE grade with 28-day outcome and 90-day outcome of AoCLD patients.

Outcome	NO HE (*N* = 3,610)	HE1 (*N* = 127)	HE2 (*N* = 137)	HE3 (*N* = 55)	HE4 (*N* = 20)
28-day adverse outcome	351 (9.7)	23 (18.1)	35 (25.5)	26 (47.3)	13 (65.0)
28-day LT	147 (4.1)	5 (3.9)	4 (2.9)	7 (12.7)	2 (10.0)
28-day die	204 (5.7)	18 (14.2)	31 (22.6)	19 (34.5)	11 (55.0)
90-day adverse outcome	594 (16.5)	36 (28.3)	52 (38.0)	32 (58.2)	14 (70.0)
90-day LT	204 (5.7)	7 (5.5)	6 (4.4)	7 (12.7)	2 (10.0)
90-day die	390 (10.8)	29 (22.8)	46 (33.6)	25 (45.5)	12 (60.0)

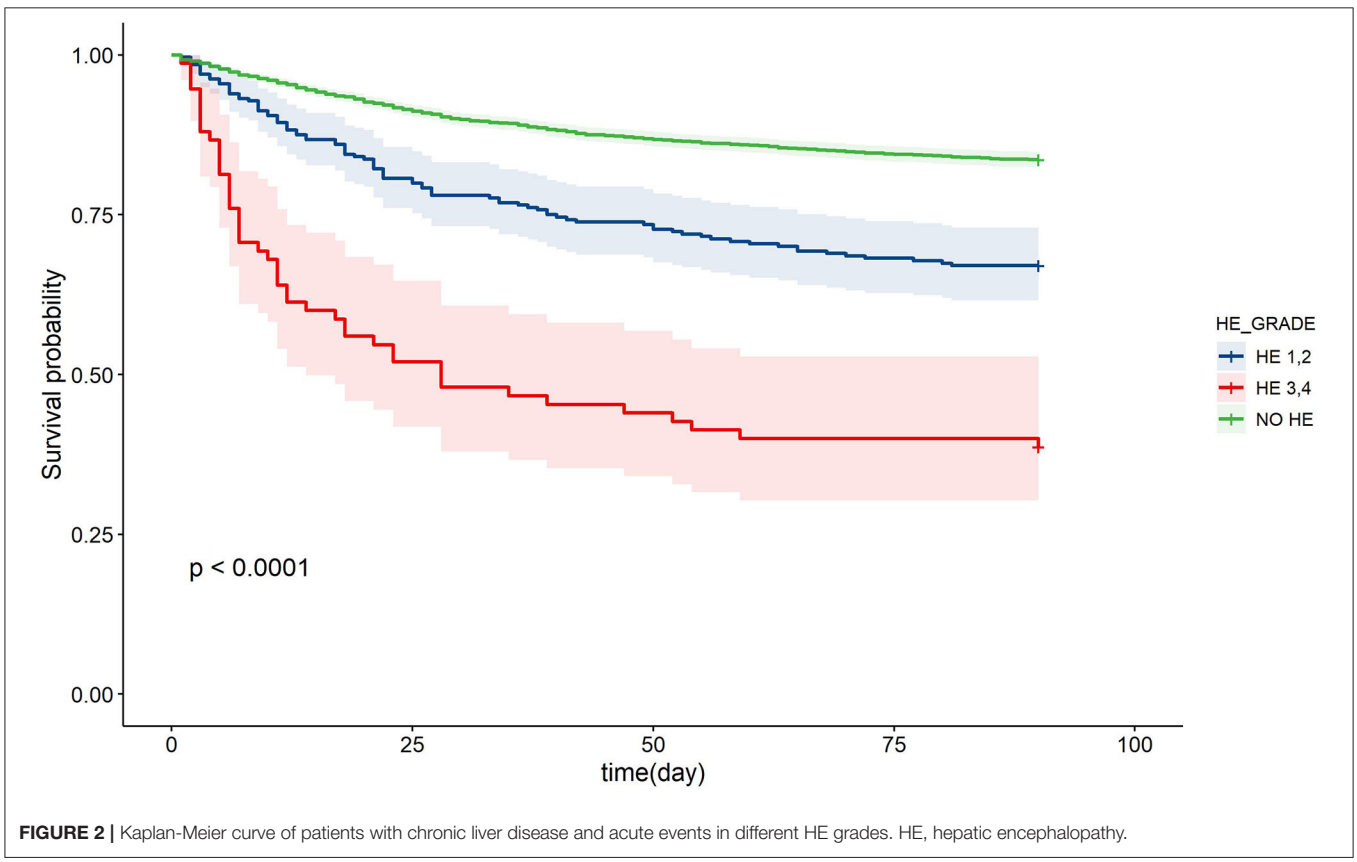


FIGURE 2 | Kaplan-Meier curve of patients with chronic liver disease and acute events in different HE grades. HE, hepatic encephalopathy.

Stratified Analysis of 28- and 90-Day Adverse Outcomes by HE Grades

To further assess the impact of HE grades on clinical adverse outcomes, we performed stratified analysis for interaction with HE grades for the individual related risk factors, including age (>50 and <50 years), TB level (>12 and <12 mg/dL), INR (>1.5 and <1.5), and HBV (HBV-related cirrhosis and non-HBV-related cirrhosis). The stratified analyses demonstrated that HE grades 3–4 but not HE grades 1–2 were independent risk factors for 28-day (**Figure 5**) and 90-day (**Figure 6**) adverse outcomes in all subgroups after fully adjusting for age, sex, etiology, cirrhosis, ascites, infection, GI bleeding, INR, and CR.

HBV was the main etiology (58.5%) in our cohort, and patients with cirrhosis constituted 71.1% of the entire group; thus, we further investigated the impact of HBV on the risk of HE in patients with cirrhosis. Multivariable logistic regression revealed that HE grades 3–4 were independent risk factors for 90-day adverse outcomes after full adjustment, both in patients with HBV-related (**Table 6**) and non-HBV-related cirrhosis (**Table 7**).

Non-cirrhotic patients were stratified into two groups according to the INR values. Non-cirrhotic patients with an INR < 1.5 were excluded because of the limited number of patients with 90-day adverse outcomes (only 10 patients). In non-cirrhotic patients with an INR > 1.5, HE grades 3–4 were also an independent risk factor for 90-day adverse outcomes after adjusting for confounders (**Table 8**).

DISCUSSION

The increasing burden of CLD with acute event worldwide should raise concerns regarding the prevention of morbidity and mortality in these patients (4). Our study's results shed light on

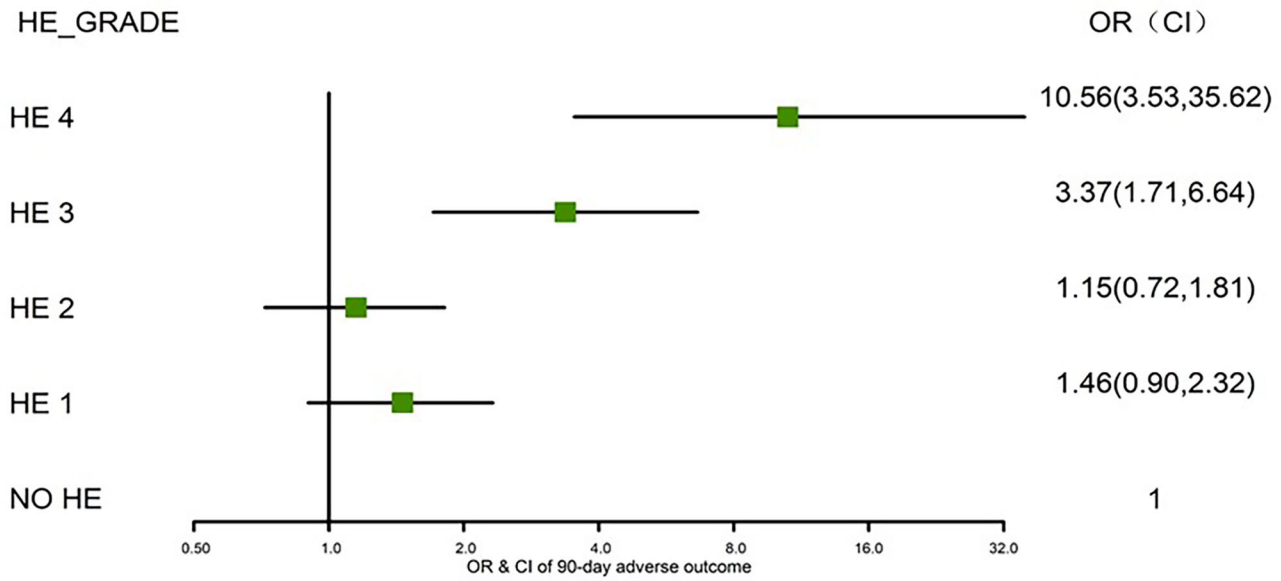


FIGURE 3 | Risks of 90-day adverse outcomes by HE grades I–IV (fully adjusted). HE, hepatic encephalopathy; OR, odds ratio; CI, confidence interval.

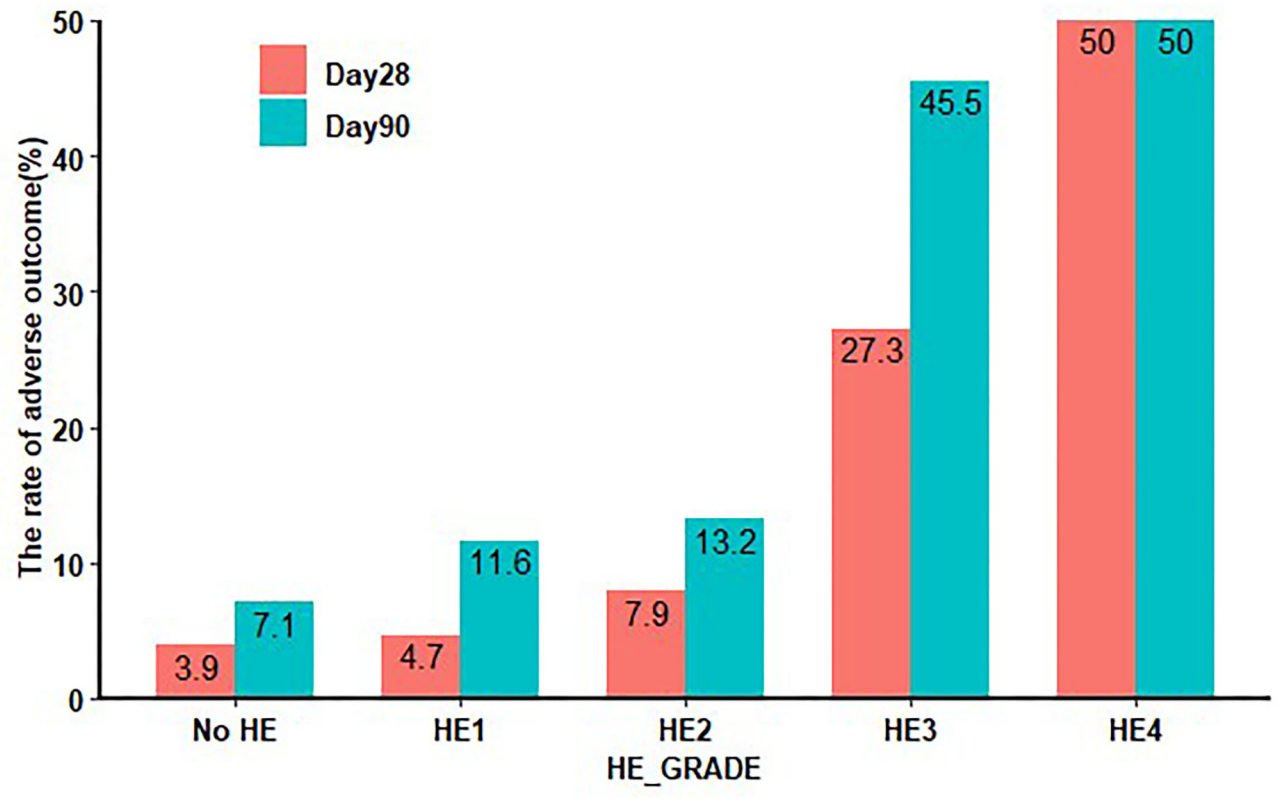


FIGURE 4 | Twenty-eight-day and 90-day clinical adverse outcomes in HE excluding other organ failure. HE: TB level < 12 mg/dL, INR < 1.5, CR < 2 mg/dL, without respiratory failure. HE, hepatic encephalopathy; TB, total bilirubin; INR, international normalized ratio; CR, creatinine.

TABLE 3 | Characteristics of HE patients with different etiologies.

Characteristics	HE			HE		
	Alcohol ($n = 69$)	HBV ($n = 171$)	*P*-value	Non-cirrhosis ($n = 42$)	Cirrhosis ($n = 297$)	*P*-value
Demographic						
Age	54.3 (48.0, 59.0)	50.5 (39.7, 56.8)	0.008	39.5 (33.5, 46.9)	53.3 (46.7, 61.1)	<0.001
Gender (male)	68 (98.6)	144 (84.2)	0.004	32 (76.2)	240 (80.8)	0.62
HE_GRADE			0.319			0.479
HE 1	23 (33.3)	62 (36.3)		17 (40.5)	110 (37.0)	
HE 2	27 (39.1)	76 (44.4)		13 (31.0)	124 (41.8)	
HE 3	16 (23.2)	23 (13.5)		8 (19.0)	47 (15.8)	
HE 4	3 (4.3)	10 (5.8)		4 (9.5)	16 (5.4)	
AD						
Infection	19 (27.5)	50 (29.2)	0.915	13 (31.0)	88 (29.6)	1
Jaundice	33 (47.8)	112 (65.5)	0.017	38 (90.5)	156 (52.5)	<0.001
Ascites	37 (53.6)	78 (45.6)	0.326	12 (28.6)	146 (49.2)	0.019
GI-Bleeding	19 (27.5)	17 (9.9)	0.001	0 (0.0)	48 (16.2)	0.01
Laboratory tests						
TB	4.77 (2.16, 10.36)	14.09 (2.83, 26.68)	<0.001	21.96 (14.61, 28.94)	5.49 (2.18, 22.58)	<0.001
INR	1.61 (1.35, 2.09)	2.14 (1.52, 2.91)	<0.001	2.62 (1.87, 3.19)	1.70 (1.40, 2.41)	<0.001
CR	0.81 (0.65, 1.05)	0.81 (0.63, 1.04)	0.63	0.87 (0.67, 1.00)	0.79 (0.64, 1.10)	0.518
BUN	6.31 (4.60, 11.76)	5.21 (3.80, 7.67)	0.012	3.90 (2.47, 6.38)	5.94 (4.13, 10.20)	<0.001
ALB	30.0 (27.40, 33.00)	30.8 (27.00, 34.75)	0.554	32.15 (29.10, 35.66)	29.90 (26.60, 33.50)	0.005
ALT	30.0 (18.00, 58.00)	81.0 (27.30, 426.35)	<0.001	287.5 (106.7, 1,183.8)	42.0 (23.0, 111.7)	<0.001
AST	51.6 (32.00, 102.22)	101.1 (48.50, 239.85)	<0.001	187.9 (122.3, 535.3)	65.0 (35.6, 146.0)	<0.001
WBC	5.98 (4.30, 8.90)	6.10 (3.77, 10.09)	0.695	8.81 (5.63, 11.22)	5.89 (3.76, 9.20)	0.001
PLT	68.0 (46.40, 99.40)	82.0 (51.20, 121.00)	0.18	121.0 (91.8, 190.0)	71.0 (45.4, 112.0)	<0.001
FIO_2	471.4 (457.1, 476.2)	471.4 (461.9, 476.2)	0.362	476.2 (466.7, 476.2)	471.4 (461.9, 476.2)	0.407
MAP	91.0 (76.67, 96.00)	89.0 (82.66, 96.84)	0.219	89.0 (83.0, 99.3)	89.0 (81.0, 96.0)	0.361
AKP	105.0 (74.5,152.7)	125.5 (98.0, 162.7)	0.024	134.5 (97.7, 168.1)	117.0 (83.0, 158.0)	0.159
γ-GT	86.8 (34.4, 190.3)	46.2 (26.3, 81.9)	<0.001	68.9 (43.8, 91.6)	48.6 (24.0, 99.0)	0.035
NL_ratio	5.13 (3.11, 7.62)	3.82 (2.26, 7.03)	0.054	4.62 (2.83, 7.71)	3.90 (2.31, 7.10)	0.176
Hemoglobin	88.0 (76.2, 115.2)	116.0 (98.0, 135.0)	<0.001	128.0 (105.8, 145.3)	106.0 (84.0, 125.0)	<0.001
Neutrophil	4.20 (2.96, 7.08)	4.23 (2.24, 8.00)	0.481	6.16 (3.88, 8.93)	3.87 (2.27, 6.36)	0.001
Lymphocyte	0.86 (0.71, 1.31)	1.10 (0.73, 1.52)	0.05	1.27 (0.97, 1.61)	1.00 (0.69, 1.45)	0.043
K	3.87 (3.48, 4.34)	3.88 (3.58, 4.27)	0.987	3.82 (3.63, 4.17)	3.83 (3.46, 4.24)	0.777
Na	136.7 (132.9, 140.5)	137.0 (133.2, 141.0)	0.702	137.8 (136.1, 140.9)	136.9 (133.0, 141.0)	0.06
Scores						
MELD	15.0 (8.8, 19.3)	25.5 (16.0, 31.0)	<0.001	29.0 (24.0, 32.0)	19.0 (13.0, 29.0)	<0.001
MELD_Na	16.0 (10.0, 23.5)	27.0 (16.0, 33.0)	<0.001	30.0 (27.0, 33.0)	21.00 (13.5, 29.0)	<0.001
IMELD	36.0 (27.0, 41.5)	43.0 (35.5, 51.0)	<0.001	43.0 (38.0, 50.0)	39.0 (33.0, 50.0)	0.049
CLIF_SOFA	7.0 (6.0, 9.3)	9.0 (7.0, 10.0)	0.09	9.0 (8.0, 11.0)	8.0 (6.0, 9.0)	0.003
SOFA	10.0 (8.0, 11.0)	10.0 (9.0, 11.0)	0.165	10.0 (9.0, 11.0)	10.0 (9.0, 11.0)	0.846
CHILD_PUGH	11.0 (9.0, 12.0)	11.0 (9.0, 12.0)	0.249	11.0 (10.0, 13.0)	11.0 (9.0, 12.0)	0.117
Outcome						
O28	9 (13.0)	56 (32.7)	0.003	18 (42.9)	79 (26.6)	0.046
O90	19 (27.5)	73 (42.7)	0.041	20 (47.6)	114 (38.4)	0.328

the characteristics and impact of HE in patients with AoCLD from two large multicenter cohorts from an area highly endemic for HBV infection. Our study indicated that HE grades 3–4 are independent risk factors for 28- and 90-day adverse outcomes, and HE grade 3 could be used as an indicator of brain failure in patients with CLDs and acute events. To our knowledge, our study is currently the largest prospective cohort of patients with acute events of CLD in the East.

The incidence of HE in patients with AoCLD was 8.61%. However, the overall prevalence and cumulative incidence of

TABLE 4 | Odds ratios and *p*-values of HE grades in the total population at day 28.

HE_GRADE	Num of 28-day adverse outcome (%)	Model I	Model II	Model III	Model IV
NO HE	351 (9.7)	1.0	1.0	1.0	1.0
1	23 (18.1)	2.05 (1.26, 3.20) 0.0024	1.99 (1.21, 3.13) 0.003	1.95 (1.18, 3.10) 0.005	1.47 (0.85, 2.46) 0.146
2	35 (25.5)	3.18 (2.11, 4.70) <0.0001	3.10 (2.04, 4.60) <0.0001	2.77 (1.82, 4.15) <0.0001	1.16 (0.68, 1.89) 0.563
3	26 (47.3)	8.32 (4.82, 14.30) <0.0001	8.76 (5.02, 15.21) <0.0001	8.80 (4.97, 15.54) <0.0001	4.03 (2.06, 7.78) <0.0001
4	13 (65.0)	17.24 (7.01, 46.15) <0.0001	18.76 (7.57, 50.58) <0.0001	19.04 (7.51, 52.44) <0.0001	16.74 (5.81, 52.31) <0.0001

Model I, unadjusted.
Model II, adjusted for age, sex, and etiology.
Model III, adjusted for model II plus cirrhosis, ascites, infection, and gastrointestinal bleeding.
Model IV, adjusted for model III plus total bilirubin, international normalized ratio, and creatinine.

TABLE 5 | Odds ratios and *p*-values of HE grades in the total population at day 90.

HE_GRADE	Num of 90-day adverse outcome (%)	Model I	Model II	Model III	Model IV
NO HE	594 (16.5)	1.0	1.0	1.0	1.0
1	36 (28.3)	2.00 (1.34, 2.97) 0.0004	1.95 (1.29, 2.88) 0.003	1.95 (1.28, 2.91) 0.0014	1.46 (0.90, 2.32) 0.11
2	52 (38.0)	3.00 (2.10, 4.30) <0.0001	2.94 (2.04, 4.20) <0.0001	2.66 (1.82, 3.83) <0.0001	1.15 (0.72, 1.81) 0.54
3	32 (58.2)	7.10 (4.14, 12.34) <0.0001	7.26 (4.21, 12.73) <0.0001	7.65 (4.35, 13.56) <0.0001	3.37 (1.71, 6.64) 0.0004
4	14 (70.0)	11.90 (4.75, 33.70) <0.0001	12.66 (5.03, 35.99) <0.0001	13.07 (5.05, 38.10) <0.0001	10.56 (3.53, 35.62) <0.0001

Model I, unadjusted.
Model II, adjusted for age, sex, and etiology.
Model III, adjusted for model II plus cirrhosis, ascites, infection, and gastrointestinal bleeding.
Model IV, adjusted for model III plus total bilirubin, international normalized ratio, and creatinine.

HE are difficult to define, depending on symptom variability, the tools used for detection and scoring, and objective bias (7). Interestingly, the MELD, MELD-Na, IMELD, SOFA, and Child-Pugh scores showed a decrease in HE grade 4 compared to HE grade 3, indicating an increasing incidence of clinical adverse outcomes. The same results have been obtained in patients waiting for liver transplantation, revealing an independent role of HE in survival (20). The incidence of HE is also affected by different etiologies and cirrhosis status. The incidence of HE was higher in patients with alcohol consumption than in patients with HBV infection, although most other clinical indicators, MELD scores, and clinical outcomes are worse in HBV groups, suggesting that HE may be an indicator of organ failure independent of other organ failure in patients with alcohol consumption.

HE is a marker of decompensated disease and is a component of the Child-Pugh-Turcotte scoring system (21). The impact of HE on mortality among patients with cirrhosis, acute-on-chronic liver failure, and end-stage liver diseases awaiting liver transplantation has been explored in several studies (9, 22–27). In the Canonic Study by the EASL, HE appeared as an isolated syndrome or as part of acute-on-chronic liver failure, with different characteristics and high mortality. In these two large experiences of multicenter cohorts, we found that higher HE grades continue to carry a poor prognosis, as expected. The same conclusion has been drawn in subgroups such as HBV-related and non-HBV-related cirrhotic patients and non-cirrhotic patients with an INR > 1.5. Our results are consistent with those of other studies showing that HE grades are positively correlated with adverse clinical outcomes (15, 25, 28). Moreover, HE grades 3 and 4 [severe HE (20)] remained independent determinants of mortality in patients with AoCLD, independent of age, sex, etiology, ascites, infection, GI bleeding, TB, INR, and CR. Thus, we elucidated the clinical importance and

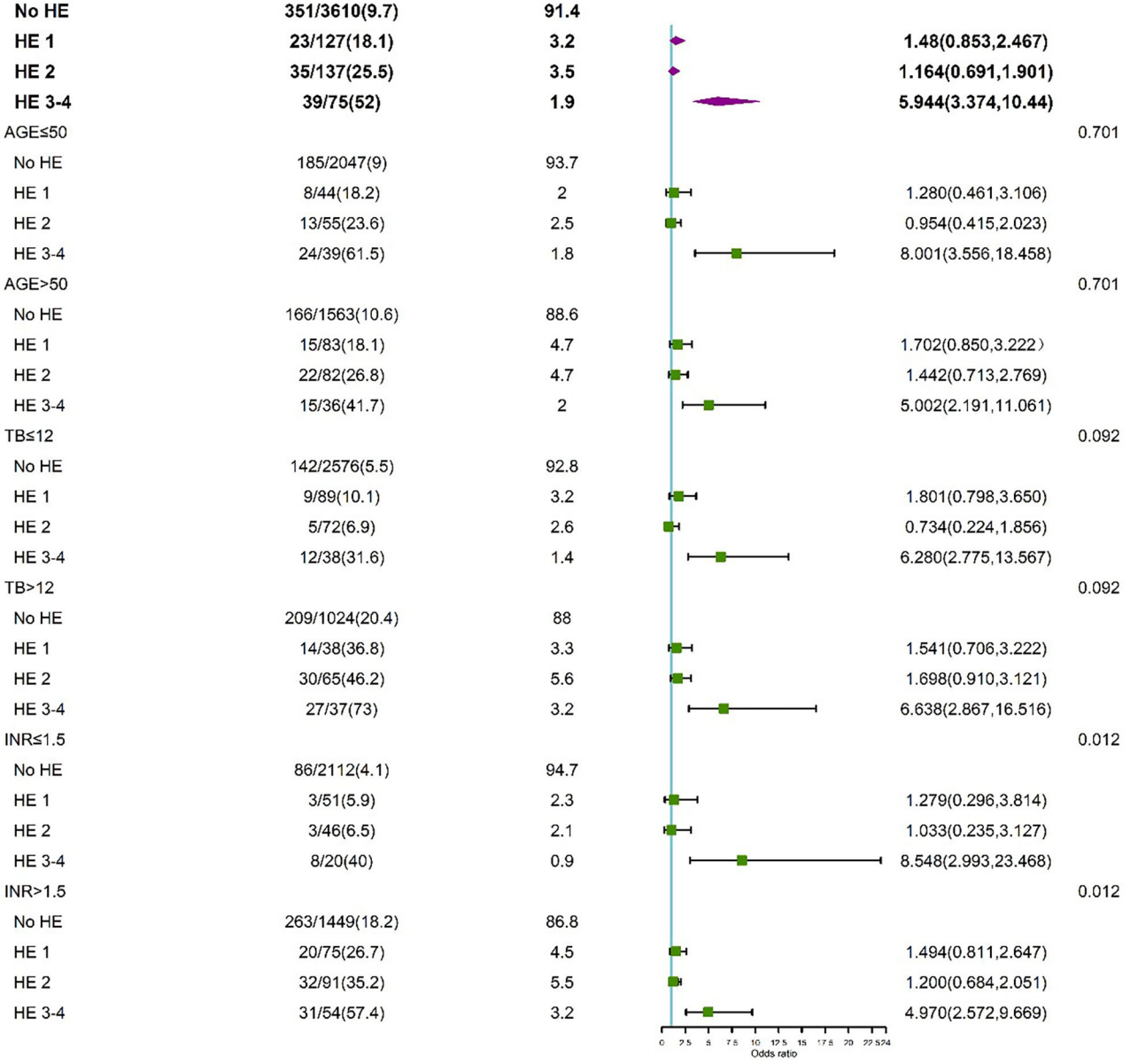


FIGURE 5 | Stratified analyses of 28-day adverse outcomes by HE grades. HE, hepatic encephalopathy; TB, total bilirubin; INR, international normalized ratio; CI, confidence interval.

prognostic significance of HE in patients with AoCLD, based on solid evidence-based proof instead of clinical experience or expert opinion.

Although there are several scoring systems for grading the severity of HE, the West Haven Criteria are most commonly used (29, 30). The potential subjectivity of HE grade identification using the West Haven Criteria has made accurate diagnosis of HE difficult in clinical settings, especially in isolating HE grade 0 from grade 1, or distinguishing grade 1 from grade 2. This is the reason HE grade 0 was not displayed in our analysis. However, the differentiation between HE grades 3–4 and 1–2 has good reliability and is routinely performed in clinical practice (31). We also chose the admission grade of HE to define HE severity, which avoided multiple grades due to individual subjectivity and reduced intra-observer variability, especially for general practitioners. However, the maximum HE grade and duration of HE in hospitalized patients significantly affect mortality and even survival rate after transplantation (9, 10, 16). Thus, it is of great

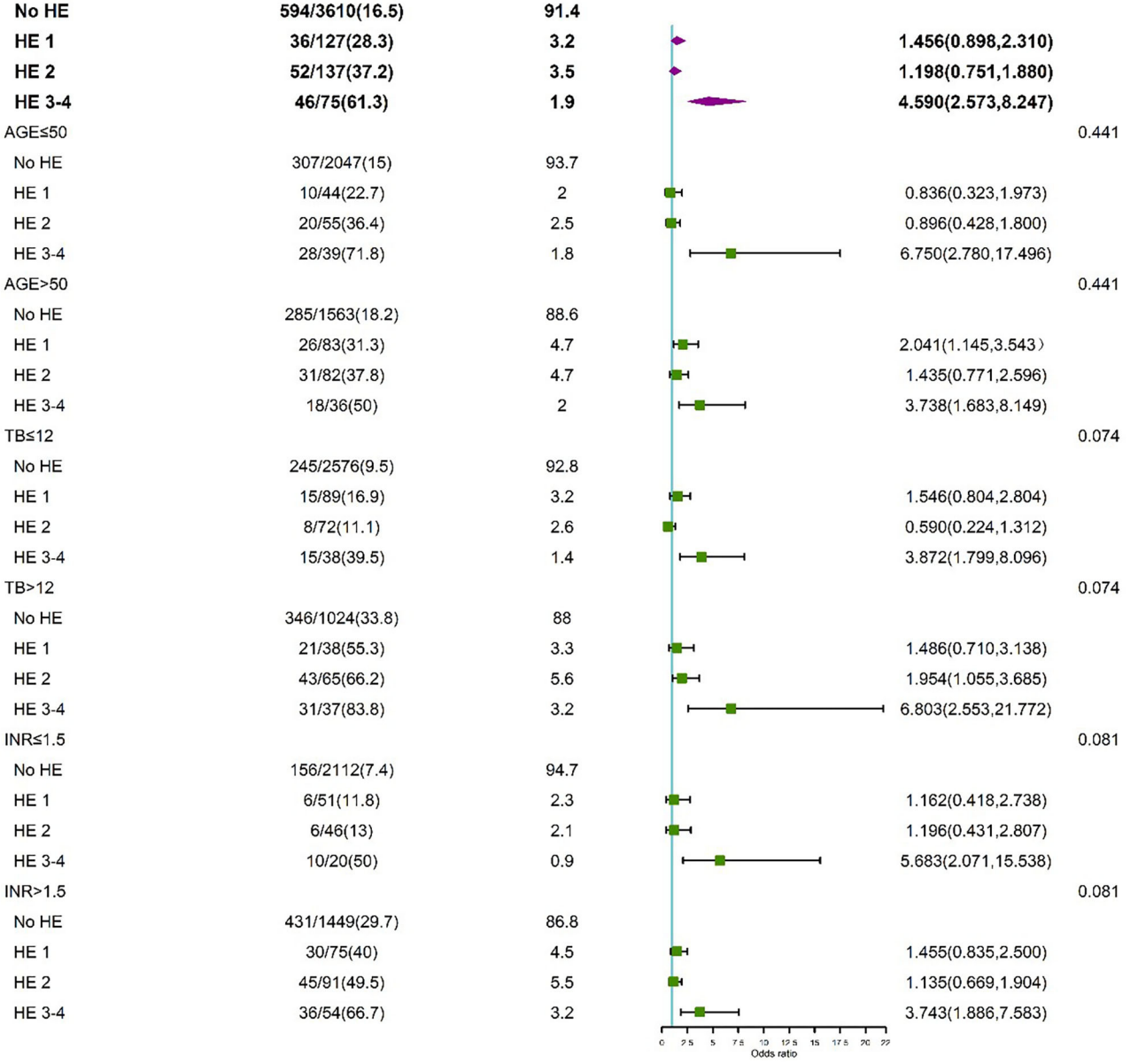


FIGURE 6 | Stratified analyses of 90-day adverse outcome by HE grades. HE, hepatic encephalopathy; TB, total bilirubin; INR, international normalized ratio; CI, confidence interval.

importance to develop a more objective tool that will improve the accuracy and reproducibility of HE severity assessment, thereby increasing the prognostic value of HE grades in clinical settings.

Since HE can exist with and without other non-HE organ failures, we further assessed the impact of HE on clinical adverse outcomes independent of other organ failures. Common non-HE organ failures include liver failure, renal failure, coagulation dysfunction, and respiratory failure. First, we adjusted the values of TB, INR, and CR in a multivariable logistic regression model and found that HE grades 3 and 4 remained independent factors in the clinical adverse outcomes of patients with AoCLD. Moreover, we found that any grade of HE, but especially the higher grades, remained associated with high clinical adverse, irrespective of non-HE-associated organ failure. This becomes even more obvious because HE grade 3 significantly worsened the mortality prognosis regardless of non-HE-associated organ failure from 7.9 to 27.3% within 28 days. Summarizing all these results, our findings indicated that after excluding patients with

TABLE 6 | Odds ratios and *p*-values of HE grades in patients with HBV-associated cirrhosis at day 90.

HE_GRADE	Num of 90-day adverse outcome (%)	Model I	Model II	Model III	Model IV
NO HE	375 (22.0)	1.0	1.0	1.0	1.0
1	19 (32.8)	1.72 (0.96, 2.98) 0.055	1.61 (0.90, 2.80) 0.094	1.70 (0.94, 2.98) 0.066	1.57 (0.79, 3.03) 0.177
2	30 (40.0)	2.36 (1.45, 3.79) 0.0003	2.34 (1.44, 3.76) 0.0004	2.34 (1.43, 3.79) 0.0005	1.00 (0.54, 1.83) 0.975
3-4	22 (73.3)	9.76 (4.48, 23.52) <0.0001	9.70 (4.44, 23.43) <0.0001	9.95 (4.52, 24.18) <0.0001	6.51 (2.42, 18.97) 0.0003

Model I, unadjusted.
Model II, adjusted for age, sex, and etiology.
Model III, adjusted for model II plus ascites, infection, and gastrointestinal bleeding.
Model IV, adjusted for model III plus total bilirubin, international normalized ratio, and creatinine.

TABLE 7 | Odds ratios and *p*-values of HE grades in non-HBV-associated cirrhosis at day 90.

HE_GRADE	Num of 90-day adverse outcome (%)	Model I	Model II	Model III	Model IV
NO HE	151 (18.8)	1.0	1.0	1.0	1.0
1	15 (28.8)	1.75 (0.91, 3.21) 0.079	1.87 (0.97, 3.46) 0.051	2.02 (1.03, 3.78) 0.032	1.68 (0.78, 3.40) 0.161
2	12 (24.5)	1.40 (0.68, 2.67) 0.327	1.58 (0.76, 3.06) 0.190	1.67 (0.79, 3.29) 0.150	1.11 (0.47, 2.39) 0.788
3–4	15 (45.5)	3.59 (1.75, 7.29) 0.0003	4.12 (1.98, 8.49) 0.0001	4.54 (2.16, 9.46) <0.0001	3.50 (1.51, 7.90) 0.002

Model I, unadjusted.
Model II, adjusted for age, sex, etiology.
Model III, adjusted for model II plus ascites, infection, and gastrointestinal bleeding.
Model IV, adjusted for model III plus total bilirubin, international normalized ratio, and creatinine.

TABLE 8 | Odds ratios and *p*-values of HE grades in non-cirrhosis with INR > 1.5 at day 90.

HE_GRADE	Num of 90-day adverse outcome (%)	Model I	Model II	Model III	Model IV
NO HE	56 (19.6)	1.0	1.0	1.0	1.0
1	2 (16.7)	0.81 (0.12, 3.21) 0.798	0.99 (0.14, 4.11) 0.992	1.03 (0.14, 4.57) 0.963	0.59 (0.08, 2.75) 0.548
2	8 (66.7)	8.17 (2.48, 31.54) 0.0008	9.24 (2.68, 37.18) 0.0006	8.23 (2.26, 34.73) 0.001	2.04 (0.41, 10.85) 0.380
3–4	9 (81.8)	18.40 (4.58, 122.93) 0.0002	22.05 (5.08, 154.13) 0.0001	26.43 (5.95, 186.50) <0.0001	7.07 (1.32, 54.05) 0.030

Model I, unadjusted.
Model II, adjusted for age, sex, etiology.
Model III, adjusted for model II plus ascites, infection, and gastrointestinal bleeding.
Model IV, adjusted for model III plus total bilirubin, international normalized ratio, and creatinine.

other organ failure, HE grade 3 remained an indicator of brain failure in referred patients. A previous study also found that added non-HE-associated organ failures increased the mortality rate but did not affect the impact of HE severity on mortality in multivariable analysis (9). Therefore, clinicians, intensivists, and hospital medicine specialists view HE grade 3 as an important risk factor for death regardless of other non-HE-associated organ failures to accurately prognosticate the patients (32). In addition, it is important for clinicians to make every effort to prevent the development of HE or its worsening over time to affect outcomes. Prevention of progression to higher grades of HE should be an important therapeutic goal.

There are some limitations to our study. First, the severity of HE is a dynamic process that changes with progression of disease or initiation of treatment, and attention should be paid to the severity of HE at several time points in the future. Second, the grading of HE severity may vary between different medical centers due to subjective bias. Despite these limitations, we conclude that HE grades 3–4 are an important determinant of 28- and 90-day adverse outcomes in patients with AoCLD, independent of other organ failures, based on solid evidence-based proof. Counseling of patients and caregivers to seek medical attention before the development of grade 3–4 HE and prevention of in-hospital development of grades 3–4 HE should continue to be an important therapeutic goal.

DATA AVAILABILITY STATEMENT

The original contributions generated for 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 Renji Hospital Ethics Committee of Shanghai Jiaotong University School of Medicine [ethics codes: (2014)148k and (2016)142k]. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LL (data curation: equal; formal analysis: lead; investigation: equal; methodology: lead; writing-original draft: equal). HL (2nd author), GD, XW, SLu, ZM, YG, FL, XLu, HY, XZ, YZ, and JC (conceptualization: equal; data curation: equal; funding acquisition: equal; investigation: equal). BL, JS, SW, SY, WT, QZ, SLuo, JZ, WY, TL, RZ, XLiu, WG, SLi, XM, HR, HL (14th author), and JL (data curation: equal; investigation: equal). ZQ (conceptualization: equal; data curation: equal; investigation: equal). RC (conceptualization: equal; writing–original draft: lead; writing–review and editing: lead). YH (conceptualization: lead; data curation: lead; investigation: lead; supervision: lead; writing–review and editing: equal). All authors read and approve the version to be published.

FUNDING

The collection, analysis, and interpretation of data and manuscript writing were supported by grants from the National Natural Sciences Foundation of China (No. 82070613 and 81970550), the National Natural Sciences Foundation of Hunan Province (No. 2019JJ30041), National Major Science and Technology Projects (2018ZX10302206 and 2018ZX10723203), and the Innovation-Driven Project of Central South University (No. 2020CX044).

ACKNOWLEDGMENTS

We thank the study participants for volunteering for this study. We thank Editage for critical reading of the manuscript.

REFERENCES

1. Blum H. A global problem: chronic liver disease. *Dtsch Med Wochenschr.* (2015) 140:1050. doi: 10.1055/s-0041-102910
2. Kim WR, Brown RS Jr, Terrault NA, El-Serag H. Burden of liver disease in the United States: summary of a workshop. *Hepatology.* (2002) 36:227–42. doi: 10.1053/jhep.2002.34734
3. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med.* (2006) 3:e442. doi: 10.1371/journal.pmed.0030442
4. Bajaj JS. Defining acute-on-chronic liver failure: will East and West ever meet. *Gastroenterology.* (2013) 144:1337–9. doi: 10.1053/j.gastro.2013.04.024
5. Clemmesen JO, Larsen FS, Kondrup J, Hansen BA, Ott P. Cerebral herniation in patients with acute liver failure is correlated with arterial ammonia concentration. *Hepatology.* (1999) 29:648–53. doi: 10.1002/hep.510290309
6. Weissenborn K, Bokemeyer M, Krause J, Ennen J, Ahl B. Neurological and neuropsychiatric syndromes associated with liver disease. *AIDS.* (2005) 19 (Suppl. 3):S93–8. doi: 10.1097/01.aids.0000192076.03443.6d
7. Montagnese S, Russo FP, Amodio P, Burra P, Gasbarrini A, Loguercio C,et al. Hepatic encephalopathy 2018: a clinical practice guideline by the Italian Association for the Study of the Liver (AISF). *Dig Liver Dis.* (2019) 51:190–205. doi: 10.1016/j.dld.2018.11.035
8. American Association for the Study of Liver Diseases, European Association for the Study of the Liver. Hepatic encephalopathy in chronic liver disease: 2014 practice guideline by the European Association for the Study of the Liver and the American Association for the Study of Liver Diseases. *J Hepatol.* (2014) 61:642–59. doi: 10.1016/j.jhep.2014.05.042
9. Bajaj JS, O'Leary JG, Tandon P, Wong F, Garcia-Tsao G, Kamath PS, et al. Hepatic encephalopathy is associated with mortality in patients with cirrhosis independent of other extrahepatic organ failures. *Clin Gastroenterol Hepatol.* (2017) 15:565–74.e4. doi: 10.1016/j.cgh.2016.09.157
10. Lucidi C, Ginanni Corradini S, Abraldes JG, Merli M, Tandon P, Ferri F, et al. Hepatic encephalopathy expands the predictivity of model for end-stage liver disease in liver transplant setting: evidence by means of 2 independent cohorts. *Liver Transpl.* (2016) 22:1333–42. doi: 10.1002/lt.24517
11. Gu WY, Xu BY, Zheng X, Chen J, Wang XB, Huang Y, et al. Acute-on-chronic liver failure in china: rationale for developing a patient registry and baseline characteristics. *Am J Epidemiol.* (2018) 187:1829–39. doi: 10.1093/aje/kwy083
12. Zhang Y, Xu BY, Wang XB, Zheng X, Huang Y, Chen J, et al. Prevalence and clinical significance of portal vein thrombosis in patients with cirrhosis and acute decompensation. *Clin Gastroenterol Hepatol.* (2020) 18:2564–72.e1. doi: 10.1016/j.cgh.2020.02.037
13. D'Amico G, Morabito A, D'Amico M, Pasta L, Malizia G, Rebora P, et al. Clinical states of cirrhosis and competing risks. *J Hepatol.* (2018) 68:563–76. doi: 10.1016/j.jhep.2017.10.020
14. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology.* (2013) 144:1426–37, 1437.e1–9. doi: 10.1053/j.gastro.2013.02.042
15. Qiao L, Wang X, Deng G, Huang Y, Chen J, Meng Z, et al. Cohort profile: a multicentre prospective validation cohort of the Chinese acute-on-chronic liver failure (CATCH-LIFE) study. *BMJ Open.* (2021) 11:e037793 doi: 10.1136/bmjopen-2020-037793
16. Amodio P. Hepatic encephalopathy: diagnosis and management. *Liver Int.* (2018) 38:966–975. doi: 10.1111/liv.13752

17. Gerok W. Hepatic encephalopathies. *Infusionsther Klin Ernahr.* (1987) 14 (Suppl. 5):43–9. doi: 10.1159/000226329
18. Yu Z, Zhang Y, Cao Y, Xu M, You S, Chen Y, et al. A dynamic prediction model for prognosis of acute-on-chronic liver failure based on the trend of clinical indicators. *Sci Rep.* (2021) 11:1810 doi: 10.1038/s41598-021-81431-0
19. Sarin SK, Kedarisetty CK, Abbas Z, Amarapurkar D, Bihari C, Chan AC, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) 2014. *Hepatol Int.* (2014) 8:453–71. doi: 10.1007/s12072-014-9580-2
20. Brandman D, Biggins SW, Hameed B, Roberts JP, Terrault NA. Pretransplant severe hepatic encephalopathy, peritransplant sodium and post-liver transplantation morbidity and mortality. *Liver Int.* (2012) 32:158–64. doi: 10.1111/j.1478-3231.2011.02618.x
21. Child CG, Turcotte JG. Surgery and portal hypertension. *Major Probl Clin Surg.* (1964) 1:1–85.
22. Montagnese S, De Rui M, Schiff S, Ceranto E, Valenti P, Angeli P, et al. Prognostic benefit of the addition of a quantitative index of hepatic encephalopathy to the MELD score: the MELD-EEG. *Liver Int.* (2015) 35:58–64. doi: 10.1111/liv.12490
23. Di Pascoli M, Ceranto E, De Nardi P, Donato D, Gatta A, Angeli P, et al. Hospitalizations due to cirrhosis: clinical aspects in a large cohort of italian patients and cost analysis report. *Dig Dis.* (2017) 35:433–8. doi: 10.1159/000458722
24. Labenz C, Wörns MA, Schattenberg JM, Huber Y, Galle PR, Labenz J. Epidemiology of hepatic encephalopathy in german hospitals - the EpHE study. *Z Gastroenterol.* (2017) 55:741–7. doi: 10.1055/s-0043-114671
25. Gadiparthi C, Cholankeril G, Yoo ER, Hu M, Wong RJ, Ahmed A. Waitlist outcomes in liver transplant candidates with high MELD and severe hepatic encephalopathy. *Dig Dis Sci.* (2018) 63:1647–53. doi: 10.1007/s10620-018-5032-5
26. Cordoba J, Ventura-Cots M, Simón-Talero M, Amorós À, Pavesi M, Vilstrup H, et al. Characteristics, risk factors, and mortality of cirrhotic patients hospitalized for hepatic encephalopathy with and without acute-on-chronic liver failure (ACLF). *J Hepatol.* (2014) 60:275–81. doi: 10.1016/j.jhep.2013.10.004
27. Garg H, Kumar A, Garg V, Sharma P, Sharma BC, Sarin SK. Clinical profile and predictors of mortality in patients of acute-on-chronic liver failure. *Dig Liver Dis.* (2012) 44:166–71. doi: 10.1016/j.dld.2011.08.029
28. Tapper EB, Aberasturi D, Zhao Z, Hsu CY, Parikh ND. Outcomes after hepatic encephalopathy in population-based cohorts of patients with cirrhosis. *Aliment Pharmacol Ther.* (2020) 51:1397–1405. doi: 10.1111/apt.15749
29. Cash WJ, McConville P, McDermott E, McCormick PA, Callender ME, McDougall NI. Current concepts in the assessment and treatment of hepatic encephalopathy. *QJM.* (2010) 103:9–16. doi: 10.1093/qjmed/hcp152
30. Atterbury CE, Maddrey WC, Conn HO. Neomycin-sorbitol and lactulose in the treatment of acute portal-systemic encephalopathy. A controlled, double-blind clinical trial. *Am J Dig Dis.* (1978) 23:398–406. doi: 10.1007/BF01072921
31. Salam M, Matherly S, Farooq IS, Stravitz RT, Sterling RK, Sanyal AJ, et al. Modified-orientation log to assess hepatic encephalopathy. *Aliment Pharmacol Ther.* (2012) 35:913–20. doi: 10.1111/j.1365-2036.2012.05038.x
32. Nadim MK, Durand F, Kellum JA, Levitsky J, O'Leary JG, Karvellas CJ, et al. Management of the critically ill patient with cirrhosis: a multidisciplinary perspective. *J Hepatol.* (2016) 64:717–35. doi: 10.1016/j.jhep.2015.10.019

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.

The handling editor declared a shared affiliation with several of the authors SLu, XZ, and SLi.

frontiers in Medicine

ORIGINAL RESEARCH
published: 05 August 2021
doi: 10.3389/fmed.2021.724224

Retrospective Analysis of the Clinical Efficacy of N-Acetylcysteine in the Treatment of Hepatitis B Virus Related Acute-on-Chronic Liver Failure

*Meng-Lan Wang, Xiu-Jun Yin, Xue-Lian Li, Fa-Da Wang, Jing Zhou, Ya-Chao Tao, Yong-Hong Wang, Dong-Bo Wu and En-Qiang Chen**

Center of Infectious Diseases, West China Hospital of Sichuan University, Chengdu, China

Objective: HBV-related acute-on-chronic liver failure (HBV-ACLF) has a high mortality due to severe intrahepatic cholestasis and coagulation dysfunction, thus new treatment measures are urgently needed to improve the therapeutic effect. This study aimed to observe the efficacy of N-acetylcysteine (NAC) in the treatment of HBV-ACLF.

Methods: The data of patients with HBV-ACLF admitted to West China Hospital from October 2019 to August 2020 were collected retrospectively, and they were divided into treatment group and control group according to whether they had received additional NAC treatment. The improvement of biochemistry, coagulation function and disease severity score after 14 days of hospitalization were analyzed between two groups.

Results: A total of 90 HBV-ACLF patients were included, including 42 patients in treatment group and 48 patients in control group. Compared with baseline, serum TBil, DBil, TBA, GGT and ALP in two groups both decreased significantly, while PTA increased significantly. Interesting, the decrease of serum TBil, DBil and TBA and the increase of PTA in treatment group were all significantly than these in control group. Additionally, more patients in treatment group than control group changed from CTP grade C to grade B. Subgroup analysis of CTP grade C patients showed that the decrease of serum TBil, DBil and TBA and the increase of PTA in treatment group were significantly than these in control group.

Conclusion: The NAC treatment may help to improve intrahepatic cholestasis and coagulation dysfunction of HBV-ACLF.

Keywords: N-acetylcysteine, hepatitis B, intrahepatic cholestasis, acute-on-chronic liver failure, clinical efficacy

OPEN ACCESS

Edited by:
Yu Shi,
Zhejiang University, China

Reviewed by:
Xiaogang Xiang,
Shanghai Jiao Tong University, China
Ma Ke,
Huazhong University of Science and Technology, China
Guohong Deng,
Army Medical University, China

****Correspondence:***
En-Qiang Chen
chenenqiang1983@hotmail.com

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *12 June 2021*
Accepted: *09 July 2021*
Published: *05 August 2021*

Citation:
Wang M-L, Yin X-J, Li X-L, Wang F-D, Zhou J, Tao Y-C, Wang Y-H, Wu D-B and Chen E-Q (2021) Retrospective Analysis of the Clinical Efficacy of N-Acetylcysteine in the Treatment of Hepatitis B Virus Related Acute-on-Chronic Liver Failure. Front. Med. 8:724224. doi: 10.3389/fmed.2021.724224

INTRODUCTION

Acute-on-chronic liver failure (ACLF) is a common syndrome, which occurs simultaneously with organ failure and has a high mortality (1). In China, nearly 50,000 people die of liver failure every year, and more than 80% of them are caused by hepatitis B virus (HBV) chronic infection (2). Severe intrahepatic cholestasis and deterioration of coagulation function are not only important clinical

features of HBV-related ACLF (HBV-ACLF), but also closely related to the prognosis of patients (1, 3). Reducing intrahepatic cholestasis and promoting the recovery of coagulation function is the key to the comprehensive treatment of HBV-ACLF (4, 5). Thus, how to promote bile excretion and quickly reduce intrahepatic cholestasis has attracted much clinical attention.

Adenosylmethionine and ursodeoxycholic acid are two important drugs in the treatment of intrahepatic cholestasis (6, 7), which can effectively improve the progress of the disease to a certain extent. However, they are not satisfactory in improving the severe intrahepatic cholestasis of HBV-ACLF, so clinicians are constantly looking for new treatments to improve this dilemma (3, 8). For example, artificial liver support therapy is used by some clinicians to try to treat severe cholestatic jaundice, but the clinical effectiveness and cost-effectiveness are not satisfactory (9). Therefore, in addition to liver transplantation and stem cell therapy (10, 11), clinicians are eager for new drugs and measures to treat intrahepatic cholestasis in HBV-ACLF patients.

Acetylcysteine (NAC) is a small single peptide molecule, which is composed of L-cysteine and acetyl group and contains sulfhydryl group. At present, it is believed that the protective and therapeutic effects of GSH on liver may be related to the maintenance and increase of GSH content in hepatocytes (12). In addition, NAC has the effects of scavenging free radicals and antioxidation, which helps to protect mitochondrial function, inhibit inflammation, and improve liver hemodynamics and microcirculation, thus promoting the repair of hepatocytes (12–14). Therefore, NAC is more and more used in the comprehensive treatment of live failure (15, 16). In this study, we will retrospectively analyze the clinical data of patients with HBV-ACLF treated with NAC to evaluate whether the addition of NAC on the basis of existing comprehensive treatment can further improve the prognosis of patients with HBV-ACLF.

PATIENTS AND METHODS

Study Design and Participants

This is a single center retrospective clinical study, which was carried out in West China Hospital of Sichuan University, with the purpose of evaluating the efficacy and safety of NAC for the treatment of patients with HBV-ACLF. The diagnostic criteria of HBV-ACLF were in accordance with consensus recommendations of the Asian Pacific association for the study of the liver (APASL) (17). This study was conformed strictly to the ethics guidelines of the 1975 Declaration of Helsinki, and approved by the ethics committee of West China Hospital of Sichuan University.

Patients with the following characteristics were deemed eligible for enrollment in this study: (a) symptoms of weak, anorexia, abdominal distension, nausea and other serious gastrointestinal symptoms; (b) laboratory evidences of serum total bilirubin (TBil) $\geq$10 $\times$ ULN μmol/L and international normalized ratio (INR) $\geq$1.5 or prothrombin activity <40%; (c) positive serum hepatitis B surface antigen (HBsAg) for more than 6 months and detectable serum HBV-DNA; (d) aged 18–65 years, regardless of gender. Patients with any of the following conditions were excluded: (a) serious complications in previous 3 months (e.g., gastrointestinal bleeding, serious infection such as sepsis); (b) other causes of active liver disease, including autoimmune liver diseases, drug-induced liver damage, alcoholic liver disease, genetic metabolic liver disease; (c) evidences of liver cancer or other malignant tumors, severe diabetes and autoimmune diseases; (d) coinfection with hepatitis A, C, D or E viruses, and/or human immunodeficiency virus; (e) important organ dysfunctions not due to liver disease; (f) pregnancy and lactation; (g) receiving artificial liver support treatment; (h) failed to receive comprehensive support treatments.

The patients in treatment group and control group were both received active comprehensive supportive treatment, including glycyrrhizic acid preparation, reduced glutathione, polyene phosphatidylcholine, adenomethionine, ursodeoxycholic acid, human albumin and plasma infusion. Patients in treatment group also received NAC treatment (produced by Hangzhou Minsheng Pharmaceutical Group Co., Ltd), with a dosage of 8 g diluted with 250 mL of 10% glucose (intravenous infusion, once a day) and the course of treatment was not <2 weeks.

Data Collection and Observation Indicators

In this study, we collected the detailed demographic data of patients (including age, gender, long course of disease, treatment before admission, etc.), and the auxiliary examination results (including blood routine, various biochemical indexes, coagulation function, blood ammonia, blood pressure, etc.) at the time of admission and during hospitalization HBV related virological indicators and various imaging examination reports, as well as adverse reactions possibly related to N-acetylcysteine reported during treatment. In this study, the blood parameter, serum biochemical and electrolyte indices were detected by automated blood cell Analyzer, automated coagulation analyzer and automatic biochemical analyzer (Olympus AU5400, Olympus Corporation, Tokyo, Japan) using the standard procedures. HBV serological markers were evaluated by electrochemiluminescence immunoassay (Elecsys; Roche Diagnostics, China).

In this study, we mainly explore whether there are significant differences between the treatment group and the control group in the indicators related to intrahepatic cholestasis (such as bilirubin, total bile acid, glutamyltranspeptidase and alkaline phosphatase), as well as prothrombin time, albumin, creatinine, MELD and CTP scores.

Statistical Analysis

Clinical and biochemical data were expressed as frequencies or median/range, as appropriate. Frequencies were compared using the Chi-square test, and the quantitative data was compared using Student's *t*-test (when values were normally distributed) or the nonparametric Mann-Whitney *U*-test. All data were processed by SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) and a value of $P < 0.05$ was considered statistically significant.

RESULTS

A total of 90 patients with HBV-ACLF were included in this study, including 42 patients in the treatment group and 48

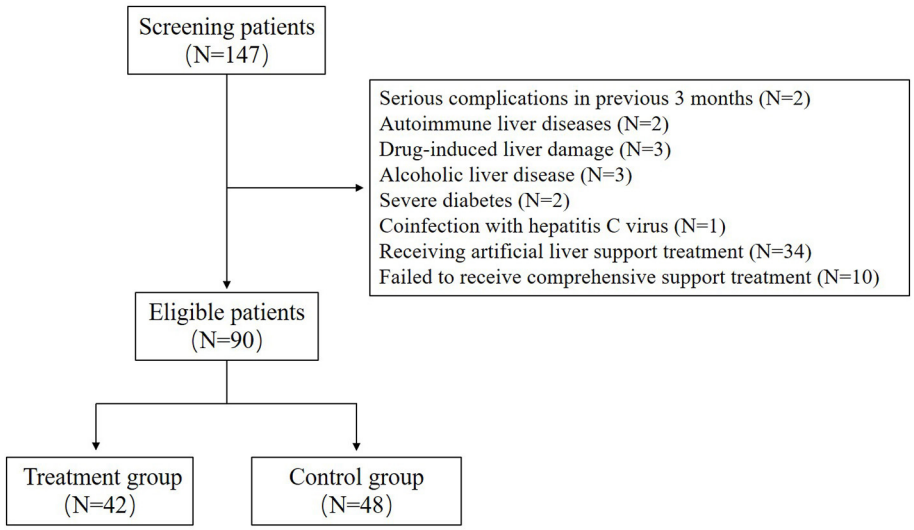


FIGURE 1 | The patient screening and enrollment flow chart.

patients in the control group (**Figure 1**). In this study, all patients in the two groups had cirrhosis, which was indicated by upper abdominal ultrasound or CT. There was no significant difference in age, gender, duration of disease and antiviral treatment before admission between the two groups; There was no significant difference in HBeAg positive rate and HBV DNA level between the two groups. There was also no significant difference in serum TBil, TBA, ALP and PT between the two groups. In addition, nearly half of the patients in each group had ascites. The detailed information is shown in **Table 1**.

During the 2-week observation period, the serum TBIL, DBIL, TBA, GGT and ALP levels of the two groups were significantly lower than those before treatment, which showed gradual downward trends (**Figure 2**). After 1 week of treatment, there was no significant difference in serum levels of TBil [304.5 (284.0–344.0)μmol/L vs. 324.0 (295.0–374.0) μmol/L, $P = 0.071$], DBil [222.5 (195.0–277.0) μmol/L vs. 253.0 (216.0–285.5) μmol/L, $P = 0.099$], TBA [178 (144–244) μmol/L vs. 211.0 (166.5–256.5) μmol/L, $P = 0.072$], and ALP [153 (134–177) IU/L vs. 174.5 (142.5–204.5) IU/L, $P = 0.085$] between the treatment group and control group; while serum GGT was significantly lower in control group than in treatment group [247.5 (188–301) IU/L vs. 220.0 (192.0–262.5) IU/L, $P = 0.047$]. However, after 2 weeks of treatment, the serum levels of DBil [153.5 (110.0–187.0) IU/L vs. 200.0 (166.0–241.5) IU/L, $P = 0.008$] and TBA [113.5 (71.0–148.0) μmol/L vs. 155.5 (123.5–195.0) μmol/L, $P = 0.006$] in the treatment group were significantly lower than those in the control group, although there was no significant difference in the distribution of serum TBIL, GGT and ALP levels. At the second week of treatment, the PT of the treatment group was lower than that of the control group [20.3 (18.3–22.5) second vs. 22.7 (20.5–31.1) seconds, $P = 0.007$] (**Figure 3A**), but there was no significant difference in serum albumin and creatinine between the two groups (**Figures 3B,C**). Although there was no significant difference in MELD score between two groups after 2 weeks of treatment (**Figure 4A**), more patients in the treatment group changed from CTP C-grade to B-grade than the control group (**Figure 4B**), and the difference was statistically significant ($P = 0.003$).

In addition, after 2 weeks of treatment, patients in the treatment group had lower blood ammonia levels than patients in the control group [44.5 (40.1–58.2) vs. 54.2 (49.6–71.9), $P < 0.001$]. After follow-up, it was found that the average length of hospital stay of the treatment group was significantly shorter than that of the control group [15.0 (13.0–17.0) vs. 21.5 (19.0–25.5), $P < 0.001$], although there was no significant difference in the short-term mortality between treatment group and control group [7.1% (3/42) vs. 10.4% (5/48) for 28-day mortality, $P = 0.719$; 9.5% (4/42) vs. 14.6% (7/48) for 90-day mortality, $P = 0.465$].

Subgroup analysis of HBV-ACLF patients with CTP C-grade showed that serum TBIL, DBIL and TB were all lower in the treatment group than those in the control group no matter in the first week or the second week after treatment, although there was no significant difference in the distribution of serum GGT and ALP between the two groups (**Figure 5**).

In the treatment group, there were 8 (19.2%) cases of chest tightness, 9 (21.4%) cases of nausea and 4 (9.5%) cases of vomiting; while 10 (23.8%) patients had vascular pain on the infusion side during the first infusion of NAC, and 6 of them had transient local skin swelling. None of the patients stopped using NAC because of the possible adverse reactions mentioned above. In the control group, 4 (8.3%) patients had chest tightness, 3 (6.3%) patients had nausea and 2 (4.2%) patients had vomiting.

DISCUSSION

HBV-ACLF is one of the common types of liver failure in China. At present, the pathogenesis of HBV-ACLF has not been

TABLE 1 | Comparison of demographic characteristics and laboratory variables between treatment group and control group.

	Control ($N = 48$)	Treatment ($N = 42$)	*p*-value
Age (median, IQR), year	46 (36–53)	44 (38–52)	0.962
Male gender (*n*, %)	27 (56.3)	24 (57.1)	0.932
Duration of disease before admission (median, IQR), day	10.0 (8.0–14.5)	12.0 (9.0–15.0)	0.234
Antiviral therapy before admission			–
Untreated/ Discontinue treatment (*n*, %)	27 (56.2)/21 (43.8)	21 (50.0)/21 (50.0)	0.553
Drug withdrawal time[#] (median, IQR), month	17.0 (12.0–22.0)	16.0 (13.0–19.0)	0.659
Serum HBVDNA (median, IQR), lg10 IU/mL	4.97 (4.16–6.65)	4.82 (4.00–5.81)	0.125
Serum HBeAg positivity (*n*, %)	22 (45.8)	18 (42.9)	0.777
Serum HBsAg (median, IQR), lg10 IU/mL	3.5 (3.2–3.9)	3.5 (3.3–3.9)	0.836
Alanine aminotransferase (median, IQR), IU/L	999.0 (878.5–1787.5)	1,128 (947–2,027)	0.194
Total bilirubin (median, IQR), μmol/L	398.0 (365.0–438.0)	397.5 (364.0–456.0)	0.372
Direct bilirubin (median,IQR), μmol/L	301.0 (279.0–357.5)	298.0 (276.0–324.0)	0.562
Total bile aci (median, IQR), μmol/L	265.5 (216.0–304.5)	237.5 (192.0–307.0)	0.170
Albumin (median, IQR)	33.4 (30.5–34.7)	32.0 (30.1–35.3)	0.635
Alkaline phosphatase	193.0 (164.5–237.5)	185.0 (145.0–221.0)	0.385
γ-glutamyltranspeptidase	253.0 (205.5–299.5)	295.0 (237.0–355.0)	0.009
Blood ammonia	80.3 (61.8–94.4)	63.9 (45.3–89.5)	0.033
Serum creatinine	84.0 (75.5–93.5)	83.0 (66.0–102.0)	0.534
Alpha fetoprotein	37.5 (18.7–72.0)	28.7 (15.8–46.2)	0.251
Prothrombin time	30.5 (28.7–36.4)	32.3 (28.9–37.4)	0.275
International Normalized Ratio	2.6 (2.4–3.1)	2.7 (2.4–3.2)	0.232
Blood sodium	140.0 (136.5–142.5)	138.5 (135.0–142.0)	0.135
Hepatic encephalopathy (*n*, %)	6 (12.5)	5 (11.9)	0.931
Spontaneous Bacterial Peritonitis (*n*, %)	18 (37.5)	14 (33.3)	0.680
Ascites (*n*, %)	23 (47.9)	20 (47.6)	0.978
Hepatorenal syndrome (*n*, %)	3 (6.3)	5 (11.9)	0.465
MELD score (median, IQR)	28.0 (26.0–31.0)	29.0 (26.0–32.0)	0.586
Child-Turcotte-Pugh classification: A/B/C (*n*, %)	0 (0.0)/9 (18.8)/39 (81.3)	0 (0.0)/13 (31.0)/29 (69.0)	0.179

Note: The symbol [#] represents the time when the patient has stopped taking antiviral drugs.

fully elucidated (3). In this kind of patients, a large number of liver parenchymal cells necrosis can lead to a significant decline in liver metabolic detoxification function, and then lead to a large dose of endotoxin into the blood without inactivation (18). Endotoxemia is an important pathogenic factor that causes or aggravates severe liver damage (19). The increase of serum endotoxin level can stimulate the synthesis and expression of pro-inflammatory cytokines, aggravate the damage of liver function, and finally form a vicious circle (20). At present, it is believed that the activation of inducible nitric oxide synthase (iNOS) and its catalytic production of nitric oxide (NO) play an important role in LPS induced hepatocyte damage in patients with HBV-ACLF (18, 20).

Previous studies have reported that the iNOS is mainly distributed in hepatocytes and Kupffer cells in the liver (21, 22). It can be induced and activated by LPS and a variety of cytokines, and then produce a large number of endogenous NO with cytotoxic effect (21). As a free radical, NO can react with superoxide anion to form peroxynitrite, which has strong oxidation ability to protein, lipid and DNA, and then participates in the pathological process of hepatocyte injury. In human body, the serious damage of hepatocytes not only shows the release of ALT caused by the damage of hepatocyte membrane, but also leads to the blocked secretion and excretion of bile acid and bilirubin, and even affects the important synthesis function of liver. Thus, blocking or inhibiting the inflammatory response mediated by iNOS may help to alleviate or reverse the occurrence and development of severe liver injury (21, 23).

As we all know, acetylcysteine is the precursor of reduced glutathione (GSH), which is an oxygen free radical scavenger *in vivo*. The mechanism of its hepatoprotective effect is not very clear. At present, it is believed that NAC can not only directly react with ROS intermediate to inactivate and maintain the integrity of antioxidant enzyme system structure and functional recovery, but also increase the concentration of intracellular GSH and induce the accumulation of GSH into cells through deacetylation, so as to promote anti-oxidation, improve the ability of intracellular detoxification and reduce the release of oxygen free radicals (24). In addition, NAC may also play a protective role in liver by improving hemodynamics and oxygen transport capacity and expanding microcirculation (12, 14). At present, NAC is well-established in the treatment of acetaminophen induced fulminant liver failure, but its efficacy

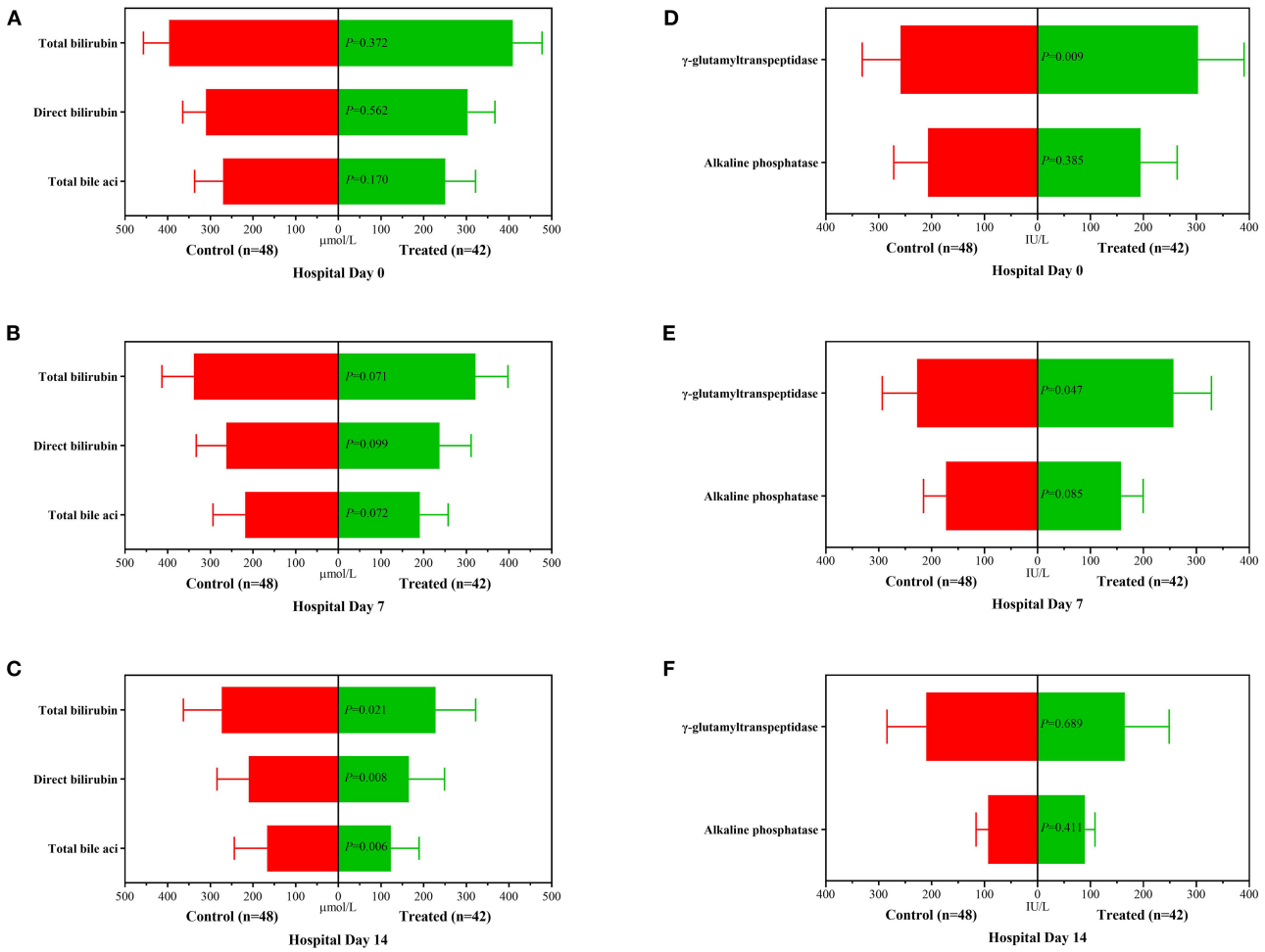


FIGURE 2 | Changes of biochemical variables before treatment, 1 and 2 weeks after treatment between the treatment group and the control group. **(A)**: Distribution of serum TBil, DBil and TBA before treatment; **(B)**: Distribution of serum TBil, DBil and TBA after 1 week of treatment; **(C)**: Distribution of serum TBil, DBil and TBA after 2 weeks of treatment; **(D)**: Distribution of serum ALP and GGT before treatment; **(E)**: Distribution of serum ALP and GGT after 1 week of treatment; **(F)**: Distribution of serum ALP and GGT after 2 weeks of treatment.

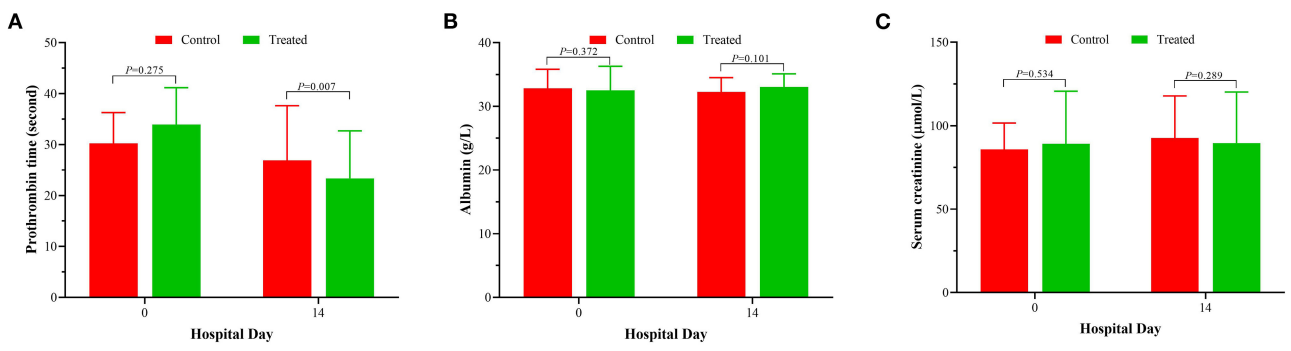


FIGURE 3 | Changes of prothrombin time **(A)**, albumin **(B)** and serum creatinine **(C)** after 2 weeks of treatment between the treatment group and the control group.

and mechanism in therapy of other forms of liver failure is unclear (16). In this study, we analyzed the efficacy and safety of adding NAC to the existing standard treatment for HBV-ACLF. Our results show that increasing NAC treatment can promote the remission of intrahepatic cholestasis and the improvement of coagulation function; and for CTP C-grade

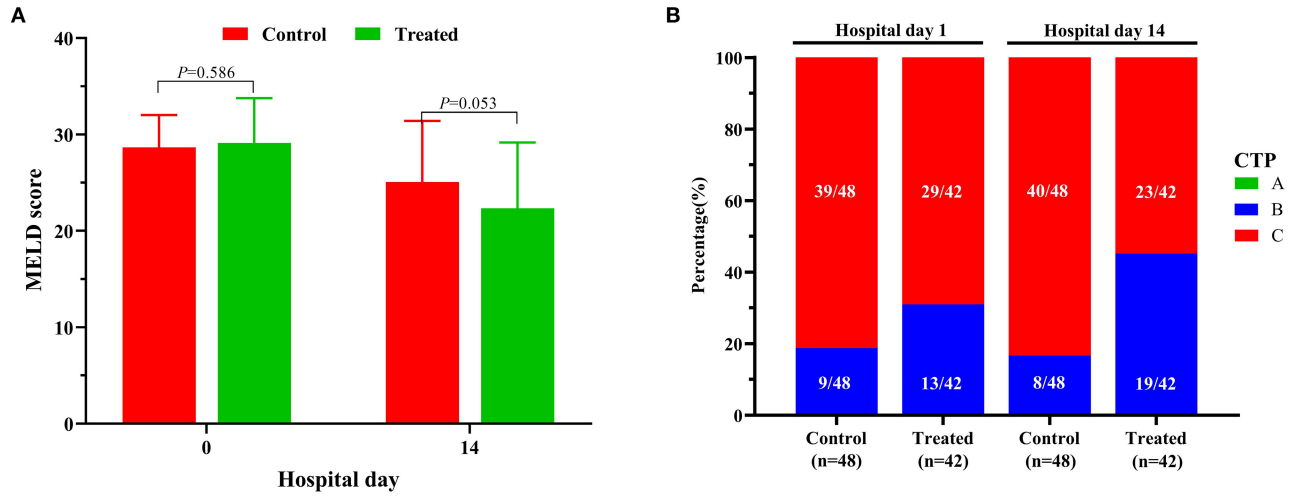


FIGURE 4 | Changes of MELD **(A)** scores and CTP grades **(B)** between the treatment group and the control group.

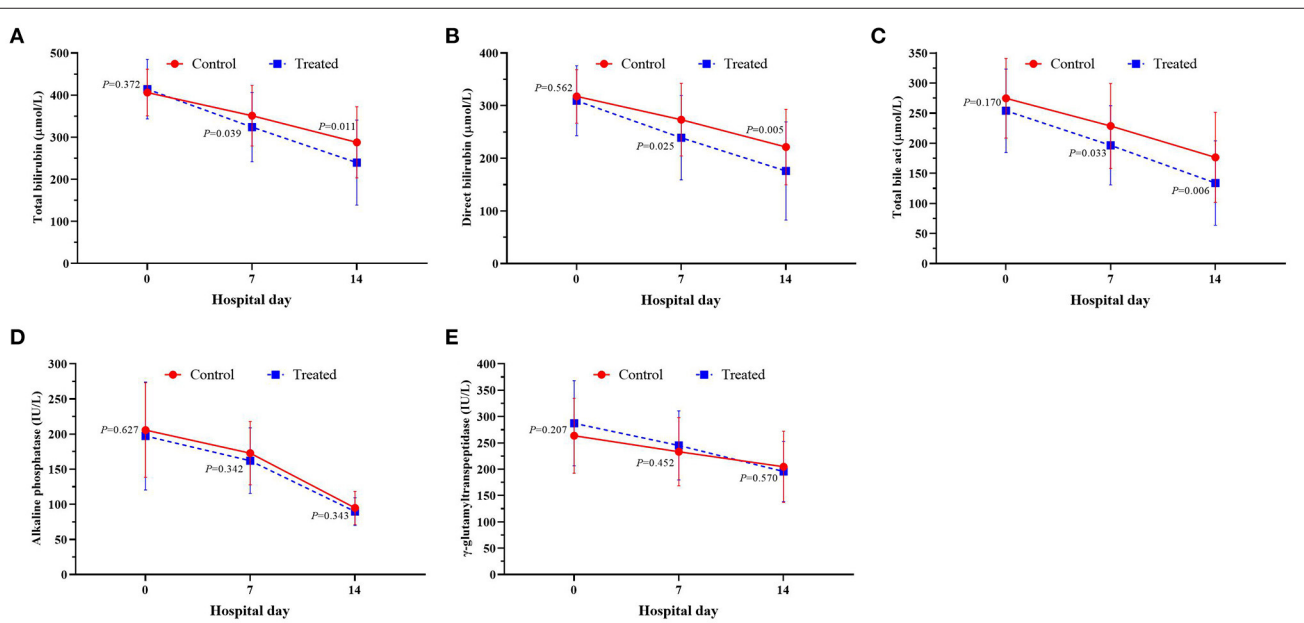


FIGURE 5 | Changes of serum serum TBil **(A)**, DBil **(B)**, TBA **(C)**, ALP **(D)** and GGT **(E)** between the treatment group and the control group.

patients, increasing NAC treatment can make their liver function recovery better. At present, some scholars believe that NAC, as an antioxidant, can inhibit the activation of iNOS, and its role in alleviating hepatocyte injury and improving tissue hypoxia may be realized by regulating the redox state of hepatocytes and vasodilation (25). It has also been reported recently that NAC application could alleviate macrophages aggregation and inflammatory response, and mitigating liver injury and cell apoptosis (26).

In fact, NAC is also mentioned in AASLD guidelines for the treatment of liver failure caused by HBV infection (27). Unfortunately, in the past, because of the side effects of NAC (28), it was not routinely used in the treatment of HBV-ACLF in China. However, with the deepening of NAC research and improvement of its preparation process, the probability of side effects of NAC in clinical application is significantly reduced, so NAC is expected to be used in the comprehensive treatment of HBV-ACLF. In this study, although some patients reported chest tightness, nausea and vomiting, most of these adverse reactions were transient. After symptomatic treatment, the discomfort of the vast majority of patients was quickly relieved or disappeared, and no patients stopped treatment because

of these possible related adverse reactions. Our preliminary results have suggested that the current clinical use of NAC has good safety. In this study, the temporary local skin swelling at the infusion site may be related to the excessive speed of intravenous infusion. If conditions permit, it is recommended to use infusion pump for intravenous infusion of NAC, which may help to reduce the probability of this potential adverse reaction.

There are some limitations in this study. In addition to the small sample size and retrospective study design, it is impossible to know whether these patients with HBV-ACLF have endotoxemia and whether they have iNOS and ROS mediated inflammatory disorder. The latter should be helpful to explain the good clinical efficacy of NAC. Therefore, it is necessary to investigate the mechanism of NAC in the treatment of HBV-ACLF in addition to a larger sample of prospective, randomized controlled studies. For a retrospective study, propensity score method is usually used for matching control group. However, due to the sample size, we did not use this routine protocol, so there is a potential bias in the grouping of patients.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of West China Hospital of Sichuan University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

M-LW, X-JY, X-LL, F-DW, JZ, Y-CT, Y-HW, and D-BW participated in the collection and analysis of data and the writing of the first draft of the article. E-QC designed the study, conducted the study supervision, and revised the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Arroyo V, Moreau R, Jalan R. Acute-on-chronic liver failure. *N Engl J Med.* (2020) 382:2137–45. doi: 10.1056/NEJMra1914900
2. Zhao RH, Shi Y, Zhao H, Wu W, Sheng JF. Acute-on-chronic liver failure in chronic hepatitis B: an update. *Expert Rev Gastroenterol Hepatol.* (2018) 12:341–50. doi: 10.1080/17474124.2018.1426459
3. Chen EQ, Shimakami T, Fan YC, Angeli P. Acute-on-chronic liver failure: from basic research to clinical applications. *Can J Gastroenterol Hepatol.* (2018) 2018:5029789. doi: 10.1155/2018/5029789
4. Feuer G, Di Fonzo CJ. Intrahepatic cholestasis: a review of biochemical-pathological mechanisms. *Drug Metabol Drug Interact.* (1992) 10:1–161. doi: 10.1515/DMDI.1992.10.1-2.1
5. Jungst C, Berg T, Cheng J, Green RM, Jia J, Mason AL, et al. Intrahepatic cholestasis in common chronic liver diseases. *Eur J Clin Invest.* (2013) 43:1069–83. doi: 10.1111/eci.12128
6. Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. *Hepatology.* (2002) 36:525–31. doi: 10.1053/jhep.2002.36088
7. Mato JM, Martinez-Chantar ML, Lu SC. S-adenosylmethionine metabolism and liver disease. *Ann Hepatol.* (2013) 12:183–9. doi: 10.1016/S1665-2681(19)31355-9
8. Liu XY, Peng F, Pan YJ, Chen J. Advanced therapeutic strategies for HBV-related acute-on-chronic liver failure. *Hepatobiliary Pancreat Dis Int.* (2015) 14:354–60. doi: 10.1016/S1499-3872(15)60338-1
9. Larsen FS. Artificial liver support in acute and acute-on-chronic liver failure. *Curr Opin Crit Care.* (2019) 25:187–91. doi: 10.1097/MCC.0000000000000584
10. Chen B, Pang L, Cao H, Wu D, Wang Y, Tao Y, et al. Autologous stem cell transplantation for patients with viral hepatitis-induced liver cirrhosis: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol.* (2019) 31:1283–91. doi: 10.1097/MEG.0000000000001455
11. Wu DB, Chen EQ, Tang H. Stem cell transplantation for the treatment of end-stage liver disease. *World J Hepatol.* (2018) 10:907–10. doi: 10.4254/wjh.v10.i12.907
12. Millea PJ. N-acetylcysteine: multiple clinical applications. *Am Fam Physician.* (2009) 80:265–9.
13. Otrubova O, Turecky L, Ulicna O, Janega P, Luha J, Muchova J. Therapeutic effects of N-acetyl-L-cysteine on liver damage induced by long-term CCl4 administration. *Gen Physiol Biophys.* (2018) 37:23–31. doi: 10.4149/gpb_2017016
14. McPheeters CM, VanArsdale VM, Weant KA. N-Acetylcysteine use in non-acetaminophen-induced acute liver failure. *Adv Emerg Nurs J.* (2016) 38:183–9. doi: 10.1097/TME.0000000000000116
15. Siu JT, Nguyen T, Turgeon RD. N-acetylcysteine for non-paracetamol (acetaminophen)-related acute liver failure. *Cochrane Database Syst Rev.* (2020) 12:CD012123. doi: 10.1002/14651858.CD012123.pub2
16. Will JS, Snyder CJ, Westerfield KL. N-acetylcysteine (NAC) for the prevention of liver failure in heat injury-mediated ischemic hepatitis. *Mil Med.* (2019) 184:565–7. doi: 10.1093/milmed/usz022
17. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the asian pacific association for the study of the liver (APASL): an update. *Hepatol Int.* (2019) 13:353–90. doi: 10.1007/s12072-019-09946-3
18. Zhang J, Gao S, Duan Z, Hu KQ. Overview on acute-on-chronic liver failure. *Front Med.* (2016) 10:1–17. doi: 10.1007/s11684-016-0439-x
19. Cai Q, Liu W, Zhu M, Sheng J. Microbial infections as a trigger for acute-on-chronic liver failure: a review. *Med Sci Monit.* (2019) 25:4773–83. doi: 10.12659/MSM.915637
20. Han DW. Intestinal endotoxemia as a pathogenetic mechanism in liver failure. *World J Gastroenterol.* (2002) 8:961–5. doi: 10.3748/wjg.v8.i6.961
21. Anavi S, Tirosh O. iNOS as a metabolic enzyme under stress conditions. *Free Radic Biol Med.* (2020) 146:16–35. doi: 10.1016/j.freeradbiomed.2019.10.411
22. Xue Q, Yan Y, Zhang R, Xiong H. Regulation of iNOS on immune cells and its role in diseases. *Int J Mol Sci.* (2018) 19:3805. doi: 10.3390/ijms19123805
23. Leifeld L, Fielenbach M, Dumoulin FL, Speidel N, Sauerbruch T, Spengler U. Inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) expression in fulminant hepatic failure. *J Hepatol.* (2002) 37:613–9. doi: 10.1016/S0168-8278(02)00271-4
24. Szakmany T, Hauser B, Radermacher P. N-acetylcysteine for sepsis and systemic inflammatory response in adults. *Cochrane Database Syst Rev.* (2012) 2012:CD006616. doi: 10.1002/14651858.CD006616.pub2

25. Caglikulekci M, Pata C, Apa DD, Dirlik M, Tamer L, Yaylak F, et al. The effect of N-acetylcysteine (NAC) on liver and renal tissue inducible nitric oxide synthase (iNOS) and tissue lipid peroxidation in obstructive jaundice stimulated by lipopolysaccharide (LPS). *Pharmacol Res.* (2004) 49:227–38. doi: 10.1016/j.phrs.2003.09.013
26. Zhou H, Sun Y, Wang Q, Li Z, Zhong W, Wang X, et al. N-acetylcysteine alleviates liver injury by suppressing macrophage-mediated inflammatory response post microwave ablation. *Int Immunopharmacol.* (2020) 85:106580. doi: 10.1016/j.intimp.2020.106580
27. Lee WM, Stravitz RT, Larson AM. Introduction to the revised American association for the study of liver diseases position paper on acute liver failure 2011. *Hepatology.* (2012) 55:965–7. doi: 10.1002/hep.25551
28. Sandilands EA, Bateman DN. Adverse reactions associated with acetylcysteine. *Clin Toxicol (Phila).* (2009) 47:81–8. doi: 10.1080/15563650802665587

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

REVIEW
published: 09 August 2021
doi: 10.3389/fmed.2021.725993

Immunological Manifestations of Hepatitis E-Associated Acute and Chronic Liver Failure and Its Regulatory Mechanisms

Jian Wu[1†], *Bai Ling*[2†], *Naizhou Guo*[3†], *Guanghua Zhai*[1], *Meifen Li*[1] *and Yurong Guo*[4*]

[1] Department of Clinical Laboratory, Gusu School, Suzhou Municipal Hospital, The Affiliated Suzhou Hospital of Nanjing Medical University, Nanjing Medical University, Suzhou, China, [2] Department of Pharmacy, The First People's Hospital of Yancheng City, The Yancheng Clinical College of Xuzhou Medical University, Yancheng, China, [3] Department of Clinical Laboratory, The First People's Hospital of Yancheng City, The Yancheng Clinical College of Xuzhou Medical University, Yancheng, China, [4] Department of Laboratory Medicine, Yancheng Hospital of Traditional Chinese Medicine, Affiliated to Nanjing University of Traditional Chinese Medicine, Yancheng, China

OPEN ACCESS

Edited by:
Tao Chen,
Huazhong University of Science and Technology, China

Reviewed by:
Xinyi Tang,
Mayo Clinic, United States
Jin Xin,
Tongde Hospital of Zhejiang Province, China

***Correspondence:**
Yurong Guo
3128954693@qq.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 16 June 2021
Accepted: 16 July 2021
Published: 09 August 2021

Citation:
Wu J, Ling B, Guo N, Zhai G, Li M and Guo Y (2021) Immunological Manifestations of Hepatitis E-Associated Acute and Chronic Liver Failure and Its Regulatory Mechanisms. Front. Med. 8:725993. doi: 10.3389/fmed.2021.725993

Hepatitis E virus (HEV) is a common cause of viral hepatitis in developing countries, most commonly transmitted through the fecal-oral route. The virus is mainly of genotypes (GT) 1 and GT2 genotypes, and patients usually show symptoms of acute hepatitis. Due to the rising trend of HEV serological prevalence in global population, HEV has become an important public health problem in developed countries. Severe hepatitis caused by HEV includes acute and chronic liver failure (ACLF). ACLF frequently occurs in developed countries and is caused by overlapping chronic liver diseases of HEV with genotypes GT3 and GT4. Because the onset of hepatitis E is closely associated with immunity, it is critical to understand the immunological mechanism of hepatitis E associated with acute and chronic liver failure (HEV-ACLF). This review discusses the immunological manifestations and mechanisms of HEV-ACLF, intrahepatic immune microenvironment and treatment, and raises outstanding questions about the immunological mechanism and treatment of the disease.

Keywords: hepatitis E virus, acute and chronic liver failure, immune manifestations, mechanism, microenvironment

INTRODUCTION

Hepatitis E is a liver infection caused by the single-stranded RNA hepatitis E virus (HEV) and usually a self-limited disease (1). However, the disease may develop into severe hepatitis in patients with altered immune responses, such as pregnant women. Recent studies have reported that HEV infection can lead to chronic hepatitis in patients with low immunity, such as HIV infected patients, organ transplant recipients and patients suffering from malignant diseases (2–5). Currently, there are four well-characterized HEV genotypes that can infect mammals, among them, genotypes (GT) 1 and 2 cause human infection, while genotypes 3 and 4 bring about zoonotic disease (6) that can infect a wide range of hosts. HEV is mainly transmitted by the fecal-oral route. Usually, an immune-capable individual can eliminate the virus spontaneously without causing complications. However, in some cases where the immune system is unable to resist the virus, HEV

infected patients will experience symptoms of acute viral hepatitis such as jaundice, hepatomegaly, vomiting, nausea, abdominal pain and fever (7). In patients with low immunity, HEV virus can cause chronic liver infection, liver failure and extrahepatic symptoms. A large number of studies have shown that the immune response, rather than the virus itself, is the key factor driving the occurrence of hepatitis E (6). As a self-limited disease, the treatment principle is supporting treatment in patients with normal immune function, while for cancer patients and patients who receive long-term immunosuppressive therapy after organ transplantation, antiviral therapy and temporary suspension of immunotherapy can alleviate the symptoms of some patients (8). HEV infection can cause acute decompensation of chronic liver disease, leading to liver failure and death of patients, known as acute chronic liver failure (ACLF). The Asia-Pacific Association for the Study of the Liver (APASL) defines ACLF as acute liver impairment characterized by jaundice and coagulation disorders, complicated by ascites or encephalopathy within 4 weeks in patients with previously diagnosed or undiagnosed chronic liver disease. ACLF is defined by the American Society of Liver Diseases as an acute worsening of preexisting chronic liver disease, usually associated with an emergency event and associated with increased mortality from multi-system and organ failure at 3 months (9). Both definitions refer to the basic characteristics of acute liver damage or acute exacerbation of chronic liver disease in patients. The superposition of HEV infection has been shown to be an important cause of liver injury in patients with chronic liver disease, who also have a higher mortality rate than patients with stable compensatory cirrhosis. Therefore, ACLF is characterized by acute deterioration of compensatory chronic liver disease in patients with stable cirrhosis, and the main clinical manifestations are hyperbilirubinemia (with clinical jaundice), hepatic encephalopathy, and decreased liver function, leading to hypoproteinemia and shortened prothrombin time (10). The genotypes of HEV causing acute and chronic liver failure in chronic liver disease are GT3 and GT4, which are different from those caused by GT1 and GT2 HEV during pregnancy. ACLF and ALF present similar clinical symptoms and signs in the acute phase and are difficult to distinguish from each other. However, the prognosis of ACLF is worse, and the mortality is also significantly higher than that of ALF (11). The early mortality of ACLF is as high as 50–90% (12–14). In recent years, a large number of clinical studies have shown that immune response plays a very important role in the occurrence and development of ACLF, but the exact pathogenesis of ACLF remains to be clarified.

In this paper, the manifestations of immune response and the regulatory mechanisms in the occurrence and development of hepatitis E associated acute and chronic liver failure (HEV-ACLF) were elaborated, and the mechanisms underlying severe hepatitis E were summarized and explored. This article will provide a new idea for clinical treatment and research of hepatitis E.

IMMUNOLOGICAL MANIFESTATIONS AND MECHANISMS OF ACUTE HEPATITIS E (AHE)

HEV usually infects susceptible populations through the fecal-oral route, and the clinical manifestations of patients vary greatly, from asymptomatic infection to uncomplicated acute viral hepatitis and severe fulminant liver failure. The reasons for the various degrees of disease severity and the pathogenesis of the disease remain unclear. However, more and more studies have found that the virus is not the driving factor of liver injury, and the main cause of liver tissue injury in AHE patients may be the host immunity (6).

Under normal conditions, the symptoms of acute hepatitis are presented after HEV infection, which are mainly manifested by the rapid increase of serum liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), as well as jaundice. Several studies in developing countries such as India have shown the occurrence of toxemia in AHE patients. Reverse transcription-polymerase chain reaction (RT-PCR) was used to detect HEV RNA in serum and feces during the onset of the disease, and the results showed that the positive rate of serum HEV RNA in the 1st week of onset was about 86.7 and 6.6% in the 6th week of the disease. Meanwhile, the excretion of HEV RNA in the stool was about 70% in the 1st week. In the 4th week, it was reduced to 20%, and it turned negative around the 5th week (15). Approximately 80% of patients have detectable anti-HEV immunoglobulin IgM, and the HEV IgM appears 1 week after the onset of symptoms. In the following 6 weeks, IgM-positive cases showed a monotonic decline. It decreased to 18.3% in the 7th week (16). Based on the statistics of the above detection indicators, anti-HEV IgM, serum HEV RNA and fecal virus disappear in a few weeks, and fecal virus disappeared first, followed by HEV RNA, and then anti-HEV IgM. The longest duration of HEV viremia was 42 days, that of HEV fecal excretion was 28 days, and IgM was 49 days (15). Several studies have found that the fecal excretion rate of the viruses is significantly lower than the detection rate of serum HEV RNA. Therefore, fecal virus shedding is not an accurate and desirable diagnostic method. The positive rate of HEV RNA was higher than that of anti-HEV IgM, suggesting that detection of HEV RNA may be a good indicator of persistent HEV infection and diagnosis (15).

A study of 60 AHE patients in a tertiary care facility in Rajasthan, India showed that among the initially tested IgM-negative patients, HEV RNA could be detected in the feces or serum of 13 patients. This finding indicated that low antibody titer may not reflect the viral load (6). Another study showed that the duration of viremia exceeded the time for transaminase to return to normal, which also indicated that liver damage may be unrelated to virus replication (15). Since the HEV itself is not associated with liver damage, it is likely that the body's own immune response causes liver damage as it clears the virus.

In order to prove that virus-induced host immune response is an important mechanism leading to hepatic cytopathic injury, many studies have explored the changes of the immunity in AHE patients through both *in vivo* and *in vitro* experiments. Immunohistochemical (IHC) staining of liver tissues from patients with acute liver failure (ALF) caused by HEV showed that the levels of CD3, CD8 (cytotoxic T cells), Granzyme B (granzyme B: Activated cytotoxic T cells and NK cell markers), CD56 (natural killer cells), CD4 (helper T cells), and CD8/CD3 and CD4/CD3 ratios were higher than those in normal liver tissue. IHC staining results showed that activated $CD8^+$ T cells and NK cells were present in liver tissues infected with HEV (1). Analysis of the peripheral blood PBMC of patients with AHE showed that the number of NK and NKT cells was significantly lower than that of the healthy control group, but the number of activated NK and NKT cells was significantly higher than that of the healthy control group, and the number and activation status of NK and NKT cells returned to normal in the recovery period of AHE (17). By studying the cells of liver and peripheral blood of AHE, it was found that $CD8^+$ T cells and their specific adaptive immune response play an important role in the pathogenesis of hepatitis E. In addition, NK cells and NKT cells play roles in the pathogenesis of acute HEV infection. NK and NKT cells constitute the main part of liver lymphocytes among which NK cells are large granule lymphocytes, accounting for 10–15% of peripheral blood mononuclear cells (18, 19), and 30% of intrahepatic lymphocytes. The interaction of perforin-granzyme and Fas-FasL can mediate cell death and activate other cells by secreting cytokines, thereby inhibiting virus replication. NKT cells are a kind of unconventional T cells that simultaneously express the cell surface characteristics of both NK cells (CD56) and T cells (CD3) (18). During the hepatotropic virus infection, the highly expressed CD1d on the surface of liver cells will cause the activation of NKT cells, and NKT cells can produce γ-interferon (IFN-γ) and IL-4 (15, 20). Comprehensive IHC study of the liver tissue and analysis of peripheral blood cells of AHE patients showed that the total number of NK cells and NKT cells in peripheral blood decreased, but its activation increased, while NK cells and NKT cells in the liver tissue increased, indicating that the increase of the number of cells illustrates the possible acute HEV infection, because the activated NK and NKT cells might migrate from peripheral blood to infiltrate the liver, and play a role in killing viruses in the liver (17).

In the study of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, it was found that cytotoxic $CD8^+$ T cells mediate direct killing of hepatocytes, while $CD4^+$ T cells inhibit virus replication and activates host macrophages by producing IFN-γ and tumor necrosis factor- α (TNF-α) and clear the virus with a non-cytopathic mechanism (21). In the absence of an effective HEV virus and cell co-culture system, it is difficult to determine whether the HEV virus directly affects the pathogenesis of host hepatocytes. Studies have shown that HEV particles are small, non-enveloped, 32–34 nm in diameter, and icosahedral symmetric. The viral gene is about 7.2 kb in length, is single-stranded, positive, and polyadenylated RNA containing three open reading frames (ORF): ORF1 encodes non-structural protein responsible for viral genome replication and multi-protein processing, while ORF2 encodes major capsid proteins. ORF3 encodes a phosphoprotein associated with the regulatory cytoskeleton (22). Normally, studies used HEV ORF2 protein (pORF2) and HEV ORF3 protein (pORF3) to stimulate patient-derived lymphocytes or transfect hepatoma cells, where ORF2 has been shown to have high immunogenicity and can effectively stimulate a specific T-cell response *in vitro* to protect primates from HEV infection (19). It was found that patients with AHE showed an acquired immune response to PORF2 and PORF3 in the form of a lymphoproliferative response. Peripheral blood mononuclear cells (PBMC) derived from patients with AHE were stimulated with pORF2 and pORF3 *in vitro*, and TNF-α^+ $CD4^+$ T cells and antigen-specific B cells that can produce IgG antibodies are significantly higher than healthy controls (21). Therefore, it is suggested that HEV is similar to HBV and HCV infection. After the onset of AHE, HEV-specific immune response occurred, and its level decreased with the decrease of anti-HEV lgM antibody titer and the normalization of liver function. Therefore, the immune response is considered to be a pathogenic factor in the development of hepatitis E, and also the main force to clear the virus (**Table 1**).

DISTRIBUTION OF IMMUNE CELLS AND RELATED CYTOKINES IN HEV-ACLF PATIENTS

Using IHC analysis of lymphocyte subsets in liver tissue infiltration and distribution, a study found that patients with ACLF had increased intrahepatic $CD4^+$ T cells, $CD8^+$ T cells, and NK cells compared with the chronic hepatitis and normal control group. Meanwhile, the study also showed that patients with ACLF had intrahepatic lymphocytes count about 50 times more than normal subjects. This finding indicated that the high concentration of intrahepatic lymphocytes in ACLF patients compared with normal people is caused by the infiltration of peripheral blood lymphocytes into the liver (1, 17). The low circulating lymphocytes in the peripheral blood of ACLF patients increases opportunistic infection and cause endotoxemia, and may activate monocytes and macrophages to release high levels of TNF-α, thereby aggravating the inflammatory damage of liver tissue (26, 27). The activation of EM cells is related to the increased expression of transcription factor T-Bet, and the activation of EM cells is mediated by IFN-γ after stimulation, the level of T-Bet increased further. The expression of T-Bet is related to the increased levels of chemokines including CXCR3, Granzyme B and CD122, which can drive EM cells to migrate from peripheral blood to liver (28). In addition to the above cytokines, IL-15, IL-18, CXCL8, CXCL9, CXCL10, and CCL2 promote the chemotaxis of immune cells to liver tissue, among which CXCL8 and CCL2 can promote the recruitment of non-adaptive immune cells such as monocytes to liver (29). The infiltration of lymphocytes into liver tissue is a multi-step process mediated by the interaction between multiple adhesion molecules, chemokines

TABLE 1 | Immune cells and cytokines in hepatitis E related diseases.

References	Country	Immune cells and cytokines	Cases
Prabhu et al. (1)	India	$CD8^+$ T cells	37 patients with acute liver failure (ALF) because of HEV infection; Acute hepatitis A ($n = 1$); Acute hepatitis B ($n = 6$); Acute hepatitis C ($n = 6$);
Srivastava et al. (17)	India	NK and NKT; cells	41 patients with acute hepatitis E
Jilani et al. (23)	India	CD4 cells; CD8 cells	Pregnant FHF ($n = 50$); Non-pregnant FHF ($n = 50$)
Moller et al. (24)	US	$CD163^+$; macrophages	ALF ($n = 100$)
Wu et al. (12)	India	Mono-macs; dendritic cell; Macrophages	AVH-E ($n = 44$); Pregnant females with AVH-E ($n = 12$); ALF-NE ($n = 5$)
Das et al. (25)	India	NK cells; IFN-γ; TNFα	Patients in the acute phase of hepatitis E infection ($n = 86$); HEV recovered individuals ($n = 101$)

and chemokine receptors released by immune cells such as lymphocytes, monocytes and macrophages (30). Since the infiltration of $CD4^+$ T cells and $CD8^+$ T cells from peripheral blood into liver tissue was also observed in acute hepatitis E and acute liver failure during pregnancy, it was inferred that the infiltration of $CD4^+$ T cells and $CD8^+$ T cells in acute and chronic hepatitis E caused by HEV might cause ALF (1, 23).

The study for the gene expression profile of liver biopsy in patients with HEV liver failure showed that compared with normal liver tissue, 1,703 genes were up-regulated and 1,674 genes were down regulated in liver tissue (31). Among them, 1,235 differential genes were related to immune system pathway, mainly related to cellular immune mechanism. such as T-cell toxic surface molecules, CTL mediated immune response, T helper cell surface molecules, costimulatory signaling in T cell activation pathway, T cell receptor signaling pathway, TCR activation signaling pathway initiated by Lck and Fyn tyrosine kinase, T cell receptor and CD3 complex signaling pathway, and IL-17 signaling pathway. qRT-PCR also showed that the mRNA levels of these genes were in good agreement with the microarray results, and the surface molecules of T cells such as Fyn and NF were found κB1, protein kinase C, and CTLA4 are over expressed in liver tissue, which indicated that activated $CD8^+$ T cells play a role in liver injury during HEV infection.

Acute and chronic hepatitis caused by hepatitis E virus and acute liver failure are also caused by the infiltration of $CD4^+$ T cells and $CD8^+$ T cells (32). In fact, the transport of lymphocytes to the liver is a major immune monitoring mechanism. Intrahepatic lymphocytes are essential for virus clearance or control of viral infection. However, continuous transport of lymphocytes to the liver may lead to extensive necrosis of hepatocytes and eventually lead to liver failure.

INTRAHEPATIC IMMUNE MICROENVIRONMENT IN HEV-ACLF PATIENTS

IHC showed that there were more $CD4^+$ T cells in the liver of AHE patients than in the healthy control group. The increase of $CD4^+$ T cells, from the peripheral blood mononuclear cell count to the IHC analysis of the cell infiltration in the liver, was observed in patients with acute and chronic hepatitis E-induced liver failure. The amplification of $CD4^+$ T cells that produce IFN-γ and TNF-α play important roles in limiting HEV replication and resolving HEV infection (1). $CD4^+$ T cells produce cytokines that promote the differentiation of $CD8^+$ T cells into cytotoxic T cells (CTL), which can directly kill infected liver cells and promote liver cell damage while helping to clear the virus. Data from liver biopsies indicate that similar infiltration of $CD8^+$ T cells can be seen in liver tissues of ALF caused by hepatitis E and other hepatitis virus infections, and the data indicate that $CD8^+$ cell population exceeds $CD4^+$ cells (1). Therefore, $CD8^+$ T cell-mediated cellular immunity, which can directly kill the hepatitis virus, plays a key role in the progression of hepatitis E.

It is well-known that adaptive immune response includes antigen presentation, activation of lymphocytes, formation of immune molecules and occurrence of immunological effects (**Figure 1**). In the stage of antigen recognition, T lymphocytes and B lymphocytes can accurately recognize antigen through TCR and BCR, respectively, while T lymphocytes can only complete subsequent activation and proliferation and kill target cells after antigen presentation by antigen presenting cells. Some studies have discussed the influence and mechanism of monocytes and macrophages in the pathogenesis of AHE. Previous studies on patients and chimpanzees infected with hepatitis E virus have shown that the presence and role of inflammatory macrophages in acetaminophen-induced ALF have been elucidated and understood (32). In addition, in the

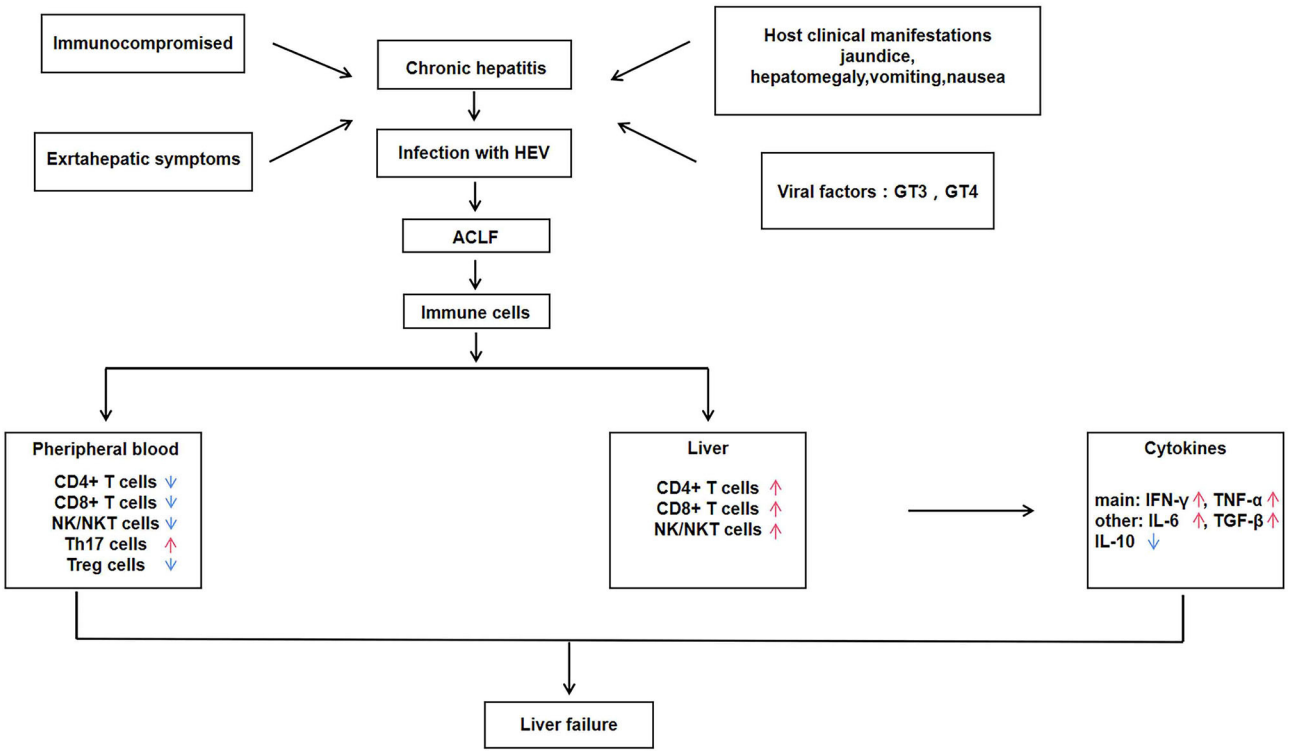


FIGURE 1 | Immune regulatory mechanisms of hepatitis E associated acute and chronic liver failure (HEV-ACLF).

subsequent model of carbon tetrachloride induced liver injury, the number of $CD11b^+$ $F4/80^+$ or $F4/80^-$ or $CD11b^+$ $CD163^+$ inflammatory macrophages was significantly increased, and the infiltration of macrophages in liver tissue led to severe liver injury (24, 33–35). Monocytes and macrophages recognize pathogens through members of the Toll receptor family TLR3 and TLR8, and then secrete reactive oxygen species (ROS) to mediate phagocytosis (12). The TNF-α secreting macrophages amplified from circulating monocytes are known as activated inflammatory M1/M2 macrophages. Activated macrophages can produce enough ROS to kill pathogens and resolve infection, while its excessive production of TNF-α leads to liver inflammation, injury, and liver functional failure (36). In addition, the activation of macrophages can also produce IFN-α, IL-12, and IL-18, which can further trigger the inflammatory immune response and lead to tissue damage (37). However, the study found that although the number of macrophages in AHE patients was significantly increased compared to the healthy control individuals, the production of ROS in macrophages was low, indicating that the impaired function of macrophages led to its dysfunction and inactivation (12). The phenomenon that the number of monocytes increases while their function is impaired is caused by the secretion of TNF-α and inhibitory cytokines such as IL-10 and TGF-β by macrophages (12). A study on the damage of TLR signaling pathway during HEV infection has explained the impairment of monocyte and macrophage function in patients with acute liver failure infected with hepatitis E. Studies have shown that macrophage function activation and HEV clearance are caused by TLR3 signaling activation of MyD88-independent pathway and TLR9 signaling activation of MyD88-dependent pathway through TRIF, TRAF3, and TRAF6. However, the expression of TLR3 and 7 was reduced in AHE patients, and the MyD88-dependent pathway activated by TLR9 signaling and the downstream signaling of TLR were damaged, which resulted in reduced ROS release and impaired phagocytosis of monocytes and macrophages as well as the release of cytokines such as TNF-α and IFN-γ, leading to inefficient treatment of HEV pathogens (12).

NK cells play a crucial part in the initial response to viral infection and other pathogens is a vital component of the immune system (38). In the process of defense against viral infection, NK cells induce production of IFN-α/β and other natural cytokines, thereby inhibiting viral replication and enhancing cytotoxicity to target cells (39). A large number of studies have shown that immune-activated $CD8^+$ T cells, NK cells, and NKT cells in peripheral blood and liver can induce severe liver injury in patients infected with HEV (26, 27). NK cells are momentous in the recognition and initial response of HEV. In the battle against HEV infection, NK cells are believed to be the first attack barrier formed and can call on T and B lymphocytes, that in turn induce an adaptive immune response (40). NKT cells represent a small number of lymphocytes and exhibit characteristics of both T cells and NK cells (41). The function of NK cells is integrated and controlled by signals from various activation and inhibition receptors, that bind to pathogens and activate NK cells (42). Among

them, the most effective NK cell activated receptors (NAR) are ADCC-mediated molecules, including CD16, NKG2D, NKP30, NKP44, and NKP46. NKT cells expressing NAR exhibit highly specialized effector memory phenotypes (43, 44). NK and NKT cells expressing NARs account for 13% of peripheral blood lymphocytes and 50% of liver lymphocytes. The distribution, activation, cytotoxicity and effector function of NK/NKT cells were studied in 86 patients with acute exacerbation of HEV infection and 54 healthy controls during the development of the disease. It was shown that the percentage of NK ($CD56^+CD3^-$) and NKT ($CD56^+CD3^+$) cells in peripheral blood of AHE patients is lower than that of healthy control individuals, while the NK and NKT cells expressing NARS (NKP44 and NKP46) are more than that of healthy control individuals (25). The number of NK and NKT cells was significantly increased in an IHC study of liver biopsies from HEV-infected patients with acute liver failure. These results demonstrate that activated NK/NKT cells migrate from the peripheral blood and infiltrate into the liver to fight against virus during acute or chronic HEV infection. IFN-γ is produced by various types of cells, including monocytes, dendritic cells, NK cells, and NKT cells, with NK and NKT cells as the main sources of IFN-γ in the liver. Therefore, the above studies on various immune cells during the pathogenesis of ACLF suggested that IFN-γ produced by cytotoxic $CD8^+$ T cells and $CD4^+$ T cells in the liver can recruit macrophages, NK cells and NKT cells during the pathogenesis of AHE patients.

Macrophages infiltrating into the liver mediate phagocytosis by releasing ROS, and NK cells play a killing role by secreting perforin. FcR on the surface of macrophages and NK cells binds to the Fc segment of antibodies binding to virus-infected cells, mediating killing of virus-infected liver cells (17). Firstly, macrophages and NK/NKT cells initially recognize and kill HEV and HEV-infected liver cells through primary immune response. Then, macrophages and NK cells present viral antigens to T and B lymphocytes in the liver. After recognizing the antigen, the lymphocytes are activated, proliferated, and differentiated to produce effector cells (such as CTL) and effector molecules (such as IFN-γ, which is the most important one in the process of HEV infection). Finally, the effector cells and effector molecules are used to eliminate the hepatitis E virus and the virus-infected hepatocytes. IFN-γ secreted by immune cells during the immune response further activates and recruits macrophages, NK cells, and NKT cells, promoting differentiation of $CD8^+$ T cells into CTL, which kills infected liver cells and promotes liver damage while helping to clear HEV. Conversely, the immune response is weakened in organ transplant patients with immunosuppressive treatment, which might lead to a decrease in $CD8^+$ T cells, $CD4^+$ T cells, and the cytokines they produced. Although it protects the liver from damage, it also causes the hepatitis E virus to be unable to be removed from the body, making hepatitis E chronic. In conclusion, the immune microenvironment of the liver plays an essential role in the development of hepatitis E virus (**Figure 2**).

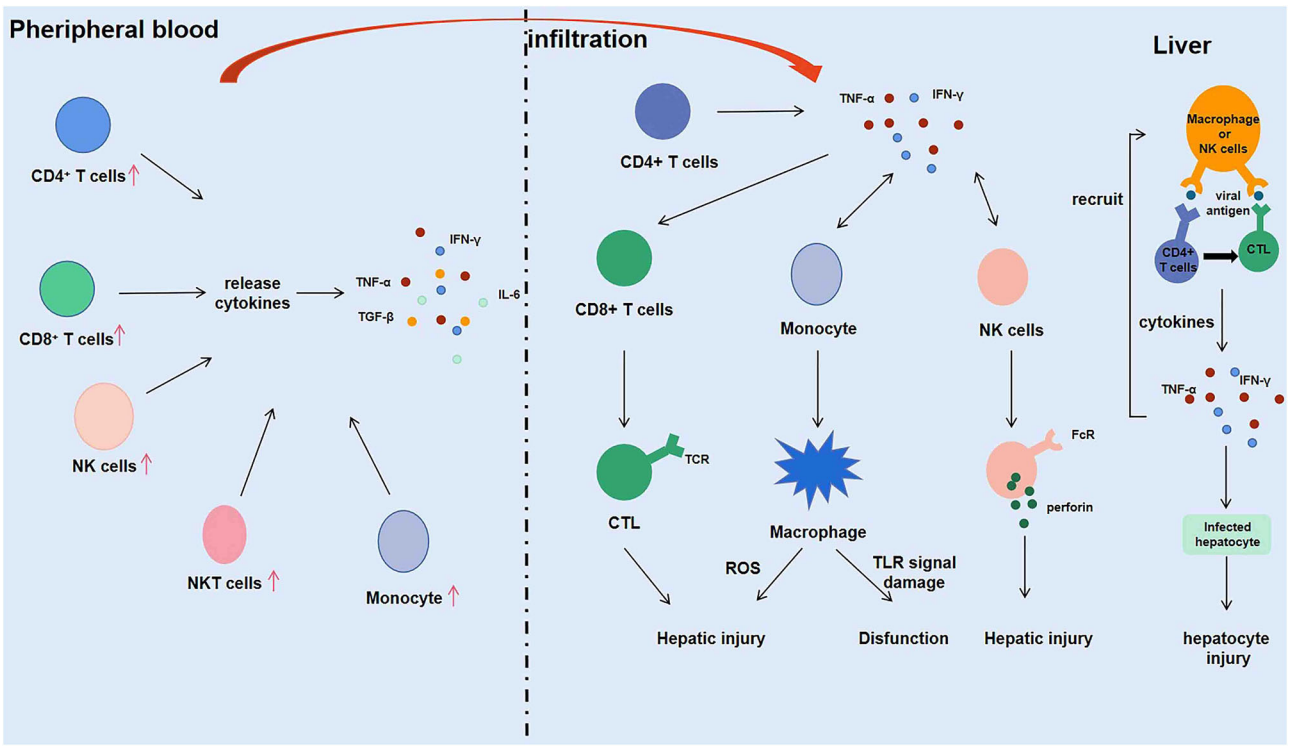


FIGURE 2 | Intrahepatic immune microenvironment in hepatitis E associated acute and chronic liver failure (HEV-ACLF) patients.

DISTRIBUTION OF OTHER IMMUNE CELLS IN HEV-ACLF PATIENTS

In addition to the above-mentioned immune cells that play a key role in the pathogenesis of HEV-ACLF, studies have shown that regulatory T cells (Treg cells) and IL-17-producing helper T cells (Th17 cells) also play a crucial role in the immune mechanism of HEV-ACLF (45). Treg cells mainly play the function of immunosuppression. Treg cells accumulate and expand in the infected site to play the immunosuppressive activity (46), and the deficiency or destruction of Treg cells will lead to autoimmune and inflammatory diseases in human and animals (47). Th17 cells are involved in the development of inflammation and autoimmune diseases by stimulating the production of a large number of inflammatory chemokines and inflammatory cytokines, and promoting the recruitment of neutrophils in tissues (48). The changes in the level of IFN-γ is similar to the trends of Th17 cells in the pathogenesis of HEV-ACLF, and high level of IFN-γ is involved in severe liver injury in patients with acute liver failure (49). The proportion of Th17 cells in peripheral blood was significantly increased in HEV-ACLF patients, suggesting that Th17 cells and IFN-γ are involved in promoting and maintaining inflammatory response and aggravating liver injury in HEV-ACLF patients. The number of Treg cell in HEV-ACLF patients showed a continuous decline and then stabilized, and the concentration of cytokine IL-10 was consistent with the dynamic changes of Treg cells. It is known that the increase of Treg cells and cytokines can inhibit the T cell response to regulate liver tissue inflammation, and the increase in their proportion may be a feedback to the increase of Th17 cells and pro-inflammatory cytokines, and suppress the immune response in the process of chronic liver failure to achieve a balance between pro-inflammatory and anti-inflammatory immunity (45).

In the pathogenesis of HEV-ACLF, pro-inflammatory immunity was significantly up-regulated, while anti-inflammatory immunity was significantly down-regulated, and the proportion of Th17 cells in peripheral blood was significantly increased. However, the number of Treg cells was decreased instead of increased, suggesting that there may be defects in the function of Treg cells. Therefore, the imbalance of Treg/Th17 is an important cause of liver function injury in patients with acute or chronic liver failure (45). The roles of T lymphocytes, regulatory T cells and helper T cells in the process of liver injury are closely related to the cytokines that they produce. It has been shown that IFN-γ and TNF-α in the liver are significantly associated with infiltration of aggregated $CD4^+$ and $CD8^+$ T cells in the liver, and that the expression of IFN-γ is unique to lymphocytes, whereas TNF-α is mainly produced by intrahepatic macrophages (12). The expression of IFN-γ and TNF-α in the liver of HEV-ACLF patients was significantly higher than that of normal controls, while the expression of anti-inflammatory cytokine IL-10 was not different between HEV-ACLF patients and normal controls.

Therefore, the upregulation of IFN-γ and TNF-α in the liver of HEV-ACLF patients is not offset by IL-10, and the imbalance of the expressions of pro-inflammatory cytokines and anti-inflammatory cytokines in the liver of patients is the important immune mechanism of HEV-ACLF liver injury. At the same time, the regulation of pro-inflammatory liver environment by cytokines may provide new ideas and strategies for the prevention and treatment of HEV-ACLF.

TREATMENT OF HEV-ACLF PATIENTS

In general, for patients with AHE with normal immunity, only symptomatic treatment is usually required because the duration of viremia is short. HEV infection can lead to chronic hepatitis in organ transplant patients who are treated with immunosuppressive agents. Studies have shown that about 30% of patients can clear the virus spontaneously by reducing immunosuppressive therapy. Clinically antiviral therapy is usually used when this approach is not successful, and the one currently used with the highest potency, efficacy and safety is ribavirin (50). Ribavirin is an effective antiviral drug that inhibits the synthesis of viral mRNA by inhibiting a variety of RNA and DNA, thus preventing viral replication (51).

Ribavirin is used to treat bronchiolitis and pneumonia caused by respiratory syncytial virus in infants and young children, chronic HCV, as well as a variety of viral infections such as viral upper respiratory tract infections. And ribavirin can be used to treat AHE and reduce the severity of the disease in patients with acute and chronic liver failure. It has shown good efficacy in the treatment of patients with chronic HEV infection, including patients with AIDS and leukemia with chronic hepatitis E, as well as transplant patients with chronic HEV infection and patients with rheumatic disease with immunosuppression. Therefore, ribavirin is the drug of choice for the treatment of HEV infection in most cases. Studies have shown that a course of 3 months is the optimal duration of ribavirin monotherapy, with longer treatment periods available for patients with severely compromised immune function. Prospective studies are needed to determine the optimal duration of ribavirin and the most beneficial therapeutic dose (52).

Although ribavirin is the preferred drug for the treatment of hepatitis E virus infection, there are many side effects of its single drug treatment. The most significant ones are severe anemia, the reduplication of the virus and the recurrence of the disease after discontinuation of treatment, and fetal deformity, Kamar et al. (50). A large number of studies have also provided solutions to the above side effects. For example, for patients with severe anemia caused by ribavirin treatment, combination therapy with another HEV inhibitor or direct acting antiviral drugs can be considered to reduce the amount of ribavirin (52). In addition, it was found that treatment with ribavirin was associated with increased heterogeneity in the open reading frame of the virus. Virus sequencing of the sera of a few patients who had failed treatment with ribavirin revealed the presence of G1634R mutation in viral polymerase. *In vitro* studies showed

that G1634R mutation increased HEV replication capacity (53). Recent data suggest that mutations in G1634R, including K1382N, Y1587F, D1384G, V1479I, and K1398R, are present in patients who relapsed during ribavirin therapy, and all of these mutations increase the replication capacity of hepatitis E virus, and increase the antiviral activity of ribavirin. Although there is currently no alternative treatment for ribavirin, *in vitro* studies have shown that the combination of sofebuvir and ribavirin may inhibit HEV replication and increase the antiviral effect of ribavirin. Sofebuvir is a nucleotide polymerase inhibitor of HCV infection that blocks HEV replication *in vitro* by inhibiting viral RNA-dependent RNA polymerase. Some patients treated with sofebuvir may develop liver fibrosis, cirrhosis, and liver failure, so the potential effect of *in vivo* two-drug combination on HEV replication needs to be determined (53).

In addition, ribavirin is contraindicated for pregnant women because of the medication-induced fetal deformities, however, untreated HEV poses a high risk to both mother and fetus. Drug trials may therefore be helpful in these patients, and acute hepatitis E often occurs in the third trimester, when fetal organ development is complete. Previous research data did not observe a clear teratogenic effect of ribavirin in humans. Nevertheless, further research on ribavirin treatment of AHE infection during pregnancy is needed (54).

Besides ribavirin, polyethylene glycol interferon alpha (PEG-IFN-α) is another drug that can be used for hepatitis E. The mechanism of action of PEG-IFN-α is that interferon binds to the specific alpha receptor on cell surface, triggers the complex signal transduction pathway in the cell and activates gene transcription, thereby regulating a variety of biological effects, including inhibition of replication of viruses in infected cells, inhibiting cell proliferation, and playing an immune regulatory role. Studies have shown that PEG-IFN-α can be used for liver transplant recipients infected with HEV, but other solid organ transplant patients such as heart transplant, lung transplant and kidney transplant are not recommended because interferon has immune-stimulating effects and can increase the risk of acute immune rejection in organ transplant patients (55).

In addition, interferon activates the body's immune system, removing the virus while damaging infected liver cells. Moreover, similar to ribavirin, PEG-IFN-α may cause fetal deformities, so it should not be used in pregnant women. Studies *in vitro* have observed a slight synergistic effect of ribavirin and interferon-α combination, which can reduce the dosage of ribavirin and potentially avoid anemia and other side effects. This finding suggested that the combination regimen can be use in the clinical setting for the treatment of hepatitis E (56). Based upon, it can be seen that safe and effective treatment of hepatitis E is urgently needed. In the case of acute HEV infection, the use of effective antiviral drugs to shorten the course of the disease can prevent the disease from progressing to fulminant liver failure and effectively prevent transmission of the virus during epidemics and outbreaks. As a high-risk group of hepatitis E, pregnant women have no therapeutic drugs and methods at present. When studying therapeutic drugs, teratogenicity and major adverse consequences should be avoided as far as possible to save the lives of pregnant women and fetuses. By the above content, the changes in hormone levels in pregnant women is one of the factors that lead to HEV infection. Therefore, modulating the hormonal system may be an effective treatment option. High concentrations of progesterone have been shown to inhibit CTV replication. Supplementation of progesterone in pregnant women with hepatitis E may be considered, and more suitable models *in vitro* and *in vivo* need to be developed for antiviral studies (57).

At present, the side effects and limitations of available therapeutic drugs promote the development of new antiviral drugs, and explore novel therapeutic targets from the perspectives of both the virus and the host. Ribavirin and PEG-IFN-α affect virus and host, respectively. Ribavirin inhibits viral replication by inhibiting the synthesis of viral mRNA. PEG-IFN-α regulates a variety of biological and immunological effects of the host and plays a role in inhibiting virus replication by binding to the specific receptors on cell surface. Antiviral drugs include inhibitors that inhibit virus entry into the host body, RNA-dependent RNA polymerase inhibitors, viral methyltransferase inhibitors, HEV helicase inhibitors, and inhibitors targeting other viral proteins (58). There are studies suggested that HEV replication may be affected by sex hormones, for example, the level of progesterone in pregnant patients may cause adverse outcomes. Some studies have found that HEV infection mainly affects men over 50 years of age in developed countries, that low testosterone may promote HEV replication, and that testosterone supplementation may be beneficial for older male patients with chronic HEV infection. Similarly, drugs that affect estrogen such as estrogen receptor modulators (tamoxifen and raloxifene) may improve the treatment of menopausal women with chronic HEV infection (59). Therefore, modulating the hormonal system might be an alternative approach to the treatment of hepatitis E. However, there is no specific treatment strategy on this approach, which requires extensive study.

CONCLUSIONS AND PERSPECTIVES

Hepatitis E is an infectious disease not only affects the underdeveloped countries, but also threads the economically developed countries. The two types of intestinal hepatitis, hepatitis E and hepatitis A, are very similar in terms of acute infection time and clinical features. Therefore, the early understanding and study on hepatitis E was based on models of hepatitis A. However, with the development of technology and in-depth research on hepatitis E, it was known that hepatitis E has similarities with hepatitis A and other types of viral hepatitis, as well as its unique characteristics. This article discusses the immunological manifestations and immune regulatory mechanisms of hepatitis E and HEV-ACLF. It is obvious that the immunological response is very important for the development and progression of hepatitis E, and the immune environment in the liver is very complicated. In addition to the immune cells (T lymphocytes, regulatory T cells, NK cells, and macrophages) and immune molecules (IFN-γ and TNF-α)

mentioned in the article, there may be other immune cells and immune molecules that may play important roles.

Currently, the protective measures and treatment methods for patients with AHE and HEV-ACLF are very limited. It is necessary to develop HEV-specific cell models and animal models, and establish three dimensional organoid liver cell culture system for further study on the immunological mechanism of hepatitis E. Such studies will be valuable for the eradication of hepatitis E.

AUTHOR CONTRIBUTIONS

JW and YG had the idea for the article. BL and GZ performed the literature search and data analysis. NG and JW drafted and critically revised the work. ML revised the draft. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Youth Medical Talent of Jiangsu Province (grant no. QNRC2016475).

ACKNOWLEDGMENTS

We thank the authors of the primary studies for their timely and helpful responses to our information requests.

REFERENCES

1. Prabhu SB, Gupta P, Durgapal H, Rath S, Gupta SD, Acharya SK, et al. Study of cellular immune response against Hepatitis E virus (HEV). *J Viral Hepat.* (2011) 18:587–94. doi: 10.1111/j.1365-2893.2010.01338.x
2. Wu J, Zhang X, Liu H, Guo N, Pan Q, Wang Y, et al. NLR and RLR in predicting liver failure and prognosis in patients with hepatitis E virus infection. *Clin Biochem.* (2019) 63:24–31. doi: 10.1016/j.clinbiochem.2018.11.012
3. Kamar N, Selves J, Mansuy JM, Ouezzani L, Peron JM, Guitard J, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med.* (2008) 358:811–7. doi: 10.1056/NEJMoa0706992
4. Ollier L, Tieulie N, Sanderson F, Heudier P, Giordanengo V, Fuzibet JG, et al. Chronic hepatitis after hepatitis E virus infection in a patient with non-Hodgkin lymphoma taking rituximab. *Ann Intern Med.* (2009) 150:430–1. doi: 10.7326/0003-4819-150-6-200903170-00026
5. Dalton HR, Bendall RP, Keane FE, Tedder RS, Ijaz S. Persistent carriage of hepatitis E virus in patients with HIV infection. *N Engl J Med.* (2009) 361:1025–7. doi: 10.1056/NEJMc0903778
6. Krain LJ, Nelson KE, Labrique AB. Host immune status and response to hepatitis E virus infection. *Clin Microbiol Rev.* (2014) 27:139–65. doi: 10.1128/CMR.00062-13
7. Wu J, Huang F, Ling Z, Liu S, Liu J, Fan J, et al. Altered faecal microbiota on the expression of Th cells responses in the exacerbation of patients with hepatitis E infection. *J Viral Hepat.* (2020) 27:1243–52. doi: 10.1111/jvh.13344
8. Halac U, Beland K, Lapierre P, Patey N, Ward P, Brassard J, et al. Chronic hepatitis E infection in children with liver transplantation. *Gut.* (2012) 61:597–603. doi: 10.1136/gutjnl-2011-300708
9. Agrawal S, Rana BS, Mitra S, Duseja A, Das A, Dhiman RK, et al. A case of acute-on-chronic liver failure (ACLF) due to an uncommon acute and chronic event. *J Clin Exp Hepatol.* (2018) 8:95–7. doi: 10.1016/j.jceh.2017.08.006
10. Kumar A, Saraswat VA. Hepatitis E and acute-on-chronic liver failure. *J Clin Exp Hepatol.* (2013) 3:225–30. doi: 10.1016/j.jceh.2013.08.013
11. Goel A, Aggarwal R. Hepatitis E: epidemiology, clinical course, prevention, and treatment. *Gastroenterol Clin North Am.* (2020) 49:315–30. doi: 10.1016/j.gtc.2020.01.011
12. Wu J, Guo Y, Lu X, Huang F, Lv F, Wei D, et al. Th1/Th2 cells and associated cytokines in acute hepatitis E and related acute liver failure. *J Immunol Res.* (2020) 2020:6027361. doi: 10.1155/2020/6027361
13. Chen P, Wang YY, Chen C, Guan J, Zhu HH, Chen Z. The immunological roles in acute-on-chronic liver failure: an update. *Hepatobiliary Pancreat Dis Int.* (2019) 18:403–11. doi: 10.1016/j.hbpd.2019.07.003
14. Hensley MK, Deng JC. Acute on chronic liver failure and immune dysfunction: a mimic of sepsis. *Semin Respir Crit Care Med.* (2018) 39:588–97. doi: 10.1055/s-0038-1672201
15. Chandra NS, Sharma A, Malhotra B, Rai RR. Dynamics of HEV viremia, fecal shedding and its relationship with transaminases and antibody response in patients with sporadic acute hepatitis E. *Virol J.* (2010) 7:213. doi: 10.1186/1743-422X-7-213
16. Nanda SK, Ansari IH, Acharya SK, Jameel S, Panda SK. Protracted viremia during acute sporadic hepatitis E virus infection. *Gastroenterology.* (1995) 108:225–30. doi: 10.1016/0016-5085(95)90028-4
17. Srivastava R, Aggarwal R, Bhagat MR, Chowdhury A, Naik S. Alterations in natural killer cells and natural killer T cells during acute viral hepatitis E. *J Viral Hepat.* (2008) 15:910–6. doi: 10.1111/j.1365-2893.2008.01036.x
18. Amon JJ, Drobeniuc J, Bower WA, Magana JC, Escobedo MA, Williams IT, et al. Locally acquired hepatitis E virus infection, El Paso, Texas. *J Med Virol.* (2006) 78:741–6. doi: 10.1002/jmv.20617
19. Masuda J, Yano K, Tamada Y, Takii Y, Ito M, Omagari K, et al. Acute hepatitis E of a man who consumed wild boar meat prior to the onset of illness in Nagasaki, Japan. *Hepatol Res.* (2005) 31:178–83. doi: 10.1016/j.hepres.2005.01.008
20. Schlauder GG, Desai SM, Zanetti AR, Tassopoulos NC, Mushahwar IK. Novel hepatitis E virus (HEV) isolates from Europe: evidence for additional genotypes of HEV. *J Med Virol.* (1999) 57:243–51. doi: 10.1002/(SICI)1096-9071(199903)57:3<243::AID-JMV6>3.0.CO;2-R
21. Srivastava R, Aggarwal R, Sachdeva S, Alam MI, Jameel S, Naik S. Adaptive immune responses during acute uncomplicated and fulminant hepatitis E. *J Gastroenterol Hepatol.* (2011) 26:306–11. doi: 10.1111/j.1440-1746.2010.06356.x
22. Majumdar M, Ratho R, Chawla Y, Singh MP. Evaluation of antigenicity and cell mediated immunity of hepatitis E virus patients: using non-radioactive MTT assay. *Indian J Med Microbiol.* (2013) 31:64–8. doi: 10.4103/0255-0857.108725
23. Jilani N, Das BC, Husain SA, Baweja UK, Chattopadhya D, Gupta RK, et al. Hepatitis E virus infection and fulminant hepatic failure during pregnancy. *J Gastroenterol Hepatol.* (2007) 22:676–82. doi: 10.1111/j.1440-1746.2007.04913.x
24. Moller HJ, Gronbaek H, Schiodt FV, Holland-Fischer P, Schilsky M, Munoz S, et al. Soluble CD163 from activated macrophages predicts mortality in acute liver failure. *J Hepatol.* (2007) 47:671–6. doi: 10.1016/j.jhep.2007.05.014
25. Das R, Tripathy A. Increased expressions of NKp44, NKp46 on NK/NKT-like cells are associated with impaired cytolytic function in self-limiting hepatitis E infection. *Med Microbiol Immunol.* (2014) 203:303–14. doi: 10.1007/s00430-014-0338-1
26. Streetz K, Leifeld L, Grundmann D, Ramakers J, Eckert K, Spengler U, et al. Tumor necrosis factor alpha in the pathogenesis of human and murine fulminant hepatic failure. *Gastroenterology.* (2000) 119:446–60. doi: 10.1053/gast.2000.9364
27. Gough DB, Fearon KC, Carter DC. Metabolic disturbance and sensitivity to endotoxin in patients with advanced cancer: relationship to lymphocyte reactivity, tumour necrosis factor (TNF) production and survival. *Clin Exp Immunol.* (1996) 105:529–36. doi: 10.1046/j.1365-2249.1996.d01-783.x

28. Hwang ES, Hong JH, Glimcher LH. IL-2 production in developing Th1 cells is regulated by heterodimerization of RelA and T-bet and requires T-bet serine residue 508. *J Exp Med.* (2005) 202:1289–300. doi: 10.1084/jem.20051044
29. El Costa H, Gouilly J, Abravanel F, Bahraoui E, Peron JM, Kamar N, et al. Effector memory CD8 T cell response elicits Hepatitis E Virus genotype 3 pathogenesis in the elderly. *PLoS Pathog.* (2021) 17:e1009367. doi: 10.1371/journal.ppat.1009367
30. Butcher EC. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell.* (1991) 67:1033–6. doi: 10.1016/0092-8674(91)90279-8
31. Naik A, Goel A, Agrawal V, Sarangi AN, Chhavi N, Singh V, et al. Changes in gene expression in liver tissue from patients with fulminant hepatitis E. *World J Gastroenterol.* (2015) 21:8032–42. doi: 10.3748/wjg.v21.i26.8032
32. Possamai LA, Antoniades CG, Anstee QM, Quaglia A, Vergani D, Thursz M, et al. Role of monocytes and macrophages in experimental and human acute liver failure. *World J Gastroenterol.* (2010) 16:1811–9. doi: 10.3748/wjg.v16.i15.1811
33. Holt MP, Cheng L, Ju C. Identification and characterization of infiltrating macrophages in acetaminophen-induced liver injury. *J Leukoc Biol.* (2008) 84:1410–21. doi: 10.1189/jlb.0308173
34. Fisher JE, McKenzie TJ, Lillegard JB Yu Y, Juskewitch JE, Nedredal GI, et al. Role of Kupffer cells and toll-like receptor 4 in acetaminophen-induced acute liver failure. *J Surg Res.* (2013) 180:147–55. doi: 10.1016/j.jss.2012.11.051
35. Zimmermann HW, Trautwein C, Tacke F. Functional role of monocytes and macrophages for the inflammatory response in acute liver injury. *Front Physiol.* (2012) 3:56. doi: 10.3389/fphys.2012.00056
36. Gordon S. Alternative activation of macrophages. *Nat Rev Immunol.* (2003) 3:23–35. doi: 10.1038/nri978
37. Soehnlein O, Lindbom L. Phagocyte partnership during the onset and resolution of inflammation. *Nat Rev Immunol.* (2010) 10:427–39. doi: 10.1038/nri2779
38. Lanier LL. NK cell recognition. *Annu Rev Immunol.* (2005) 23:225–74. doi: 10.1146/annurev.immunol.23.021704.115526
39. Biron CA, Brossay L. NK cells and NKT cells in innate defense against viral infections. *Curr Opin Immunol.* (2001) 13:458–64. doi: 10.1016/S0952-7915(00)00241-7
40. Seaman WE. Natural killer cells and natural killer T cells. *Arthritis Rheum.* (2000) 43:1204–17. doi: 10.1002/1529-0131(200006)43:6<1204::AID-ANR3>3.0.CO;2-I
41. Van Dommelen SL, Degli-Esposti MA. NKT cells and viral immunity. *Immunol Cell Biol.* (2004) 82:332–41. doi: 10.1111/j.0818-9641.2004.01261.x
42. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. *Nat Immunol.* (2008) 9:503–10. doi: 10.1038/ni1582
43. Raulet DH. Roles of the NKG2D immunoreceptor and its ligands. *Nat Rev Immunol.* (2003) 3:781–90. doi: 10.1038/nri1199
44. Moretta A, Bottino C, Vitale M, Pende D, Cantoni C, Mingari MC, et al. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. *Annu Rev Immunol.* (2001) 19:197–223. doi: 10.1146/annurev.immunol.19.1.197
45. Ni S, Li S, Yang N, Tang X, Zhang S, Hu D, et al. Deregulation of regulatory T cells in acute-on-chronic liver failure: a rat model. *Mediators Inflamm.* (2017) 2017:1390458. doi: 10.1155/2017/1390458
46. Tripathy AS, Das R, Rathod SB, Gurav YK, Arankalle VA. Peripheral T regulatory cells and cytokines in hepatitis E infection. *Eur J Clin Microbiol Infect Dis.* (2012) 31:179–84. doi: 10.1007/s10096-011-1291-1
47. Oldenhove G, de Heusch M, Urbain-Vansanten G, Urbain J, Maliszewski C, Leo O, et al. $CD4^+$ $CD25^+$ regulatory T cells control T helper cell type 1 responses to foreign antigens induced by mature dendritic cells *in vivo*. *J Exp Med.* (2003) 198:259–66. doi: 10.1084/jem.20030654
48. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. *Nat Immunol.* (2003) 4:330–6. doi: 10.1038/ni904
49. Kolls JK, Linden A. Interleukin-17 family members and inflammation. *Immunity.* (2004) 21:467–76. doi: 10.1016/j.immuni.2004.08.018
50. Kamar N, Izopet J, Tripon S, Bismuth M, Hillaire S, Dumortier J, et al. Ribavirin for chronic hepatitis E virus infection in transplant recipients. *N Engl J Med.* (2014) 370:1111–20. doi: 10.1056/NEJMoa1215246
51. Patterson JL, Fernandez-Larsson R. Molecular mechanisms of action of ribavirin. *Rev Infect Dis.* (1990) 12:1139–46. doi: 10.1093/clinids/12.6.1139
52. Dalton HR, Kamar N. Treatment of hepatitis E virus. *Curr Opin Infect Dis.* (2016) 29:639–44. doi: 10.1097/QCO.0000000000000316
53. Todt D, Gisa A, Radonic A, Nitsche A, Behrendt P, Suneetha PV, et al. *In vivo* evidence for ribavirin-induced mutagenesis of the hepatitis E virus genome. *Gut.* (2016) 65:1733–43. doi: 10.1136/gutjnl-2015-311000
54. Khuroo MS, Khuroo MS, Khuroo NS. Hepatitis E: discovery, global impact, control and cure. *World J Gastroenterol.* (2016) 22:7030–45. doi: 10.3748/wjg.v22.i31.7030
55. Rostaing L, Izopet J, Baron E, Duffaut M, Puel J, Durand D. Treatment of chronic hepatitis C with recombinant interferon alpha in kidney transplant recipients. *Transplantation.* (1995) 59:1426–31. doi: 10.1097/00007890-199505270-00012
56. Debing Y, Emerson SU, Wang Y, Pan Q, Balzarini J, Dallmeier K, et al. Ribavirin inhibits *in vitro* hepatitis E virus replication through depletion of cellular GTP pools and is moderately synergistic with alpha interferon. *Antimicrob Agents Chemother.* (2014) 58:267–73. doi: 10.1128/AAC.01795-13
57. Debing Y, Winton J, Neyts J, Dallmeier K. Cutthroat trout virus as a surrogate *in vitro* infection model for testing inhibitors of hepatitis E virus replication. *Antiviral Res.* (2013) 100:98–101. doi: 10.1016/j.antiviral.2013.07.013
58. Debing Y, Neyts J. Antiviral strategies for hepatitis E virus. *Antiviral Res.* (2014) 102:106–18. doi: 10.1016/j.antiviral.2013.12.005
59. Murakami Y, Fukasawa M, Kaneko Y, Suzuki T, Wakita T, Fukazawa H. Selective estrogen receptor modulators inhibit hepatitis C virus infection at multiple steps of the virus life cycle. *Microbes Infect.* (2013) 15:45–55. doi: 10.1016/j.micinf.2012.10.003

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 31 August 2021
doi: 10.3389/fmed.2021.726950

Baseline Neutrophil-to-Lymphocyte Ratio Is Independently Associated With 90-Day Transplant-Free Mortality in Patients With Cirrhosis

Jing Liu[1,2†], Hai Li[2,3†], Jie Xia[2,4†], Xianbo Wang[2,5†], Yan Huang[2,6†], Beiling Li[2,7†], Zhongji Meng[2,8], Yanhang Gao[2,9], Zhiping Qian[2,10], Feng Liu[2,11], Xiaobo Lu[2,12], Junping Liu[2,13], Guohong Deng[2,4], Yubao Zheng[2,14], Huadong Yan[2,15], Liang Qiao[2,3], Xiaomei Xiang[2,4], Qun Zhang[2,5], Ruochan Chen[2,6], Jinjun Chen[2,7], Sen Luo[2,8], La Gao[2,9], Liujuan Ji[2,10], Jing Li[2,11], Xinyi Zhou[2,12], Haotang Ren[2,16], Sihong Lu[1,2], Sumeng Li[1,2], Weituo Zhang[17] and Xin Zheng[1,2]*

[1] Department of Infectious Diseases, Institute of Infection and Immunology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [2] Chinese Chronic Liver Failure Consortium, Shanghai, China, [3] Department of Gastroenterology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, [4] Department of Infectious Diseases, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China, [5] Center of Integrative Medicine, Beijing Ditan Hospital, Capital Medical University, Beijing, China, [6] Department of Infectious Disease, Hunan Key Laboratory of Viral Hepatitis, Xiangya Hospital, Central South University, Hunan, China, [7] Hepatology Unit, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China, [8] Department of Infectious Diseases, Hubei Clinical Research Center for Precise Diagnosis and Treatment of Liver Cancer, Taihe Hospital, Hubei University of Medicine, Shiyan, China, [9] Department of Hepatology, First Hospital of Jilin University, Jilin, China, [10] Department of Liver Intensive Care Unit, Shanghai Public Health Clinical Centre, Fudan University, Shanghai, China, [11] Department of Infectious Diseases and Hepatology, The Second Hospital of Shandong University, Shandong, China, [12] Liver Disease Center, First Affiliated Hospital of Xinjiang Medical University, Xinjiang, China, [13] Department of Infectious Diseases, Henan Provincial People's Hospital, Henan, China, [14] Department of Infectious Diseases, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, [15] Department of Hepatology, Ningbo No. 2 Hospital, Ningbo, China, [16] State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Disease, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, [17] Clinical Research Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China*

OPEN ACCESS

Edited by:
Cornelius Engelmann, Charité – Universitätsmedizin Berlin, Germany

Reviewed by:
Michael Praktiknjo, University Hospital Bonn, Germany
Dominik Bettinger, University of Freiburg Medical Center, Germany

***Correspondence:**
Xin Zheng
xin11@hotmail.com

[†]These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *17 June 2021*
Accepted: *04 August 2021*
Published: *31 August 2021*

Citation:
Liu J, Li H, Xia J, Wang X, Huang Y, Li B, Meng Z, Gao Y, Qian Z, Liu F, Lu X, Liu J, Deng G, Zheng Y, Yan H, Qiao L, Xiang X, Zhang Q, Chen R, Chen J, Luo S, Gao L, Ji L, Li J, Zhou X, Ren H, Lu S, Li S, Zhang W and Zheng X (2021) Baseline Neutrophil-to-Lymphocyte Ratio Is Independently Associated With 90-Day Transplant-Free Mortality in Patients With Cirrhosis. Front. Med. 8:726950. doi: 10.3389/fmed.2021.726950

Background: Patients with cirrhosis have an increased risk of short-term mortality, however, few studies quantify the association between neutrophil-to-lymphocyte ratio (NLR) and 90-day transplant-free mortality in cirrhotic patients.

Methods: We prospectively analyzed 3,970 patients with chronic liver diseases from two multicenter cohorts in China (January 2015 to December 2016 and July 2018 to January 2019). Restricted cubic splines (RCS) were used to analyze the relation of NLR and all-causes 90-day transplant-free mortality in cirrhosis.

Results: A total of 2,583 cirrhotic patients were enrolled in our study. Restricted cubic splines showed that the odds ratio (OR) of all causes 90-day transplant-free mortality started to increase rapidly until around NLR 6.5, and then was relatively flat (p for non-linearity <0.001). The risk of 90-day transplant-free mortality in cirrhotic patients with NLR < 6.5 increased with an increment of 23% for every unit increase in NLR (p < 0.001). The patients with NLR < 4.5 had the highest risk (OR: 2.34, 95% CI 1.66–3.28). In multivariable-adjusted stratified analyses, the increase in the incidence of 90-day transplant-free mortality with NLR increasing was consistent (OR >1.0) across

all major prespecified subgroups, including infection group (OR: 1.04, 95% CI 1.00–1.09) and non-infection (OR: 1.06, 95% CI 1.02–1.11) group. The trends for NLR and numbers of patients with organ failure varied synchronously and were significantly increased with time from day 7 to day 28.

Conclusions: We found a non-linear association between baseline NLR and the adjusted probability of 90-day transplant-free mortality. A certain range of NLR is closely associated with poor short-term prognosis in patients with cirrhosis.

Keywords: neutrophil-to-lymphocyte ratio, short-term mortality, cirrhosis, acute decompensation, acute-on-chronic liver failure

INTRODUCTION

Acute decompensation (AD) is characterized by a rapid deterioration in patients with cirrhosis, leading to the development of acute-on-chronic liver failure (ACLF), a syndrome distinguished by organ failure and high short-term mortality, precipitating events that cause AD in patients with cirrhosis was significantly associated with surrogates of systemic inflammation and increased 90-day mortality (1).

Systemic inflammation plays a crucial role in the pathophysiological pathways leading to the deterioration of disease (2, 3) and was shown to be associated with organ dysfunction and disease progression in patients with AD and ACLF (4–6), inhibition of inflammatory signaling pathway (e.g., LPS-TLR4 axis) ameliorates organ injury and systemic inflammation, preventing disease progression (7). Systemic inflammation was related to alterations in peripheral blood leukocytes that could be captured by simple leucocyte ratio such as neutrophil-to-lymphocyte ratio (NLR) (8). Neutrophil-to-lymphocyte ratio reflects a systemic inflammatory response and shows a significant independent correlation with advanced inflammation diseases (e.g., severe alcoholic hepatitis and non-alcoholic fatty liver diseases) (9, 10). In patients with severe end-stage liver diseases (e.g., ACLF), NLR was identified as an effective biomarker for hospital mortality prediction (11). A previous study reported that increased NLR independently predicted the short-term mortality in cirrhotic patients with AD (12). A nomogram including NLR was used for individual risk stratification and selection of therapeutic strategies (13). The prior systemic review summarized the prognostic ability of NLR in predicting outcomes of cirrhotic patients (14). However, small sample sizes and the heterogeneity of the NLR thresholds used in these studies makes the clinical utility of the NLR somewhat difficult, and the utmost significance of further work should be considered in an attempt to quantify the effect of NLR at a certain range in the clinical setting, and elucidate exact threshold of NLR as well as applicable population.

Based on these considerations, we performed prospective cohorts to analyze the relation of NLR and short-term prognosis, and seek the optimal threshold in which to apply the NLR in stratifying risk, targeting interventions, and assessing resource utilization, contributing to the management of patients with cirrhosis.

PATIENTS AND METHODS

Study Population

Total 3,970 patients with chronic liver diseases were recruited from two prospective multicenter cohorts (NCT02457637, NCT03641872), with 2,600 and 1,370 patients in the development cohorts (from January 2015 to December 2016) and validation cohorts (from July 2018 to January 2019), respectively (15). We collected the patients' demographic characteristics, clinical manifestations, and laboratory measurements from the electronic medical record system. A visualization of the missing values of our data was shown in **Supplementary Figure 1**. This study was approved by the Ethics Committee of Renji Hospital, Shanghai Jiaotong University School of Medicine, in China, and the ethical committees of each center.

The exclusion criteria were as follows: (1) age >80; (2) NLR value missing; (3) non-cirrhosis; (4) liver transplantation. Therefore, the current study was carried out on 2,583 patients (**Figure 1**).

Definitions

The diagnosis of liver cirrhosis was confirmed by computed tomography (CT) and magnetic resonance imaging (MRI). Chronic liver disease was defined as liver cirrhosis or a history of liver dysfunction lasting more than 6 months. Acute liver injury was diagnosed by aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >3 × upper limit of normal, or total bilirubin > 2 × upper limits of normal within 1 week before enrollment (15, 16). Acute decompensation was defined by the acute development of hepatic encephalopathy (HE), bacterial infection, overt ascites, gastrointestinal hemorrhage, or any combination of these (**Supplementary Table 1**), and ACLF was diagnosed according to the European Association for the Study of Liver-Chronic Liver Failure (EASL-CLIF) criteria (17). Definitions related to infections: spontaneous bacteremia, spontaneous bacterial peritonitis, pneumonia, urinary tract infection, and other infections: include cellulitis, acute gastroenteritis, and cholangitis were referred to other studies (18). The MELD score was computed from baseline laboratory parameters based on the standard formula as 3.78 × ln (bilirubin [mg/dl]) + 9.57 × ln (creatinine [mg/dl]) + 11.20 × ln (international normalized ratio) + 6.43 × (0 if cholestatic or alcoholic, 1 otherwise) (19), MELD-Na score

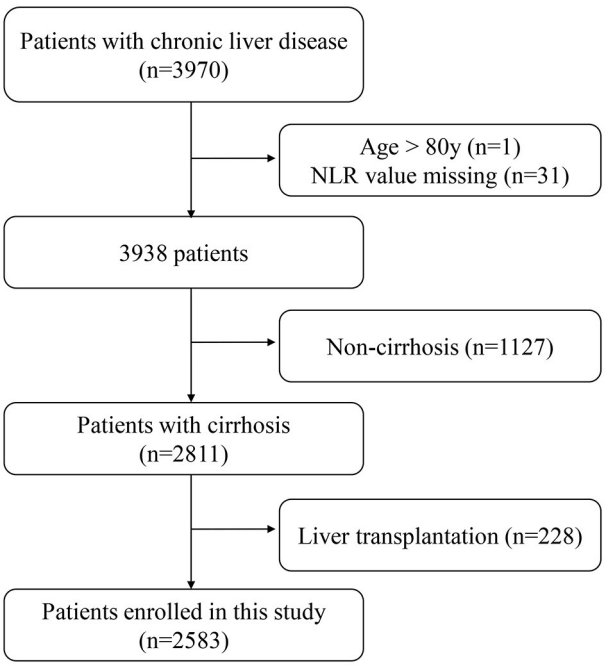


FIGURE 1 | Flow chart of patients enrolled in this study.

was calculated as MELD + (140 – Na [mmol/L]) – 0.025 × MELD × (140 – Na [mmol/L]) (20), and Child-Turcotte-Pugh (CTP) CLIF-C AD, and CLIF-C ACLF scores were calculated as described previously (21–23). Neutrophil-to-lymphocyte ratio was calculated from the ratio of peripheral blood absolute neutrophil and lymphocyte counts. The primary outcome of the study was 90-day transplant-free mortality.

Statistical Analysis

Categorical variables are expressed as frequencies or percentages and significance was tested by χ^2 or Fisher's exact test. Parametric quantitative variables are expressed as means ± standard deviation and a *t*-test was used to test the significance. Non-parametric quantitative variables are expressed as median and quartile intervals and the significance was detected by *Kruskal-Wallis* or *Mann-Whitney U*-test. *Kaplan-Meier* analysis was used to assess survival and survival curves were compared using the Log-Rank test.

We also used restricted cubic splines (RCS) with four knots at the 5^{th}, 35^{th}, 65^{th}, and 95^{th} centiles to flexibly model the association of NLR with 90-day transplant-free mortality. In the spline models, age, gender, etiology (HBV, Alcohol, Autoimmune), HE, infection, ascites, gastrointestinal bleeding, total bilirubin (TB), international normalized ratio (INR), and serum creatinine (Scr) were adjusted. We tested for potential non-linearity by using a likelihood ratio test comparing the model with only a linear term against the model with linear and cubic spline terms (24, 25). The diagnostic values of selected parameters were assessed by receiver operating characteristic (ROC) and the area under the ROC curve (AUC).

P-value <0.05 was considered statistically significant. SPSS statistical software (Macintosh version 26·0, IBM Corp., Armonk, NY, USA) and R package were used for statistical analysis.

RESULTS

A total of 2,583 patients with cirrhosis were enrolled in the present study. Based on the NLR quartile, the enrolled patients were divided into four groups, baseline characteristics of the four groups of the patients were listed in **Table 1**. The average age of the cohort patients was 51.64 ± 11.44 years old and 72.90% of them were male. The main etiology of cirrhosis was chronic infection with HBV (53.93%), followed by alcohol abuse (11.61%), autoimmune-related (9.45%), HBV coupled with alcohol abuse (8.83%), and others. Regarding the complications, ascites were the most common complication with an occurrence rate of 61.87%, while only 692 (26.79%) and 513 (19.86%) patients developed an infection and gastrointestinal bleeding, respectively. Total bilirubin, INR, white blood cells (WBC) levels increased with increasing of NLR, however, there was a significantly decreasing trend in the levels of albumin (ALB), platelet (PLT), hemoglobin, lymphocyte count. For patients with cirrhosis, we found the higher NLR was associated with the higher MELD or MELD-Na scores and 90-day transplant-free mortality.

In univariate analysis, cirrhosis, HE grades, infection, ascites, gastrointestinal bleeding, WBC, TB, INR, BUN, ALT, PLT, hemoglobin, Scr, NLR, Na, K, ALB, MELD, and MELD-Na showed a significant positive correlation with 90-day transplant-free mortality ($p < 0.05$) (data not shown). Multiple regression equations were constructed to further analyze potential relationships between NLR and 90-day transplant-free mortality in different models. Notably, the results revealed that NLR still showed a significant positive relationship with 90-day transplant-free mortality in patients with cirrhosis after adjusting for variables [Model II: age, gender, etiology (HBV, Alcohol, Autoimmune), HE grades, infection, ascites, gastrointestinal bleeding, TB, INR, and Scr ($p < 0.001$)]. To find out the association between subgroup NLR and 90-day transplant-free mortality, we modeled various forms of NLR by multiple regression equation including NLR quartile (≤1.74, >1.74, ≤2.77, >2.77, ≤4.89, >4.89). The results show that the NLR (>4.89) [unadjusted (odds ratio) OR = 7.24, 95% CI 5.05–10.37, $p < 0.001$] and NLR (>2.77, ≤4.89) (unadjusted OR =3.05, 95% CI 2.08–4.46, $p < 0.001$) were related to the incidence of 90-day transplant-free mortality in the univariate analysis compared NLR (≤1.74). It was further confirmed in the multivariate model that the NLR (>4.89) and NLR (>2.77, ≤4.89) were closely associated with 90-day transplant-free mortality adjusted for all variables in Model II (**Table 2**). When a baseline NLR value of 1.74, 2.77, and 4.89 was used as the cut-off values, patients with NLR >4.89 or NLR >2.77, ≤4.89 groups had a significantly lower 90-day survival probability compared to patients with NLR ≤1.74 or NLR >1.74, ≤2.77 groups ($p < 0.001$) (**Supplementary Figure 2**).

TABLE 1 | Baseline characteristics in all participants.

Variables	Total $n = 2,583$	>4.89 $n = 652$	(2.77,4.89] $n = 638$	(1.74,2.77] $n = 648$	(0,1.74] $n = 645$	*P*-value
Gender (male, %)	1,883 (72.90%)	505 (77.45)	465 (72.88)	472 (72.84)	441 (68.37)	0.004
Age (year)	51.64 (11.44)	52.65 (11.27)	51.52 (11.26)	51.08 (11.73)	51.31 (11.44)	0.065
Etiology (%)						
HBV	1,393 (53.93)	331 (50.77)	345 (54.08)	360 (55.56)	357 (55.35)	0.279
Alcohol	300 (11.61)	101 (15.49)	81 (12.70)	59 (9.10)	59 (9.15)	<0.001
Autoimmune	244 (9.45)	44 (6.75)	60 (9.40)	61 (9.41)	79 (12.25)	0.009
HBV and Alcohol	228 (8.83)	63 (9.66)	59 (9.25)	60 (9.26)	46 (7.13)	0.368
HBV and HEV	37 (1.43)	10 (1.53)	8 (1.25)	13 (2.01)	6 (0.93)	0.415
Others	381 (14.75)	102 (15.64)	86 (13.48)	95 (14.66)	98 (15.19)	0.720
HE grade (%)						<0.001
1	105 (4.07)	30 (4.60)	23 (3.61)	27 (4.17)	25 (3.88)	
2	118 (4.57)	50 (7.67)	32 (5.02)	20 (3.09)	16 (2.48)	
3	40 (1.55)	21 (3.22)	10 (1.57)	5 (0.77)	4 (0.62)	
4	15 (0.58)	8 (1.23)	6 (0.94)	1 (0.15)	0 (0.00)	
Infection (%)	692 (26.79)	267 (40.95)	181 (28.37)	136 (20.99)	108 (16.74)	<0.001
Ascites (%)	1,598 (61.87)	458 (70.25)	414 (64.89)	371 (57.25)	355 (55.04)	<0.001
GI Bleeding (%)	513 (19.86)	151 (23.16)	145 (22.73)	121 (18.67)	96 (14.88)	<0.001
TB (mg/dl)	3.83 (1.54–13.10)	8.84 (2.06–22.68)	4.95 (1.56–17.92)	3.67 (1.49–11.61)	2.44 (1.34–5.14)	<0.001
INR	1.48 (1.25–1.86)	1.63 (1.39–2.15)	1.48 (1.25–1.88)	1.44 (1.24–1.79)	1.38 (1.20–1.63)	<0.001
CR (mg/dl)	0.78 (0.64–0.97)	0.86 (0.68–1.21)	0.78 (0.64–0.99)	0.75 (0.63–0.90)	0.75 (0.62–0.88)	<0.001
BUN (μmol/L)	5.00 (3.72–7.28)	7.00 (4.60–11.10)	5.10 (3.74–7.31)	4.60 (3.50–6.19)	4.40 (3.40–5.70)	<0.001
ALB (g/L)	30.66 (6.05)	29.70 (5.69)	31.07 (6.34)	31.16 (5.81)	30.70 (6.27)	<0.001
ALT (U/L)	53.00 (26.00–155.00)	48.00 (24.00–144.25)	52.05 (25.77–156.50)	59.60 (28.00–186.40)	50.00 (27.00–131.00)	0.013
AST (U/L)	75.00 (39.00–173.93)	75.00 (34.90–176.30)	78.15 (37.00–179.00)	75.90 (39.20–179.30)	71.00 (43.22–159.07)	0.442
PLT (G/L)	76.00 (50.00–116.00)	77.00 (49.00–117.50)	76.00 (50.00–113.75)	77.00 (52.00–116.25)	75.00 (50.00–116.00)	0.666
AKP (μmol/L)	123.00 (86.00–170.00)	118.00 (78.97–168.00)	120.50 (84.00–170.25)	130.00 (89.00–176.00)	123.00 (90.75–163.00)	0.010
γ-GT (U/L)	64.00 (31.00–126.00)	58.30 (27.00–113.00)	62.00 (31.00–118.40)	70.70 (33.00–147.72)	63.00 (32.75–126.00)	0.004
White blood cell (G/L)	4.74 (3.31–6.92)	7.29 (5.16–10.74)	4.90 (3.50–6.81)	4.21 (2.98–5.90)	3.74 (2.70–4.94)	<0.001
Hemoglobin (g/L)	107.15 (26.81)	104.49 (27.64)	104.57 (27.90)	109.10 (26.77)	110.43 (24.31)	<0.001
Neutrophil count (G/L)	2.99 (1.90–4.85)	5.84 (4.01–9.05)	3.47 (2.42–4.82)	2.58 (1.79–3.63)	1.72 (1.19–2.36)	<0.001
Lymphocyte count (G/L)	1.06 (0.71–1.53)	0.73 (0.49–1.03)	0.96 (0.70–1.30)	1.18 (0.83–1.64)	1.46 (1.09–2.04)	<0.001
K (mmol/L)	3.82 (3.50–4.17)	3.95 (0.75)	3.82 (0.59)	3.81 (0.53)	3.83 (0.53)	<0.001
Na (mmol/L)	137.19 (5.18)	134.95 (6.12)	136.70 (5.12)	138.08 (4.32)	139.04 (3.92)	<0.001
MELD	17.00 (8.25)	21.00 (9.63)	18.00 (8.28)	17.00 (7.19)	14.00 (5.93)	<0.001
MELD-Na	19.00 (10.33)	25.00 (12.51)	20.00 (10.13)	18.00 (8.23)	15.00 (6.93)	<0.001
Child-pugh	9.00 (1.78)	9.00 (1.73)	9.00 (1.75)	9.00 (1.71)	8.00 (1.77)	<0.001
CLIF-C AD	19.00 (3.51)	20.00 (3.46)	19.00 (3.43)	18.00 (3.46)	18.00 (3.41)	<0.001

HE, hepatic encephalopathy; GI bleeding, gastrointestinal bleeding; TB, total bilirubin; INR, international normalized ratio; CR, serum creatinine; BUN, blood urea nitrogen; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PLT, platelet; AKP, alkaline phosphatase; γ-GT, γ-glutamyltransferase; K, blood potassium; Na, serum sodium.

We used RCS to flexibly model and visualize the relation of predicted NLR and with all-cause OR of 90-day transplant-free mortality in patients with cirrhosis (**Figure 2**). The OR-values started to increase rapidly until around 6.5 and then were relatively flat (p for non-linearity <0.001). There were 417 cases with NLR >6.5, the fraction of these patients was 16.14% (417/2,583). We next validated the different effects of NLR on OR between the NLR <6.5 and the NLR ≥6.5 groups and found that the threshold-effect in NLR <6.5 group was more obvious than that in NLR ≥6.5 group (**Supplementary Figure 3**). To assess the exact value-effect of NLR on the 90-day transplant-free mortality, two piece-wise linear regression models were constructed, one for the NLR <6.5 part and the other one for the NLR ≥6.5 part of the trajectory of 90-day transplant-free mortality, as divided by 6.5. Results showed that the risk of 90-day transplant-free mortality in cirrhotic patients with NLR <6.5 increased with an increment of the growth rate of 23% per unit (OR:1.23, 95% CI 1.14–1.32) ($p < 0.001$), similar results were also observed in patients with AD and ACLF (**Table 3**). To further differentiate low-range NLR populations, we dichotomized the

TABLE 2 | Relationship between NLR and 90-day transplant-free mortality.

Exposure	No. of 90-day mortality (%)	Model I (Unadjusted)		Model II (Fully Adjusted)	
		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
NLR continuous	423 (16.38)	1.14 (1.11, 1.16)	<0.001	1.05 (1.02, 1.08)	<0.001
NLR quartiles					
≤1.74	40 (6.20)	1.0 (Reference)		1.0 (Reference)	
>1.74, ≤2.77	65 (10.03)	1.69 (1.12, 2.54)	0.013	1.14 (0.73, 1.78)	0.551
>2.77, ≤4.89	107 (16.77)	3.05 (2.08, 4.46)	<0.001	1.35 (0.88, 2.08)	0.172
>4.89	211 (32.36)	7.24 (5.05, 10.37)	<0.001	2.46 (1.62, 3.72)	<0.001

OR, odds ratio; CI, confidence interval; Model I, unadjusted; Model II, adjust age, gender, etiology (HBV, alcohol, autoimmune), hepatic encephalopathy grades, infection, ascites, gastrointestinal bleeding, total bilirubin, international normalized ratio, serum creatinine.

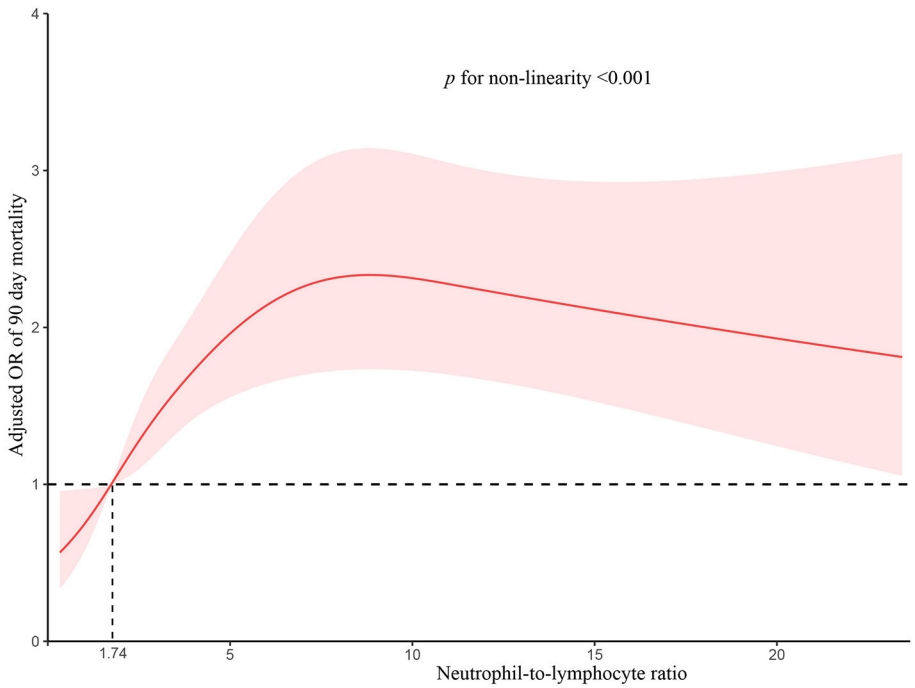


FIGURE 2 | Association of adjusted odds ratio between neutrophil-to-lymphocyte ratio and 90-day transplant-free mortality. The median NLR level within the lowest NLR quartile (1.74) served as the referent value (odds = 1.0). The model was adjusted for age; gender; etiology (HBV, alcohol, autoimmune); cirrhosis; hepatic encephalopathy grades, infection, ascites, gastrointestinal bleeding; total bilirubin; international normalized ratio; serum creatinine. The solid red line represents the multivariable-adjusted odds of mortality as a function of the measured NLR level and 95% confidence intervals are indicated by shaded areas. The *p*-values for non-linearity were <0.001. NLR, neutrophil-to-lymphocyte ratio.

cohort (patients with NLR <6.5) as "low" vs. "not low" using various cut points in increments of 0.5 units. This yields the remarkable result that there is a statistically significant difference in the adjusted probability of 90-day transplant-free mortality for thresholds as low as 1.5 and as high as 5.5, especially for patients with NLR <4.5 (OR:2.34, 95% CI 1.66–3.28) (**Figure 3**).

Association of the NLR with 90-day transplant-free mortality in patients with cirrhosis was analyzed using logistic regression models across different subgroups, and the results are presented in **Figure 4**. After adjustment for variables, the increase in the rate of 90-day transplant-free mortality with NLR increasing was consistent (OR >1.0) across all major prespecified subgroups, especially for patients with infection (OR:1.04, 95% CI 1.00–1.09) and non-infection (OR:1.06, 95% CI 1.02–1.11) (**Figure 4**). Furthermore, we detected no significant interaction for most of the pre-specified baseline factors ($p > 0.05$ for all comparisons).

To investigate the longitudinal association between the NLR and organ failure (liver failure, kidney failure, cerebral failure, coagulation failure, circulatory failure, and respiratory failure), we analyzed the kinetic changes of NLR in patients who did not have organ failure at baseline and subsequently developed organ failure during hospitalization, results showed that the

trends for NLR and numbers of patients with organ failure varied synchronously, and were significantly increased with time during day 7 to day 28 (**Figure 5**).

TABLE 3 | Threshold effect analysis of neutrophil-to-lymphocyte ratio on the 90-day transplant-free mortality using two piece-wise linear regression.

Neutrophil-to-lymphocyte ratio (NLR)	Crude OR (95% Confidence interval) *p*-value	Adjusted OR (95% Confidence interval)* *p*-value
All patients		
NLR < 6.5	1.47 (1.38, 1.57) <0.001	1.23 (1.14, 1.32) <0.001
NLR ≥ 6.5	0.99 (0.95, 1.03) 0.648	0.98 (0.93, 1.02) 0.321
AD patients		
NLR < 6.5	1.44 (1.35, 1.54) <0.001	1.23 (1.13, 1.33)<0.001
NLR ≥ 6.5	0.99 (0.96, 1.03) 0.742	0.97 (0.93, 1.02) 0.275
Non-AD patients		
NLR < 6.5	1.63 (1.33, 1.98) <0.001	1.31 (0.99, 1.73) 0.058
NLR ≥ 6.5	0.93 (0.74, 1.16) 0.497	1.01 (0.78, 1.31) 0.924
ACLF patients		
NLR < 6.5	1.30 (1.14, 1.49) 0.001	1.23 (1.06, 1.44) 0.007
NLR ≥ 6.5	0.98 (0.92, 1.05) 0.555	0.95 (0.89, 1.02) 0.195
Non-ACLF patients		
NLR < 6.5	1.36 (1.26, 1.47) <0.001	1.19 (1.08, 1.30) <0.001
NLR ≥ 6.5	0.98 (0.92, 1.03) 0.433	1.00 (0.94, 1.07) 0.940

**Adjusting for age, gender, etiology (HBV, alcohol, autoimmune), hepatic encephalopathy grades, infection, ascites, gastrointestinal bleeding, total bilirubin, international normalized ratio, serum creatinine.*

Next, we examined the possibilities of using parameters as prognostic factors for identifying 90-day transplant-free mortality in patients with AD or ACLF. Neutrophil-to-lymphocyte ratio, MELD, MLED-Na, CLIF-C AD, CLIF-C ACLF, and NLR coupled with MELD, MELD-Na, CLIF-C AD, and CLIF-C ACLF were selected as potential prognostic factors for further detailed statistical analysis. The ROC curve and AUC, which were calculated by R package "pROC," were performed to assess the diagnostic value of these five selected and federated parameters. We used the CLIF-C AD score and CLIF-C ACLF score in patients with AD and ACLF, respectively. Results showed that MELD (0.814) and MELD-Na (0.821) showed better prediction than CLIF-C AD (0.628), and NLR + MELD-Na with a higher AUC (0.826) than NLR + MELD (0.821), MELD-Na (0.821), MELD (0.814) in patients with AD (**Figure 6A**), however, MELD (0.683), MELD-Na (0.689), and CLIF-C-ACLF (0.677) showed roughly equivalent prognostic power in patients with ACLF (**Figure 6B**). Additionally, we substituting the WBC in CLIF-C-AD and CLIF-C-ACLF with NLR (CLIF-C ADNLR and CLIF-C ACLFNLR), results showed that it didn't significantly improve score performance [CLIF-C AD vs. CLIF-C ADNLR: 0.628 vs. 0.633 (**Figure 6A**); CLIF-C ACLF vs. CLIF-C ACLFNLR: 0.677 vs. 0.681 (**Figure 6B**)].

DISCUSSION

Our large, multi-center cohort study has firstly confirmed that the value of NLR 6.5 is a feasible cut-off value across clinical settings in patients with cirrhosis, patients with NLR below 6.5 were the optimal population in which to apply NLR in stratifying risk, with a 23% increment in risk of 90-day transplant-free mortality for every unit increase in NLR.

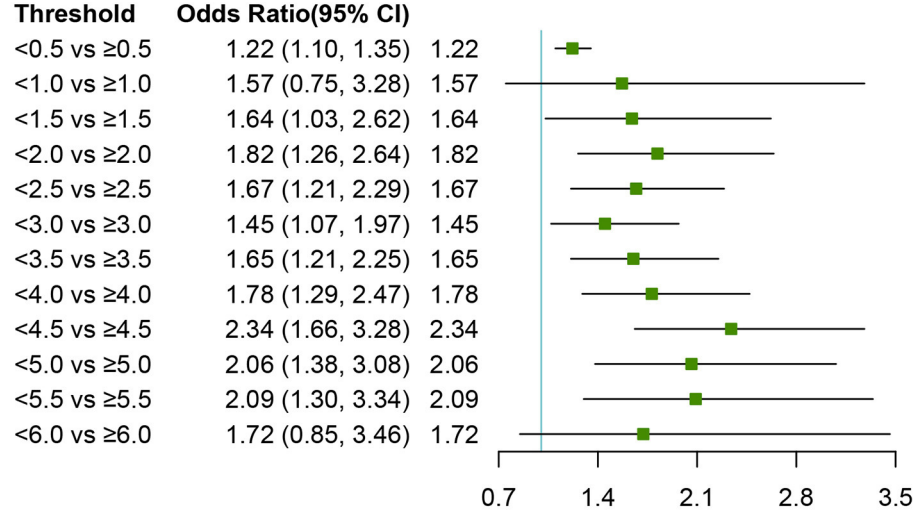


FIGURE 3 | Wide-ranged neutrophil-to-lymphocyte ratio thresholds and adjusted odds ratios of 90-day transplant-free mortality. The cohort of patients (NLR < 6.5) whose NLR was dichotomized into "low" vs. "not low" groups using various cut points in increments of 0.5. Logistic regression was used to estimate the odds ratio of death between the two groups, adjusting for age; gender; etiology (HBV, alcohol, autoimmune); cirrhosis; hepatic encephalopathy grades, infection, ascites, gastrointestinal bleeding; total bilirubin; international normalized ratio; serum creatinine. Squares indicate estimated adjusted odds ratios, and error bars indicate 95% CIs. NLR, neutrophil-to-lymphocyte ratio; OR, Odds Ratios.

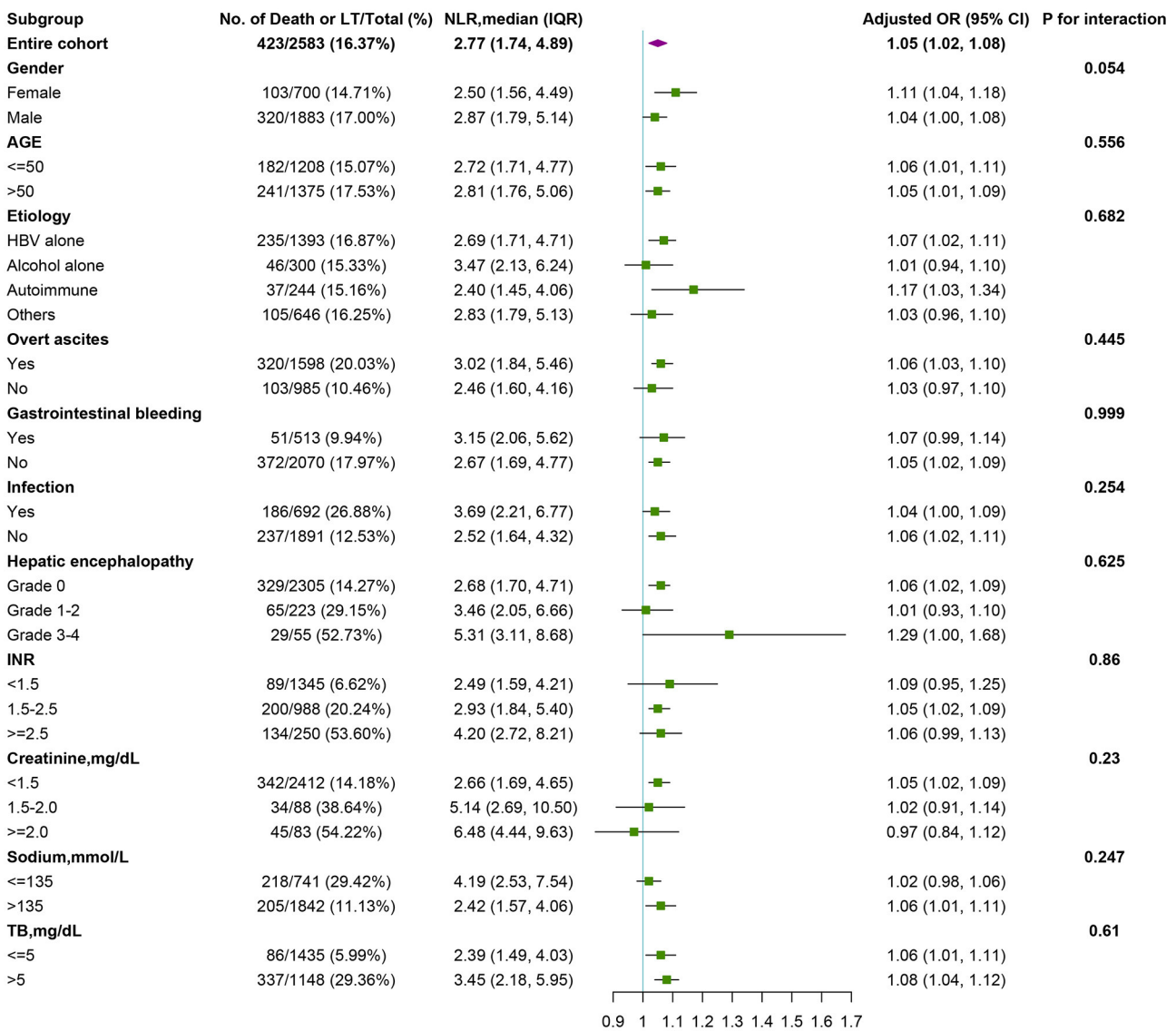


FIGURE 4 | Stratified analyses of risk of death in pre-specified and exploratory subgroups in each subgroup. The multivariable-adjusted odds ratios (95% confidence intervals) of death per unit increment in the standard deviation of NLR level is plotted for the entire cohort and according to strata of baseline covariates. The model was adjusted for age; gender; etiology (HBV, alcohol, autoimmune); cirrhosis; hepatic encephalopathy grades, infection, ascites, gastrointestinal bleeding; total bilirubin; international normalized ratio; serum creatinine. In each case, the model is not adjusted for the stratification variable. A test for interaction between NLR and strata of baseline covariates is also performed. NLR, neutrophil-to-lymphocyte ratio.

Neutrophil-to-lymphocyte ratio has been studied as a prognostic marker in cirrhotic patients on the basis that it reflects the levels of systemic inflammation driving disease progression in cirrhosis (26, 27). More recently, more and more evidence shows that NLR is a clinically relevant prognostic parameter in patients with liver cirrhosis. A propensity score matching study revealed that NLR over 8.9 may serve as a robust cutoff value for identifying cirrhotic patients at high risk of 90-day mortality (12). Rice et al. (28) revealed that 2.84-folds risk of death was demonstrated in cirrhotic patients with NLR >9 when compared to NLR <3. However, previous studies recruited a unique population, who were predisposed to infection and development of AD or ACLF, representing high mortality, it's unclear if NLR had the same prognostic value of mortality in patients with cirrhosis. In the current study, we found that NLR >4.89 was associated with more than 7-folds risk of 90-day transplant-free mortality compared with the lowest quartile (NLR ≤1.74) in patients with cirrhosis. More importantly, we found that NLR showed a positive relationship with 90-day transplant-free mortality regardless of the presence or absence of infection. These results demonstrated that NLR was an independent risk factor of mortality in hospitalized patients with cirrhosis including AD or non-AD, ACLF or non-ACLF, infection or non-infection patients, suggesting some of the same mechanisms involved in

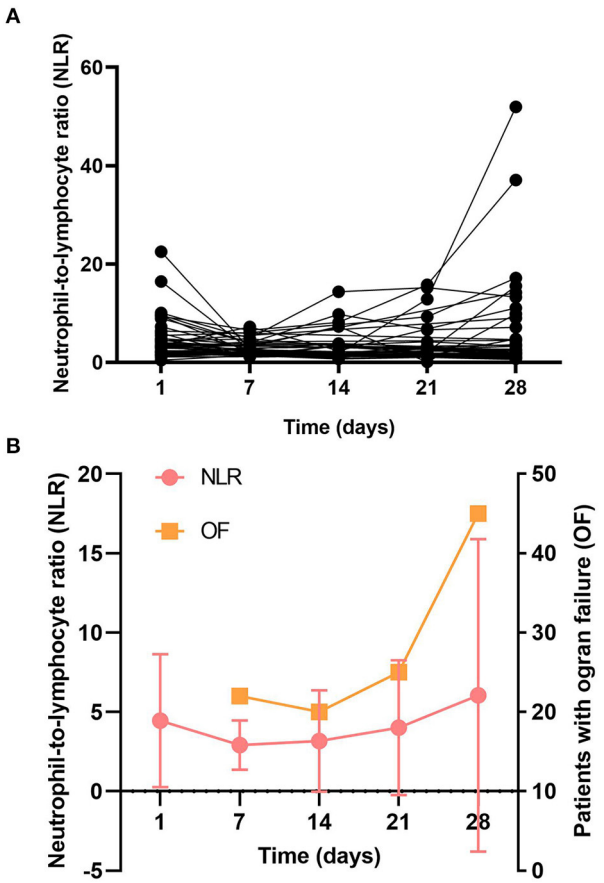


FIGURE 5 | The longitudinal association of NLR and organ dysfunction. Patients were dichotomized into those who did not have organ failure at baseline and subsequently developed organ failure. **(A)** The longitudinal curves of NLR over time; **(B)** Kinetic analysis of the number of organ failure and NLR.

systemic inflammation and eventually resulting in various acute insults. These findings corroborate and extend previous research on the applicable population of NLR.

More recent works on cancer diseases suggested that every unit increase in NLR was associated with a 10–17% increase in 12-month mortality (29, 30). Although NLR has been suggested as an independent risk factor for transplant-free survival and overall survival in liver cirrhosis (11, 26, 28, 31, 32), however, it is still unknown whether there is a linear relationship between the NLR and the risk of death in cirrhosis. Our study provided further results and showed that when an NLR cutoff at approximate 6.5, the increasing trend of OR of 90-day transplant-free mortality with increased NLR occurred significant non-linear relationship or threshold effect, and the risk of 90-day transplant-free mortality in cirrhotic patients with NLR below 6.5 increased with an increment of the growth rate of 23% every unit increase in NLR, especially for patients with AD, ACLF, and non-ACLF. However, the effect was not obvious in NLR over 6.5 group. These results probably indicated that NLR was not enough for comprehensively reflecting the severity of diseases in cirrhotic patients with NLR over 6.5. It's reported that high NLR was not independently associated with overall survival in patients with severe diseases (e.g., advanced nasopharyngeal carcinoma) (33). Therefore, for these patients, we can choose to measure more specific diagnostic markers, such as MELD, a well-known highly specific score that is used to predict short-term survival in patients with a wide spectrum of liver disease (34). Our results also demonstrated that NLR combined with MELD or MELD-Na could improve the effectiveness of predicting mortality.

To our knowledge, MELD scores have been used to predict the mortality of cirrhotic patients awaiting liver transplantation (35), MELD-Na were shown to better predict mortality compared with the MELD score (20, 36), however, predictive significance comparisons among the NLR, MELD, and MELD-Na in cirrhotic patients with large sample size have not been reported before. Therefore, in our study, the original MELD score and the MELD-Na score were included for comparison with other scoring systems. Our results showed that the capacity of MELD-Na and MELD was good in predicting 3-month transplant-free mortality of patients with AD, although the predictive performance of NLR weakened than the other two models, compared MELD-Na and MELD alone, MELD-Na and MELD performed better prediction ability when combined with NLR. However, CLIF-C AD and CLIF-C ACLF didn't show well-predictive performance in our cohort. The heterogeneity of patients enrolled for analysis probably contributes to the difference in predictive accuracy. It's reported that the CLIF-C AD score is more accurate than other liver scores in predicting the prognosis (22) and the CLIF-C ACLF at ACLF diagnosis is superior to the MELD and MELD-Na in predicting mortality (23). However, patients enrolled in these two studies were mainly alcohol-related cirrhosis (49.0 and 54.7%), and the fraction of patients with ascites was high (63.9 and 80.2%), renal insufficiency in these patients were more prone to be observed. In our study, the main etiology of cirrhosis was chronic infection with HBV (53.9%), apart from ascites, 692 (26.79%) and 513 (19.86%) patients developed an infection and gastrointestinal bleeding, respectively. The fraction of patients with liver failure in our cohort was 27.80% (718/2,583), while only 3.2% (83/2,583) patients with renal failure were observed. These results may be indicated that CLIF-C AD or CLIF-C ACLF wasn't the best option for predicting 90-day transplant-free mortality in our cohort.

There are several limitations to our study. First, Although we found that the level of NLR and numbers of patients with organ failure increased synchronously with time during day 7 to day 28, we didn't analyze the correlation between the dynamic change of NLR and short-term mortality, future work needs to focus on providing more powerful evidence. Second, patients with cirrhosis in our multicenter cohorts received varieties of therapies involving different types, doses, and duration of drugs, it's very difficult to homogenize the potential effects of these covariates, thus, we didn't analyze the impacts of antibiotics on the lymphocyte and neutrophil counts, however, stratified analyses showed that infection was not an interaction factor for the impact of NLR on 90-day transplant-free mortality in our cohort, in other words, NLR showed a positive relationship with 90-day transplant-free mortality regardless of the presence or

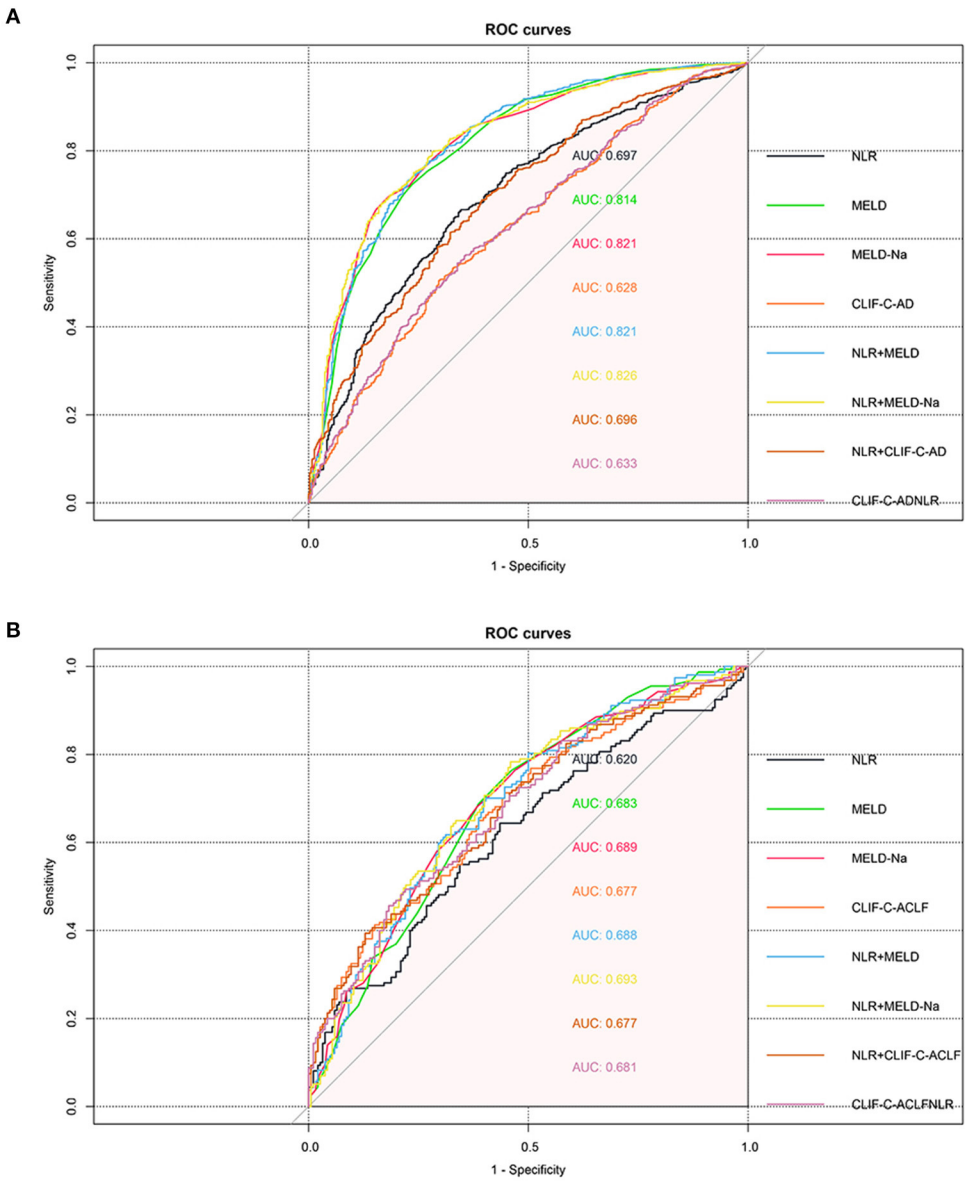


FIGURE 6 | Predictive ability of parameters for 90-day transplant-free mortality. ROC curve and AUC were calculated for neutrophil-to-lymphocyte ratio (NLR), MELD, MELD-Na, CLIF-C AD, CLIF-C ACLF, and and federated parameters by using the R package "pROC." The cirrhotic patients with AD **(A)** and ACLF **(B)**.

absence of infection, patients with infection are more likely to be treated with antibiotics, these probably indicated NLR was not significantly affected by antibiotics. Furthermore, the NLR threshold of 6.5 has been adjusted for infection.

CONCLUSION

Our study concludes that NLR is independently associated with 90-day transplant-free mortality in patients with cirrhosis. Neutrophil-to-lymphocyte ratio >6.5 may serve as a robust cut-off value, and patients with NLR below 6.5 were the optimal population in which to apply the NLR in stratifying risk.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s at: aclf_group@163.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Renji Hospital, Shanghai Jiaotong University School of Medicine. The patients/participants provided their written informed consent

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

AUTHOR CONTRIBUTIONS

All authors had access to the data and their role in writing the manuscript. JLiu and XZ: study concept and design. JLiu, HL, JX, XbW, YH, and BlL: contributed equally to this manuscript. HL, GHD, XbW, YH, BlL, ZjM, YHg, ZpQ, FL, XL, JpL, LQ, XmX, QZ, RcC, JjC, SL, LG, LjJ, JLi, XyZ, HtR, ShL, and SmL: data acquisition. WtZ: data analysis. JLiu and XZ: drafted the manuscript. All authors offered critical revision and approved the final draft of the manuscript.

FUNDING

This work was supported by the National Natural Science Foundation of China (grant numbers 81271884 and 81461130019), the National Key Research and Development Program of China (grant numbers 2017YFC0908100 and 2017YFC0908104), and the National Science and Technology Major Project (grant numbers 2018ZX10723203 and 2018ZX10302206).

ACKNOWLEDGMENTS

We thank the Chinese Acute-on-chronic liver failure Consortium members and participants for the contributions to this study: Department of Hepatology, First Hospital of Jilin University—Zhang Yanan; Center of Integrative Medicine, Beijing Ditan Hospital, Capital Medical University—Huang Yunyi; Department of Liver Intensive Care Unit, Shanghai Public Health Clinical Centre, Fudan University—Zou Ying; Hepatology Unit, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University—Renjie Ouyang; Department of Infectious Disease, Hunan Key Laboratory of Viral Hepatitis, Xiangya Hospital, Central South University—Zheng Yixiang; Department of Infectious Disease, Taihe Hospital, Hubei University of Medicine—Wang Chen; Liver Disease Center, First Affiliated Hospital of Xinjiang Medical University—Zhu Yanfang; Department of Infectious Diseases, Southwest Hospital, Third Military Medical University (Army Medical University)—Yi Zhou; Department of Infectious Diseases and Hepatology, The Second Hospital of Shandong University—Tao Li; Department of Gastroenterology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University—Zhang Yan.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.726950/full#supplementary-material

REFERENCES

1. Trebicka J, Fernandez J, Papp M, Caraceni P, Laleman W, Gambino C, et al. PREDICT identifies precipitating events associated with the clinical course of acutely decompensated cirrhosis. *J Hepatol.* (2021) 74:1097–108. doi: 10.1016/j.jhep.2020.11.019
2. Monteiro S, Grandt J, Uschner FE, Kimer N, Madsen JL, Schierwagen R, et al. Differential inflammasome activation predisposes to acute-on-chronic liver failure in human and experimental cirrhosis with and without previous decompensation. *Gut.* (2021) 70:379–87. doi: 10.1136/gutjnl-2019-320170
3. Praktiknjo M, Schierwagen R, Monteiro S, Ortiz C, Uschner FE, Jansen C, et al. Hepatic inflammasome activation as origin of Interleukin-1α and Interleukin-1β in liver cirrhosis. *Gut*. (2021) 70:1799–1800. doi: 10.1136/gutjnl-2020-322621
4. Bernardi M, Moreau R, Angeli P, Schnabl B, Arroyo V. Mechanisms of decompensation and organ failure in cirrhosis: from peripheral arterial vasodilation to systemic inflammation hypothesis. *J Hepatol*. (2015) 63:1272–84. doi: 10.1016/j.jhep.2015.07.004
5. Arroyo V, Angeli P, Moreau R, Jalan R, Claria J, Trebicka J, et al. The systemic inflammation hypothesis: towards a new paradigm of acute decompensation and multiorgan failure in cirrhosis. *J Hepatol.* (2021) 74:670–85. doi: 10.1016/j.jhep.2020.11.048
6. Praktiknjo M, Monteiro S, Grandt J, Kimer N, Madsen JL, Werge MP, et al. Cardiodynamic state is associated with systemic inflammation and fatal acute-on-chronic liver failure. *Liver Int*. (2020) 40:1457–66. doi: 10.1111/liv.14433
7. Engelmann C, Sheikh M, Sharma S, Kondo T, Loeffler-Wirth H, Zheng YB, et al. Toll-like receptor 4 is a therapeutic target for prevention and treatment of liver failure. *J Hepatol.* (2020) 73:102–12. doi: 10.1016/j.jhep.2020.01.011
8. Zahorec R. Ratio of neutrophil to lymphocyte counts–rapid and simple parameter of systemic inflammation and stress in critically ill. *Bratisl Lek Listy*. (2001) 102:5–14.
9. Khoury T, Mari A, Nseir W, Kadah A, Sbeit W, Mahamid M. Neutrophil-to-lymphocyte ratio is independently associated with inflammatory activity and fibrosis grade in nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol.* (2019) 31:1110–5. doi: 10.1097/MEG.0000000000001393
10. Forrest EH, Storey N, Sinha R, Atkinson SR, Vergis N, Richardson P, et al. Baseline neutrophil-to-lymphocyte ratio predicts response to corticosteroids and is associated with infection and renal dysfunction in alcoholic hepatitis. *Aliment Pharmacol Ther*. (2019) 50:442–53. doi: 10.1111/apt.15335
11. Bernsmeier C, Cavazza A, Fatourou EM, Theocharidou E, Akintimehin A, Baumgartner B, et al. Leucocyte ratios are biomarkers of mortality in patients with acute decompensation of cirrhosis and acute-on-chronic liver failure. *Aliment Pharmacol Ther*. (2020) 52:855–65. doi: 10.1111/apt.15932
12. Deng Y, Fan X, Ran Y, Xu X, Lin L, Cui B, et al. Prognostic impact of neutrophil-to-lymphocyte ratio in cirrhosis: a propensity score matching analysis with a prespecified cut-point. *Liver Int*. (2019) 39:2153–63. doi: 10.1111/liv.14211
13. Lin L, Yang F, Wang Y, Su S, Su Z, Jiang X, et al. Prognostic nomogram incorporating neutrophil-to-lymphocyte ratio for early mortality in decompensated liver cirrhosis. *Int Immunopharmacol*. (2018) 56:58–64. doi: 10.1016/j.intimp.2018.01.007
14. Peng Y, Li Y, He Y, Wei Q, Xie Q, Zhang L, et al. The role of neutrophil to lymphocyte ratio for the assessment of liver fibrosis and cirrhosis: a systematic review. *Expert Rev Gastroenterol Hepatol.* (2018) 12:503–13. doi: 10.1080/17474124.2018.1463158
15. Qiao L, Wang X, Deng G, Huang Y, Chen J, Meng Z, et al. Cohort profile: a multicentre prospective validation cohort of the Chinese

Acute-on-Chronic Liver Failure (CATCH-LIFE) study. *BMJ Open.* (2021) 11:e037793. doi: 10.1136/bmjopen-2020-037793
16. Zhang Y, Xu BY, Wang XB, Zheng X, Huang Y, Chen J, et al. Prevalence and clinical significance of portal vein thrombosis in patients with cirrhosis and acute decompensation. *Clin Gastroenterol Hepatol.* (2020) 18:2564.e1–72.e1. doi: 10.1016/j.cgh.2020.02.037
17. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology.* (2013) 144:1426–37, 1437.e1–9. doi: 10.1053/j.gastro.2013.02.042
18. Fernandez J, Acevedo J, Wiest R, Gustot T, Amoros A, Deulofeu C, et al. Bacterial and fungal infections in acute-on-chronic liver failure: prevalence, characteristics and impact on prognosis. *Gut.* (2018) 67:1870–80. doi: 10.1136/gutjnl-2017-314240
19. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, et al. A model to predict survival in patients with end-stage liver disease. *Hepatology.* (2001) 33:464–70. doi: 10.1053/jhep.2001.22172
20. Kim WR, Biggins SW, Kremers WK, Wiesner RH, Kamath PS, Benson JT, et al. Hyponatremia and mortality among patients on the liver-transplant waiting list. *N Engl J Med.* (2008) 359:1018–26. doi: 10.1056/NEJMoa0801209
21. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg.* (1973) 60:646–9. doi: 10.1002/bjs.1800600817
22. Jalan R, Pavesi M, Saliba F, Amoros A, Fernandez J, Holland-Fischer P, et al. The CLIF Consortium Acute Decompensation score (CLIF-C ADs) for prognosis of hospitalised cirrhotic patients without acute-on-chronic liver failure. *J Hepatol.* (2015) 62:831–40. doi: 10.1016/j.jhep.2014.11.012
23. Jalan R, Saliba F, Pavesi M, Amoros A, Moreau R, Gines P, et al. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. *J Hepatol.* (2014) 61:1038–47. doi: 10.1016/j.jhep.2014.06.012
24. Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med.* (1989) 8:551–61. doi: 10.1002/sim.4780080504
25. Govindarajulu US, Spiegelman D, Thurston SW, Ganguli B, Eisen EA. Comparing smoothing techniques in Cox models for exposure-response relationships. *Stat Med.* (2007) 26:3735–52. doi: 10.1002/sim.2848
26. Kalra A, Wedd JP, Bambha KM, Gralla J, Golden-Mason L, Collins C, et al. Neutrophil-to-lymphocyte ratio correlates with proinflammatory neutrophils and predicts death in low model for end-stage liver disease patients with cirrhosis. *Liver Transpl.* (2017) 23:155–65. doi: 10.1002/lt.24702
27. Dirchwolf M, Ruf AE. Role of systemic inflammation in cirrhosis: from pathogenesis to prognosis. *World J Hepatol.* (2015) 7:1974–81. doi: 10.4254/wjh.v7.i16.1974
28. Rice J, Dodge JL, Bambha KM, Bajaj JS, Reddy KR, Gralla J, et al. Neutrophil-to-lymphocyte ratio associates independently with mortality in hospitalized patients with cirrhosis. *Clin Gastroenterol Hepatol.* (2018) 16:1786.e1–91.e1. doi: 10.1016/j.cgh.2018.04.045
29. Matsuki T, Okamoto I, Fushimi C, Sawabe M, Kawakita D, Sato H, et al. Hematological predictive markers for recurrent or metastatic squamous cell carcinomas of the head and neck treated with nivolumab: a multicenter study of 88 patients. *Cancer Med.* (2020) 9:5015–24. doi: 10.1002/cam4.3124
30. Liu F, Zhou S, Tan L, Jiang H, Huang Y. A retrospective cohort study on pretreated neutrophil-to-lymphocyte ratio and prognosis of small cell lung cancer: evidence of effect modification by chemotherapy regimen. *Cancer Manage Res.* (2020) 12:10341–52. doi: 10.2147/CMAR.S263863
31. Biyik M, Ucar R, Solak Y, Gungor G, Polat I, Gaipov A, et al. Blood neutrophil-to-lymphocyte ratio independently predicts survival in patients with liver cirrhosis. *Eur J Gastroenterol Hepatol.* (2013) 25:435–41. doi: 10.1097/MEG.0b013e32835c2af3
32. Leithead JA, Rajoriya N, Gunson BK, Ferguson JW. Neutrophil-to-lymphocyte ratio predicts mortality in patients listed for liver transplantation. *Liver Int.* (2015) 35:502–9. doi: 10.1111/liv.12688
33. Chua ML, Tan SH, Kusumawidjaja G, Shwe MT, Cheah SL, Fong KW, et al.Neutrophil-to-lymphocyte ratio as a prognostic marker in locally advanced nasopharyngeal carcinoma: a pooled analysis of two randomised controlled trials. *Eur J Cancer.* (2016) 67:119–29. doi: 10.1016/j.ejca.2016.08.006
34. Said A, Williams J, Holden J, Remington P, Gangnon R, Musat A, et al. Model for end stage liver disease score predicts mortality across a broad spectrum of liver disease. *J Hepatol.* (2004) 40:897–903. doi: 10.1016/j.jhep.2004.02.010
35. Wiesner R, Edwards E, Freeman R, Harper A, Kim R, Kamath P, et al. Model for end-stage liver disease (MELD) and allocation of donor livers. *Gastroenterology.* (2003) 124:91–6. doi: 10.1053/gast.2003.50016
36. Biggins SW, Bambha K. MELD-based liver allocation: who is underserved? *Semin Liver Dis.* (2006) 26:211–20. doi: 10.1055/s-2006-947291

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 08 September 2021
doi: 10.3389/fmed.2021.729030

Testosterone and Estradiol as Novel Prognostic Indicators for HBV-Related Acute-on-Chronic Liver Failure

*Shuning Sun, Baoyan Xu, Wenting Tan, Xiaomei Xiang, Yi Zhou, Yunjie Dan, Yanzhi Guo, Zhaoxia Tan and Guohong Deng**

Department of Infectious Diseases, Southwest Hospital, Third Military Medical University (Amy Medical University), Chongqing, China

Background: HBV-related acute-on-chronic liver failure (HBV-ACLF) has a high short-term mortality and urgently needs an early warning system with simplicity and high accuracy. Previous studies show that sex hormones play potential roles in the progression of HBV-related liver diseases.

Aims: To explore the effect of testosterone and estradiol on the occurrence and prognosis of HBV-ACLF.

Methods: A prospective cohort of 300 chronic hepatitis B (CHB) patients was enrolled among which 108 were diagnosed with HBV-ACLF at admission and 20 developed to HBV-ACLF during hospitalization. We compared the level of serum testosterone and estradiol of patients with varied ACLF background, disease severity and cirrhosis conditions and analyzed the predictive ability of short-term prognosis. A novel prognostic model involving testosterone was developed and further validated in the HBV-ACLF group.

Results: The baseline estradiol level of HBV-ACLF group was significantly higher while testosterone was lower than that of non-ACLF group. The estradiol level increased while the testosterone level decreased as the number of organ failures increased. Testosterone had high accuracy in predicting the short-term mortality in HBV-ACLF (AUROC = 0.726) and estradiol did better in predicting the occurrence of ACLF during hospitalization (AUROC = 0.695). The novel prognostic model involving testosterone (TATIM model) was proved to have considerable prediction efficiency in HBV-ACLF cohort with or without cirrhosis.

Conclusion: Testosterone could be utilized as short-term prognostic indicator for HBV-related ACLF and estradiol can help to predict its occurrence. TATIM model is a novel prognostic model for HBV-related ACLF with simplicity and good performance irrespectively of liver cirrhosis.

Clinical Trial Registration Number: This study was based on a sub-cohort from the prospective multicenter cohort (NCT02457637).

Keywords: ACLF (acute-on-chronic liver failure), hepatitis B, mortality, prognosis, testosterone (androgen), estradiol

OPEN ACCESS

Edited by:
Yu-Chen Fan,
Shandong University, China

Reviewed by:
Yanan Du,
Shanghai Jiao Tong University, China
Jia Lin,
Capital Medical University, China

****Correspondence:***
Guohong Deng
gh_deng@hotmail.com

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *22 June 2021*
Accepted: *16 August 2021*
Published: *08 September 2021*

Citation:
Sun S, Xu B, Tan W, Xiang X, Zhou Y, Dan Y, Guo Y, Tan Z and Deng G (2021) Testosterone and Estradiol as Novel Prognostic Indicators for HBV-Related Acute-on-Chronic Liver Failure. Front. Med. 8:729030. doi: 10.3389/fmed.2021.729030

LAY SUMMARY

Sexual disparity exists in acute-on-chronic liver failure and the level of sexual hormones has significant difference between patients with and without liver failure. Testosterone and estradiol could be utilized as novel prognostic indicators irrespective of liver cirrhosis.

INTRODUCTION

Acute-on-chronic liver failure (ACLF) is a rapid-developing clinical syndrome with a high short-term mortality (1). Hepatitis B virus ranks the first in the etiology of ACLF in East Asia and other high-HBV-prevalence regions (2, 3). HBV-related ACLF leads to approximately 120 000 deaths every year and takes up massive medical resources. Even though it has poor outcomes, timely and aggressive clinical intervention could save a considerable proportion of patients' lives (4, 5). Therefore, early warning of ACLF is necessary and urgently needed.

Previous studies have shown that males are dominated in most chronic liver diseases and liver failure (6, 7). The level of sexual hormones has significant changes in the course of end-stage liver diseases (8, 9) and is associated with prognosis of cirrhosis (10), non-alcoholic fatty liver disease (NAFLD) (11) and hepatocellular carcinoma(HCC) (12). Further studies reveal that sexual hormones affect the HBV-related liver diseases via a comprehensive way including promoting or inhibiting the HBV replication (13), affecting the expression of sex hormone receptors or related transcription factors (14) and regulating the inflammatory or protective immune response (15–17). However, most of the existing studies focus on cirrhosis, NAFLD or HCC, it remains unclear whether sexual hormones are associated with HBV-ACLF.

As for early warning of liver failure, Child-Turcotte-Pugh score, Model for End-Stage Liver Disease (MELD) (18, 19), the integrated MELD (iMELD) and Sequential Organ Failure Assessment (SOFA) are the most common prognostic models for severe liver diseases but they lack the pertinence for ACLF. The Chronic Liver Failure Consortium ACLF score (CLIF-C ACLF) proposed by European Association for the Study of the Liver(EASL) in 2013 is proved to have superior predictive performance for short-term mortality in the Consortium Acute-On-Chronic Liver Failure in Cirrhosis (CANONIC) study (20). However, the major etiology of their cohort is differing from that of ACLF population in Asia-Pacific regions. The Chinese Group on the Study of Severe Hepatitis B (COSSH) proposed the COSSH-ACLF score in 2017, which have higher accuracy for predicting HBV-ACLF outcomes (3). Nevertheless, both CLIF-C ACLF and COSSH-C ACLF are based on complicated evaluation of organ failure and a simpler and more practicable scoring model is the most needed at present.

Based on the potential roles of sex hormone in HBV-related liver diseases and the urgent need of early-warning in HBV-related ACLF, we conducted this research to investigate whether testosterone and estradiol could be novel prognostic indicators for HBV-related ACLF. Firstly, we compared the level of serum testosterone and estradiol of patients with varied ACLF background, disease severity and cirrhosis conditions and then analyzed their predictive ability respectively. Finally, we used OPLS-DA and logistic regression to screen the candidate indicators and developed a testosterone-related prognostic model-TATIM, which was further validated and proved to be efficient.

Abbreviations: HBV, hepatitis B virus; ACLF, acute-on-chronic liver failure; CHB, chronic hepatitis B; AUROC, area under receiver operating characteristic curve; MELD, Model for End-Stage Liver Disease; iMELD, the integrated MELD; SOFA, Sequential Organ Failure Assessment; CLIF-C, Chronic Liver Failure Consortium; EASL, European Association for the Study of the Liver; CANONIC, Consortium Acute-On-Chronic Liver Failure in Cirrhosis; COSSH, Chinese Group on the Study of Severe Hepatitis B; NAFLD, non-alcoholic fatty liver disease; HCC, hepatocellular carcinoma; OPLS-DA, Orthogonal Partial Least Squares Discrimination Analysis; CLD, chronic liver disease; UGIB, upper gastrointestinal bleeding; HE, hepatic encephalopathy; HRS, hepatorenal syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBil, total bilirubin; PT, prothrombin activity; CRF, case report form; CT, computerized tomography; HBeAg, hepatitis B e antigen; E2, estradiol; TT, testosterone; INR, international normal ratio; VIF, variance inflation factor; TSH, thyroid-stimulating hormone; TRH, thyrotropin-releasing hormone; ALD, alcoholic liver disease; AIH, autoimmune hepatitis.

MATERIALS AND METHODS

Definitions

In this study, Chronic liver disease (CLD) included chronic hepatitis B (CHB), chronic hepatitis C, chronic hepatitis E, alcoholic liver disease, non-alcoholic steatohepatitis, chronic drug-induced liver disease, autoimmune liver disease, schistosomiasis, metabolic liver disease and cryptogenic liver disease. Events of acute decompensation included ascite, bacterial infection, upper gastrointestinal bleeding (UGIB), hepatic encephalopathy (HE) and hepatorenal syndrome (HRS). Acute liver injury was defined as alanine aminotransferase (ALT) >3 NL, aspartate aminotransferase (AST) >3 NL or total bilirubin (TBil) ≥ 2 NL.

The exclusion criteria included: (1) age>80 or <15; (2) with chronic extra-hepatic dysfunction; (3) hepatocellular carcinoma (HCC) or carcinoma found in other organs; (4) applying immunosuppressive agents; (5) pregnancy.

According to the APASL 2014 consensus (4) and the updated proposals (1, 21, 22), ACLF in this study was defined as acute hepatic insult manifesting as jaundice (total bilirubin [TBil] $\geq$5mg/dl) and coagulopathy (international normalized ratio [INR] $\geq$1.5), complicated by ascites and/or HE within 4 weeks in patients with previously diagnosed or undiagnosed CLD. The cirrhosis patients with or without a history of acute decompensation who met the above criteria were diagnosed as ACLF as well.

Patients

This study is based on a sub-cohort of prospective multicenter ACLF cohort [CATCH-LIFE: NCT02457637 (23, 24), NCT03641872]. A total of 348 patients with CLD were prospectively enrolled for acute decompensation or acute liver injury from January 1, 2015 to December 31, 2016 in Southwest Hospital (Chongqing, China). Data including demographic information, clinical procedures, laboratory tests, imaging tests,

hospitalization records at admission and follow-up information was collected by electronic case report form (CRF).

According to the etiological data, 300 patients were selected as CHB cohort, among which 108 patients were diagnosed with ACLF at admission while 192 were non-ACLF. Moreover, during hospitalization 20 patients progressed into ACLF while 172 did not until discharge. The flow chart of enrollment is shown in **Supplementary Figure 1**.

Sexual Hormone Testing

The baseline level of serum testosterone and estradiol were tested using Cobas Testosterone II kits and Cobas Estradiol III kits on the Roche Elecsys 2010 Electrochemical Luminescent immune Analyzer (Roche Diagnostics, Rotkreauz, Switzerland).

Model Calculation

The Child-Turcotte-Pugh (18), MELD, iMELD (19), SOFA, CLIF-C ACLF (20) and COSSH ACLF (3) scores were calculated using the baseline data. All the prognostic models were described in the references previously.

Statistical Analysis

In this study, statistical analysis was conducted using IBM SPSS Statistics (ver. 22.0; SPSS Inc.), MedCalc(ver.19.0; MedCal Software) and SIMCA(ver.14.1;Umetrics AB). Two-tailed P values < 0.05 were statistically significant.

Quantitative variables are presented as mean ± SD or median (IQR) and categorical variables are presented as percentage. Differences between groups were compared by Student's t test, Mann-Whitney U test or one-way ANOVA for continuous variables and χ 2-test for categorical variables. For candidate parameters, orthogonal partial least squares discriminant analysis (OPLS-DA) was conducted to compare their predictive ability of short-term prognosis in HBV-ACLF cohort (22, 25). Multivariate logistic regression was conducted to identify the independent risk factors of 28 day mortality using the backward LR method, with entry and removal probabilities of 0.05 and 0.10, respectively. The TATIM prognostic model was developed by multivariate logistic regression. The Kaplan-Meier cumulative survival curves were compared with log-rank test. The area under receiver operating characteristics curves (AUROCs) were used to evaluate the predictive utility for 28 day and 90 day mortality and DeLong's tests were utilized to compare the AUROCs of varied scoring systems.

Study Approval

This study fulfilled the principles of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of our institution. Written informed consent was obtained from all patients or their legal representatives.

RESULTS

Baseline Characteristics of Cohort

The baseline characteristics of the CHB cohort are summarized in **Table 1**. Data of the HBV-ACLF cohort ($N = 108$) and non-ACLF group ($N = 192$) are shown in **Table 2** and **Supplementary Table 1**, respectively.

Among patients with ACLF, 87.0% were males, 52.8% had previous liver cirrhosis (diagnosed by radiological evidence, CT images or clinical evidence) and 49.0% were HBeAg-positive. The top five precipitating events were bacterial infection (43.5%), HBV reactivation (13.9%), recent alcohol intake (8.3%), hepatotoxic drug (7.4%) and fatigue (7.4%). The average stay of ACLF and non-ACLF patients in hospital were 25.10 ± 18.07 days and 19.46 ± 11.78 days respectively. For these newly-developed ACLF patients, the median time from admission to ACLF is 4 days ranged from 4 days to 28 days.

Screening for the Best Candidate Prognostic Indicators

We performed univariate analysis, multivariate logistic regression and OPLS-DA to identify the best prognostic parameters for ACLF.

As shown in **Table 2**, the non-survivors within 28 days were significantly older than survivors and had higher ratio of bacterial infection (66.7 vs. 6.9%, $P = 0.009$) in terms of precipitating events. In regular laboratory tests, the non-survivors had significantly higher TBil and INR than survivors. In sexual hormone tests, the serum estradiol (E2) was significant higher in the non-survivor group, whereas survivor group had higher serum testosterone (TT) level.

Before the multivariate logistic regression, all the six factors above were taken into a linear regression analysis to disregarding the effect of multi-collinearity and all their variance inflation factors (VIF) were less than 10 with tolerance greater than 0.1. The multivariate logistic regression indicated that TT and INR were independent prognostic factors ($P < 0.05$, **Table 2**). Age, TBil and E2 were also suggested as potential indicator with their p values < 0.1.

For further exploration of candidate indicators, OPLS-DA was used to rank all their predictive ability. Three dimensional scatter plot showed that non-survivors were unambiguously distinguished from survivors (**Figure 1A**). Loading scatter plot (**Figure 1B**) evaluated the candidate variables and VIP plot (**Figure 1C**) ranked their importance of prediction. The top 12 parameters were ln (TT), ln (INR), HE, INR, TT, age, ln (age), TBil, Bacterial infection, ln (TBil), HBeAg positive, and ln (E2) (all VIPpred >1.0, **Figure 1C**).

Combing the suggestion of multivariate logistic regression and OPLS-DA, we finally selected TT, INR, age and TBil as the top four prognostic indicators.

Impact of Testosterone and Estradiol on ACLF

Given that both univariate analysis and OPLS-DA suggested TT and E2 had remarkable predictive ability for 28 day mortality in HBV-ACLF patients, we further investigated their impact on disease severity, 28 day cumulative survival rate, 90 day cumulative survival rate and cumulative occurrence rate of ACLF during hospitalization.

The baseline estradiol level of HBV-ACLF group was significantly higher while testosterone was lower than those of non-ACLF group (**Figures 2A,B**). As for subtypes of ACLF, there was no significant difference of sexual hormone level among type A (non-cirrhotic), type B (compensated cirrhotic)

TABLE 1 | Baseline characteristics of CHB cohort.

Variables	Total (n = 300)	ACLF (n = 108)	non-ACLF (n = 192)	p value
Clinical characteristics				
Age (year)	45.0 ± 12.0	46.18 ± 11.89	44.40 ± 11.99	0.217
Male, *n* (%)	255 (85.0)	94 (87.0)	161 (83.9)	0.459
HBV reactivation (%)	65 (21.7)	15 (13.9)	50 (26.0)	0.014
Alcohol intaking, *n* (%)	24 (8.0)	9 (8.3)	15 (7.8)	0.873
Heptoxic drug, *n* (%)	17(5.7)	8 (7.4)	9 (4.7)	0.328
Fatigue, *n* (%)	14 (4.7)	8 (7.4)	6 (3.1)	0.091
UGIB, *n* (%)	6(2.0)	3 (2.8)	3 (1.6)	0.470
Cirrhosis, *n* (%)	153 (51.0)	57 (52.8)	96 (50.0)	0.644
Ascite, *n* (%)	102 (34.0)	37 (34.3)	65 (33.9)	0.943
HE, *n* (%)	2(0.7)	2 (1.9)	0 (0)	0.059
HBeAg +, *n* (%)	147 (49.0)	43 (42.2)	104 (54.2)	0.455
Treatment data				
Average Stay time (days)	21.49 ± 14.59	25.10 ± 18.07	19.46 ± 11.78	<0.001
Telipressin therapy, *n* (%)	4 (1.3)	4 (3.7)	0 (0)	0.016
Artificial liver support, *n* (%)	31 (10.3)	23 (21.3)	8 (4.2)	<0.001
NH3 reduction therapy, *n* (%)	24 (8.0)	21 (19.4)	3 (1.6)	<0.001
Diuretics therapy, *n* (%)	85 (28.3)	32 (29.6)	53 (27.6)	0.709
Laboratory parameters				
WBC (10∧9/L)	5.8 ± 4.0	7.17 ± 5.19	5.08 ± 2.82	<0.001
Neutrophil (10∧9/L)	4.19 ± 4.05	5.36 ± 4.47	3.54 ± 3.65	<0.001
Leukomonocyte (10∧9/L)	0.56 ± 2.13	0.90 ± 3.48	0.38 ± 0.46	0.122
TBil (mg/dL)	10.91 ± 9.02	18.33 ± 7.94	6.72 ± 6.57	<0.001
ALT (IU/L)	558.83 ± 749.02	631.39 ± 835.16	518.02 ± 694.90	0.209
AST(IU/L)	419.34 ± 502.22	475.67 ± 584.46	387.95 ± 448.53	0.148
AKP(IU/L)	136.42 ± 52.49	141.12 ± 55.69	134.26 ± 50.97	0.322
Alb (g/L)	32.5 ± 8.9	30.38 ± 5.08	33.62 ± 10.32	0.003
INR	1.58 ± 0.58	2.18 ± 0.53	1.24 ± 0.23	<0.001
Sodium (mmol/L)	137.07 ± 8.90	135.99 ± 6.16	137.67 ± 10.08	0.119
Kalium (mmol/L)	4.02 ± 0.57	4.06 ± 0.62	4.72 ± 9.81	0.419
Creatine (mg/dL)	0.80 ± 0.44	0.84 ± 0.57	0.77 ± 0.34	0.303
Log HBV-DNA(IU/ml)	4.14 ± 2.75	3.94 ± 2.76	4.26 ± 2.75	0.348
Sexual hormones				
E2 (pg/ml)	98.81 ± 82.11	144.22 ± 76.82	73.15 ± 73.60	<0.001
TT (ng/ml)	3.33 ± 3.31	1.59 ± 1.69	4.32 ± 3.58	<0.001
Scoring systems				
Child-Pugh	8.86 ± 1.92	10.51 ± 0.98	7.93 ± 1.69	<0.001
MELD	14.33 ± 7.76	21.30 ± 5.91	10.27 ± 5.45	<0.001
iMELD	2.80 ± 0.96	3.59 ± 0.86	2.35 ± 0.69	<0.001
SOFA	4.90 ± 2.26	7.22 ± 0.98	3.58 ± 1.63	<0.001
CLIF-C ACLF	32.22 ± 8.92	38.38 ± 8.49	28.76 ± 7.12	<0.001
COSSH ACLF	5.08 ± 0.97	5.99 ± 0.88	4.55 ± 0.52	<0.001

AKP, phosphatase alkaline; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLIF-C ACLF, European Association for the Study of Chronic Liver Failure; COSSH ACLF, ACLF model of Chinese Group on the Study of Severe Hepatitis B; E2,estradiol; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HE, hepatic encephalopathy; INR, international normalized ratio; MELD, Model for End-stage Liver Disease; SOFA, sequential organ failure assessment; TT, testosterone; UGIB, upper gastrointestinal hemorrhage; WBC, white blood count.

and type C (decompensated cirrhotic) (**Figures 2E,F**). The E2 level at admission increased while the TT level decreased as the number of organ failures increased from 0 to 2 (**Figures 2C,D**). The E2 level was positively correlated with MELD, SOFA and CLIF-C ACLF scores (r = 0.569, 0.593 and 0.484, all $P < 0.001$, **Figure 2G**) while the TT level was only negatively correlated with CLIF-C ACLF score (r = −0.3613, $P < 0.001$, **Figure 2H**).

The AUROC of E2 for 28-day mortality was 0.664 (**Figure 3A**), with a sensitivity of 0.773 and specificity of

TABLE 2 | Analysis of baseline data of HBV-ACLF cohort.

Variables	Total (n = 108)	28day Non-survivors (n = 24)	28day Survivors (n = 84)	Univariate analysis		Multivariate analysis	
				OR (95% CI)	p value	OR (95% CI)	p value
Clinical characteristics							
Age (year)	46.18 ± 11.89	52.17 ± 10.81	44.46 ± 11.68	1.060 (1.016–1.105)	0.005	12.48 (0.932–166.9)	0.068
Male, *n* (%)	94 (87.0)	23 (95.8)	71 (84.5)	4.211 (0.522–33.966)	0.146		
Bacteria infection, *n* (%)	47 (43.5)	16 (66.7)	31 (6.9)	3.419 (1.313–8.907)	0.009	2.165 (0.722–6.491)	0.130
HBV reactivation, *n* (%)	15 (13.9)	2 (8.3)	13 (15.5)	0.497 (0.104–2.371)	0.372		
Alcohol intaking, *n* (%)	9 (8.3)	1 (4.2)	8 (8.5)	0.413 (0.049–3.478)	0.680		
Heptoxic drug, *n* (%)	8 (7.4)	3 (12.5)	5 (6.0)	2.257 (0.499–10.218)	0.280		
Fatigue, *n* (%)	8 (7.4)	2 (8.3)	6 (7.1)	1.182 (0.223–6.270)	0.844		
UGIB, *n* (%)	3 (2.8)	0 (0)	3 (3.6)		1.000		
Cirrhosis, *n* (%)	57 (52.8)	13 (54.2)	44 (52.4)	1.074 (0.432–2.669)	0.877		
Ascite, *n* (%)	37 (34.3)	5 (20.8)	32 (38.1)	0.428 (0.145–1.258)	0.116		
HE, *n* (%)	2 (1.9)	2 (1.9)	0 (0)		0.048		
HBeAg +, *n* (%)	43 (42.2)	7 (29.2)	36 (46.2)	2.091 (0.244–17.886)	0.492		
Treatment characteristics							
Telipressin therapy, *n* (%)	4 (3.7)	3 (12.5)	1 (1.2)		0.034		
Artificial liver support, *n* (%)	23 (21.3)	6 (25.0)	17 (20.2)		0.615		
NH3 reduction therapy, *n* (%)	21 (19.4)	10 (41.7)	11 (13.1)		0.002		
Diuretics therapy, *n* (%)	32 (29.6)	5 (20.8)	27 (32.1)		0.285		
Laboratory parameters							
WBC (10∧9/L)	7.17 ± 5.19	8.06 ± 3.76	6.92 ± 5.52	1.037 (0.959–1.121)	0.346		
Neutrophil (10∧9/L)	5.36 ± 4.47	6.06 ± 3.44	5.16 ± 4.73	1.040 (0.950–1.139)	0.383		
Leukomonocyte (10∧9/L)	0.90 ± 3.48	0.72 ± 0.87	0.95 ± 3.93	0.975 (0.820–1.160)	0.774		
TBil (mg/dL)	18.33 ± 7.94	22.78 ± 8.47	17.06 ± 7.34	1.099 (1.033–1.170)	0.002	3.105 (0.545–17.70)	0.091
ALT (IU/L)	631.39 ± 835.16	466.16 ± 711.37	678.60 ± 865.33	1.000 (0.999–1.000)	0.274		
AST (IU/L)	475.67 ± 584.46	427.74 ± 587.11	488.79 ± 586.57	1.000 (0.999–1.001)	0.659		
AKP (IU/L)	141.12 ± 55.69	118.75 ± 46.50	146.39 ± 56.66	0.989 (0.977–1.001)	0.074		
Alb (g/L)	30.38 ± 5.08	28.73 ± 5.53	30.86 ± 4.87	0.918 (0.837–1.008)	0.070		
INR	2.18 ± 0.53	2.38 ± 0.46	2.12 ± 0.54	2.444 (1.053–5.672)	0.033	8.791 (0.706–109.4)	0.023
Sodium (mmol/L)	135.99 ± 6.16	133.61 ± 9.20	136.68 ± 4.78	0.927 (0.861–0.998)	0.127		
Kalium (mmol/L)	4.06 ± 0.62	4.15 ± 0.77	4.03 ± 0.57	0.987 (0.918–1.061)	0.160		
Creatine (mg/dL)	0.84 ± 0.57	1.12 ± 0.93	0.76 ± 0.38	2.695 (1.110–+6.543)	0.077		
Log HBV-DNA (IU/ml)	3.94 ± 2.76	3.82 ± 2.88	3.97 ± 2.75	0.981 (0.824–1.169)	0.833		
Sexual hormones							
E2 (pg/ml)	144.22 ± 76.82	182.08 ± 99.21	134.06 ± 66.78	1.007 (1.001–1.013)	0.041	1.188 (0.260–5.435)	0.098
TT (ng/ml)	1.59 ± 1.69	0.80 ± 0.51	1.80 ± 1.83	0.317 (0.134–0.751)	<0.001	0.492 (0.214–1.133)	0.004

(Continued)

TABLE 2 | Continued

Variables	Total (n = 108)	28day Non-survivors (n = 24)	28day Survivors (n = 84)	Univariate analysis		Multivariate analysis	
				OR (95% CI)	p value	OR (95% CI)	p value
Prognostic scoring systems							
Child-Pugh	10.51 ± 0.98	10.71 ± 0.91	10.45 ± 1.00	1.313 (0.817–2.108)	0.262		
MELD	21.30 ± 5.91	24.29 ± 7.48	20.43 ± 5.10	1.129 (1.034–1.233)	0.004		
iMELD	3.59 ± 0.86	4.24 ± 1.04	3.41 ± 0.71	3.929 (1.921–8.035)	<0.001		
SOFA	7.22 ± 0.98	7.96 ± 1.20	7.01 ± 0.80	2.933 (1.633–5.265)	<0.001		
CLIF-C ACLF	38.38 ± 8.49	44.71 ± 5.61	36.57 ± 8.32	1.193 (1.090–1.305)	<0.001		
COSSH-ACLF	5.99 ± 0.88	6.83 ± 0.82	5.77 ± 0.78	4.615 (2.326–9.158)	<0.001		

AKP, phosphatase alkaline; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLIF-C ACLF, European Association for the Study of Chronic Liver Failure; COSSH ACLF, ACLF model of Chinese Group on the Study of Severe Hepatitis B; E2,estradiol; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HE, hepatic encephalopathy; INR, international normalized ratio; MELD, Model for End-stage Liver Disease; SOFA, sequential organ failure assessment; TT, testosterone; UGIB, upper gastrointestinal hemorrhage; WBC, white blood count.

0.537 at a cut-off value of 126.4 pg/ml. The AUROC of TT for 28-day mortality was 0.726 (**Figure 3D**), with a sensitivity of 0.864 and specificity of 0.586 at cut-off value of 1.16 ng/ml.

According to their cut-off values, patients were allocated into two series: higher E2 group vs. standard E2 group and lower TT group vs. standard TT group. The 28d and 90d cumulative survival rate of the higher E2 group was significantly lower than those of standard E2 group, respectively. ($P = 0.023$, $P = 0.009$ by log-rank test, **Figures 3B,C**) Also, the 28d and 90d cumulative survival rate of the lower TT group was significantly lower than those of standard TT group, respectively. ($P < 0.001$, $P < 0.001$ by log-rank test, **Figures 3E,F**).

We conducted ROC analysis in ACLF subtype and found that the optimal TT cut-off value for Type A and Type C was both 1.16 ng/ml (Type A: $n = 51$, AUROC = 0.732, $p = 0.009$, sensitivity = 0.80, specificity = 0.667; Type C: $n = 42$, AUROC = 0.658, $p = 0.097$, sensitivity = 0.90, specificity = 0.433),while it was 0.6 ng/ml for Type B (Type B: $n = 15$, AUROC = 0.885, $p = 0.003$, sensitivity = 1.00, specificity = 0.769).

We used the prospective data of 192 non-ACLF patients at admission to evaluate the impact of E2 and TT on occurrence rate of ACLF within hospitalization. Only E2 showed significant predictive ability for occurrence of new ACLF. ($P = 0.002$, **Figure 3G**) The AUROC of E2 for predicting ACLF's occurrence within 28 days was 0.695, with a sensitivity of 0.684 and specificity of 0.667 at a cut-off value of 69.79 pg/ml. According to the cut-off value, the patients of non-ACLF at admission were allocated into higher E2 group and standard E2 group. The 28d cumulative ACLF occurrence rate of the higher E2 group was significantly higher than that of standard E2 group. ($P = 0.0032$ by log-rank test, **Figure 3H**).

Considering the definition of sub-type is derived from ACLF patients and the sample size of newly-developed ACLF is small ($n = 20$), we did not conduct E2's ROC analysis in sub-groups.

TATIM Model's Establishment and Validations

Based on the top four prognostic indicators (TT, INR, age and TBil) and Gender (male =1, female = 0), we established a novel prognostic model by multivariate logistic regression:

TATIM score = 0.056*TBil + 0.057*Age-1.148*TT + 0.742*INR + 1.963*Male-7.378. The TATIM score of non-survivors was significantly higher than that of survivors (−0.428 ± 1.146 vs. −2.504 ± 1.936, $P < 0.001$, **Figure 4A**) and the TATIM score increased as the number of organ failures increased from 0 to 2 (**Figure 4B**).

The TATIM score had positive correlation with the Child-Pugh, MELD, iMELD, SOFA, CLIF-C ACLF and COSSH ACLF scores (r = 0.3022, 0.4473, 0.5424, 0.5198, 7087 and 0.7470, respectively; all P < 0.05; **Figures 4C–H**).

The AUROC of TATIM score for 28-day mortality was 0.828 ($p < 0,001$), with a sensitivity of 0.955 and a specificity of 0.610 at an optimal cut-off value of −1.855. The TATIM score showed higher predictive power for 28 day mortality in HBV-ACLF patients than the MELD, iMELD and SOFA, (AUROC = 0.661, 0.725 and 0.714 respectively) and comparable predictive power

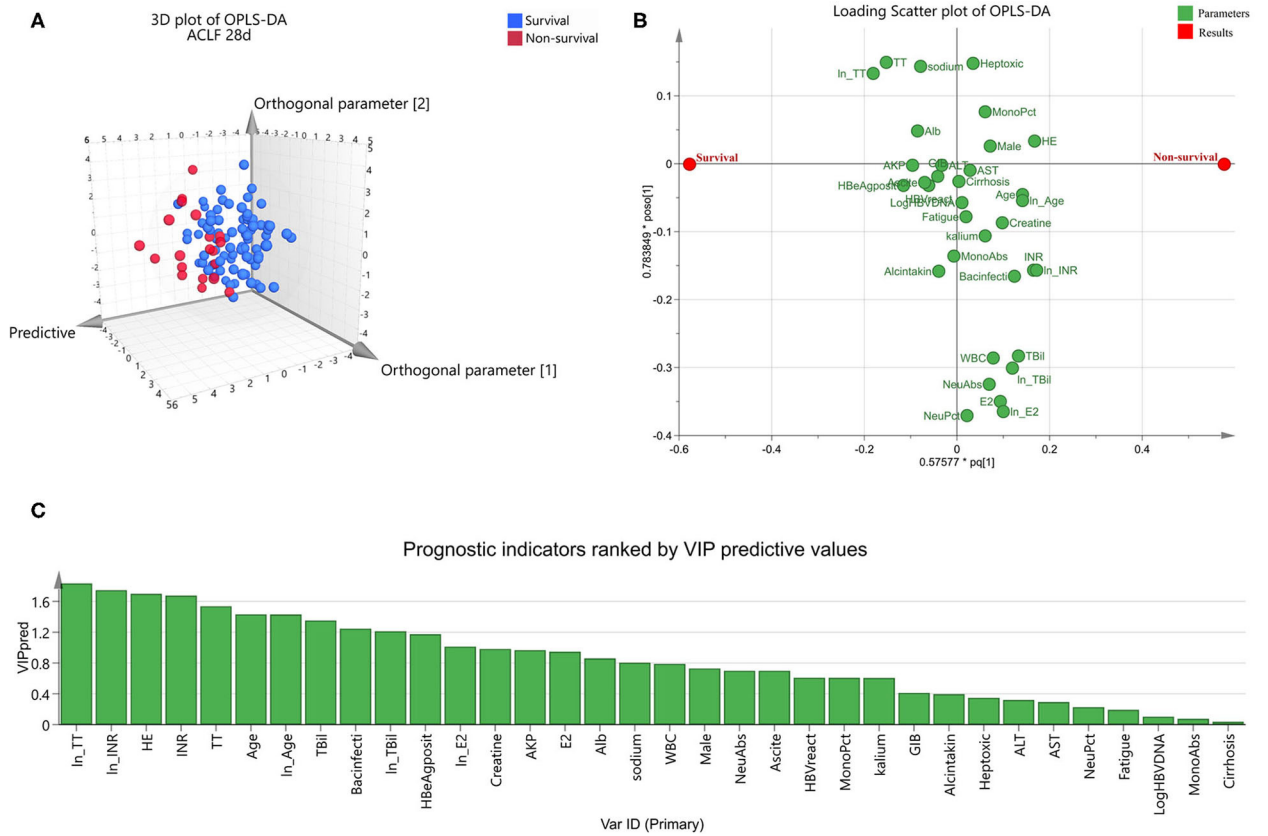


FIGURE 1 | Orthogonal partial least squares discriminant analysis to identify optimal short-term prognostic parameters for hepatitis-B-related acute-on-chronic liver failure. OPLS-DA was conducted in the ACLF group ($n = 108$). Three dimensional scatter plot **(A)** distinguished non-survivors from survivors. Loading scatter plot **(B)** was used to compare the impact of each parameters on survival. Values in prediction were ranked in VIP plot **(C)**. VIPpred > 1.0 was considered to have significant contributions to the outcome.

with CLIF-C ACLF and COSSH ACLF score. (AUROC = 0.797 and 0.796 respectively; **Figure 5A**) The sensitivity and specificity of CLIF-C ACLF were 1.00 and 0.506 while those of COSSH ACLF were 1.00 and 0.405. The detailed parameters of other models were listed in **Table 3**.

According to the optimal TATIM cut-off value patients were divided into high-TATIM group and low-TATIM group. The 28d and 90d cumulative survival rate of high-TATIM group were significantly lower than those of low-TATIM group, respectively ($P < 0.001$ by log-rank test, **Figures 5B,C**).

TATIM Score in ACLF Subtypes

Regardless of the ACLF subtypes, TATIM score had high prediction accuracy for the 28-day mortality. The AUROCs of type-A ($n = 51$), type-B ($n = 15$) and type-C ($n = 42$) were 0.829 ($p < 0.001$), 1.000 ($p < 0.001$) and 0.773 ($p < 0.001$), respectively (**Figures 6A–C**). The sensitivity and specificity of Type-A were 1.00 and 0.641 at cut-off value −1.855, those of Type-B were 1.00 and 1.00 at cut-off value −0.131 and those of Type-C were 1.00 and 0.467 at cut-off value −2.092. Compared with CLIF-C ACLF score, TATIM score performed better in predicting the 28-day outcomes for subtype-A ACLF (AUROC = 0.829 vs. 0.768) and subtype-B ACLF (AUROC = 1.000 vs. 0.958). Compared with COSSH ACLF score, TATIM score performed better in predicting the 28-day outcomes for subtype-B ACLF (AUROC = 1.000 vs. 0.958) and subtype-C ACLF (AUROC = 0.773 vs. 0.700).

DISCUSSIONS

Early warning of ACLF is necessary and urgent. In clinical practice, not only the short-term mortality of ACLF patients, but also the disease progression of non-ACLF patients are supposed to be emphasized. For the patients who are diagnosed with ACLF at admission, mortality is deserved to be focused on, which guide medical staff to take aggressive and timely measures to save patients' lives. And for the baseline non-ACLF patients whose liver function have already been impaired, we need a timely judgment whether it will get worse or even develop into ACLF. This study explored in both of the two aspects and revealed the predictive ability of testosterone and estradiol respectively. Testosterone was a novel predictor of short-term mortality in HBV-ACLF irrespective of the ACLF subtypes, while estradiol did better in predicting the progression to HBV-ACLF.

A qualified prognostic model is supposed to meet three criteria. Firstly, it has a high accuracy, by which the short-term

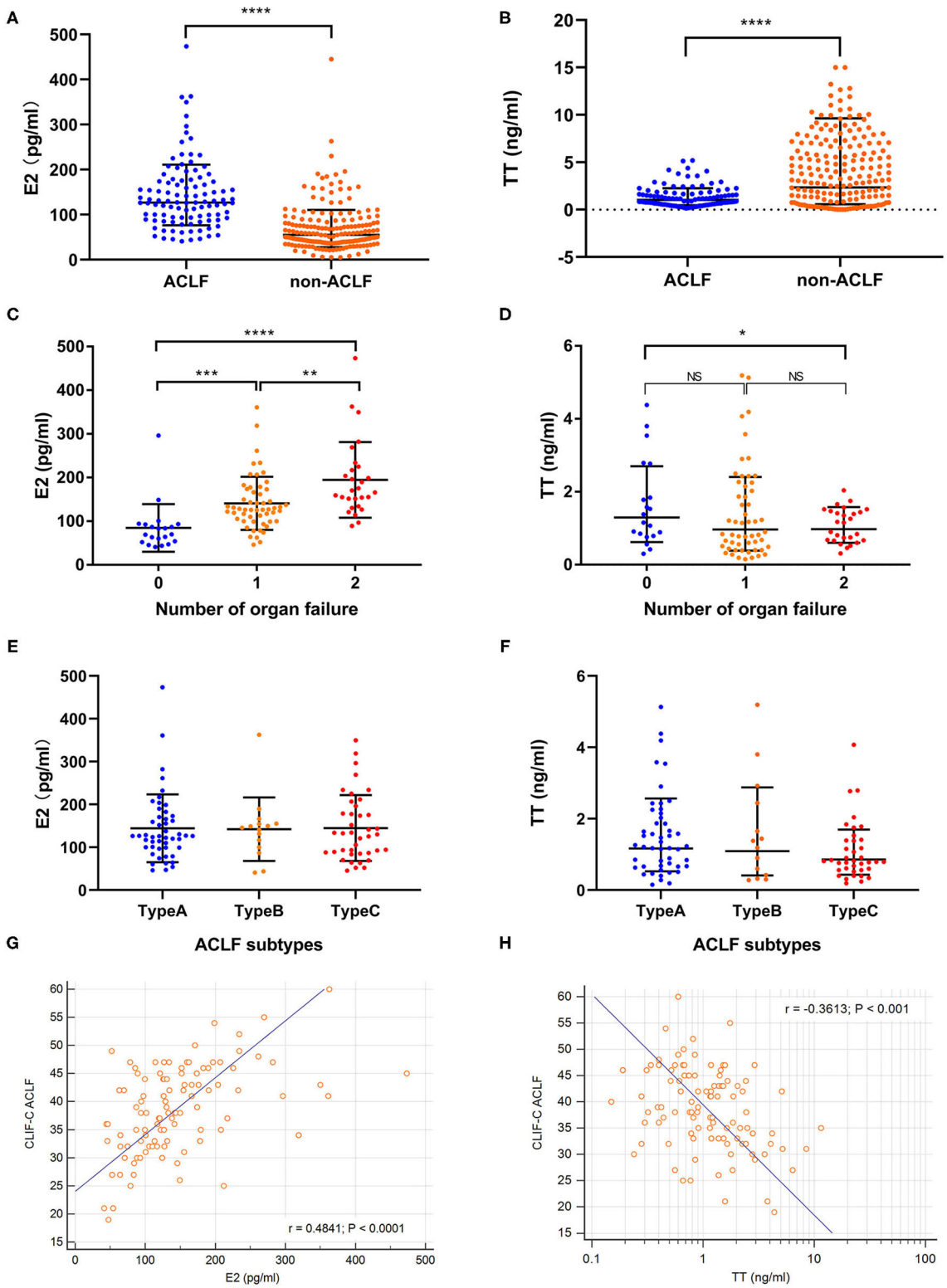


FIGURE 2 | Comparison of sexual hormone levels. Comparison of sexual hormone levels were conducted in CHB cohort ($n = 300$) and HBV-ACLF group ($n = 108$). **(A,B)** Showed the baseline estradiol and testosterone levels in hepatitis B related acute-on-chronic liver failure (HBV-ACLF, $n = 108$) and non-ACLF group ($n = 192$). **(C,D)** Revealed the changes of estradiol and testosterone according to the number of organ failures. **(E,F)** Suggested that there was no significant difference of estradiol and testosterone levels among ACLF subtypes. **(G)** Showed the linear correlation between estradiol levels and traditional prognostic scores. **(H)** Showed the linear correlation between testosterone levels and traditional prognostic scores. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

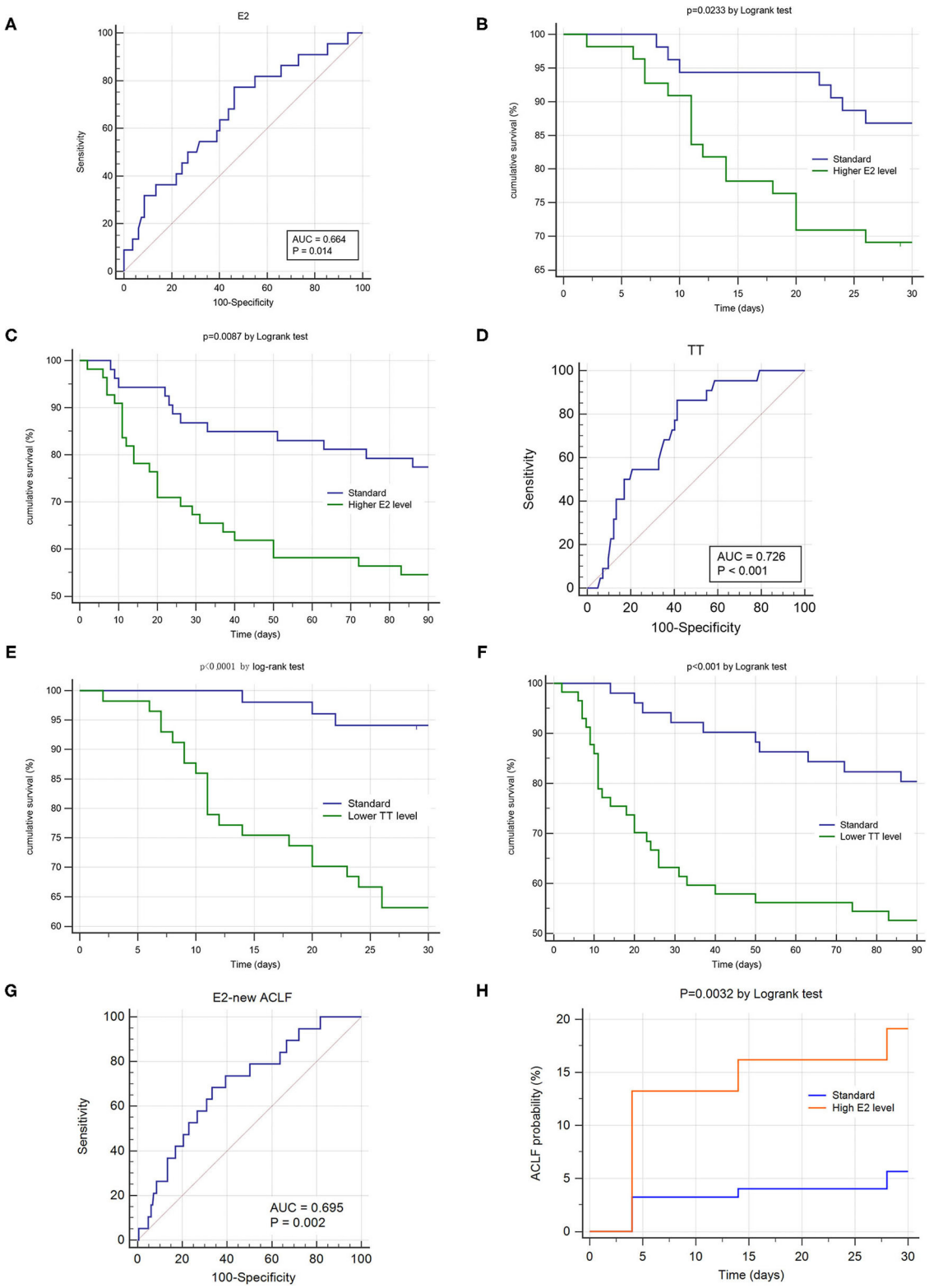


FIGURE 3 | Sexual hormone's impact on prognosis and occurrence of hepatitis B related acute-on-chronic liver failure (HBV-ACLF). ROC and K-M survival analysis were conducted in the ACLF group (n = 108, **A–F**) and non-ACLF group (n = 192, **G,H**). **(A,D)** Showed the area under receiver operating characteristic cure (AUROC) of estradiol and testosterone for 28 day survival. **(B,C)** Demonstrated the differences of 28 day and 90 day cumulative survival rates according to the estradiol levels (cut-off value = 126.6 pg/ml). **(E,F)** Demonstrated the differences of 28 day and 90 day cumulative survival rates according to the testosterone levels (cut-off value = 1.16 ng/ml). **(G)** Showed the area under receiver operating characteristic cure (AUROC) of estradiol for predicting ACLF's occurrence within 28 days. **(H)** Demonstrated the differences of 28 day cumulative ACLF occurrence rate according to the estradiol levels (cut-off value = 69.79 pg/ml).

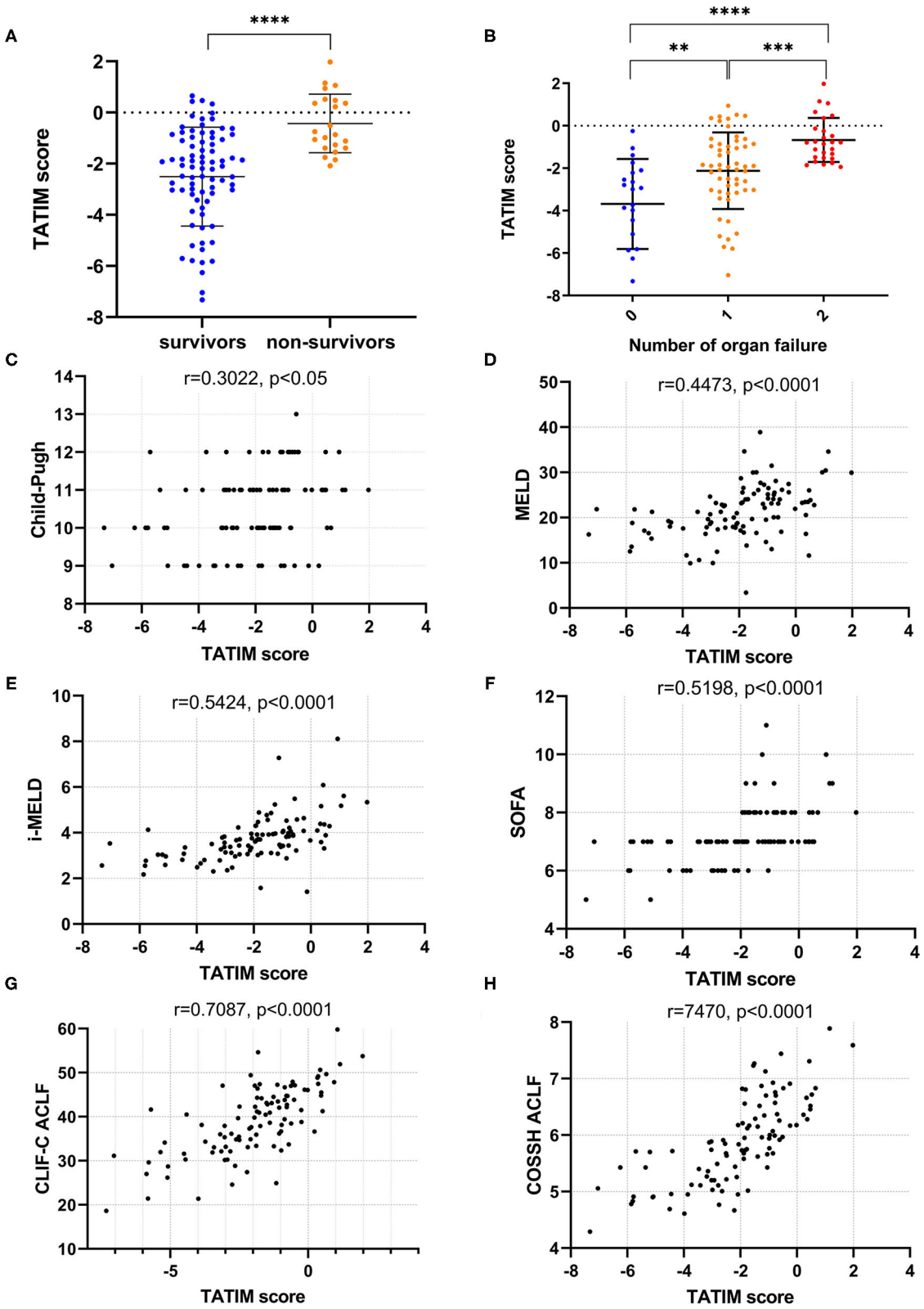


FIGURE 4 | TATIM model's correlation with disease severity. Correlation analysis was conducted in the ACLF group ($n = 108$). **(A)** Showed the TATIM scores in hepatitis B related acute-on-chronic liver failure (HBV-ACLF, $n = 108$) and non-ACLF group ($n = 192$). **(B)** Revealed the changes of TATIM scores according to the number of organ failures. **(C–H)** Showed the linear correlation between TATIM scores and previous prognostic scores. $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

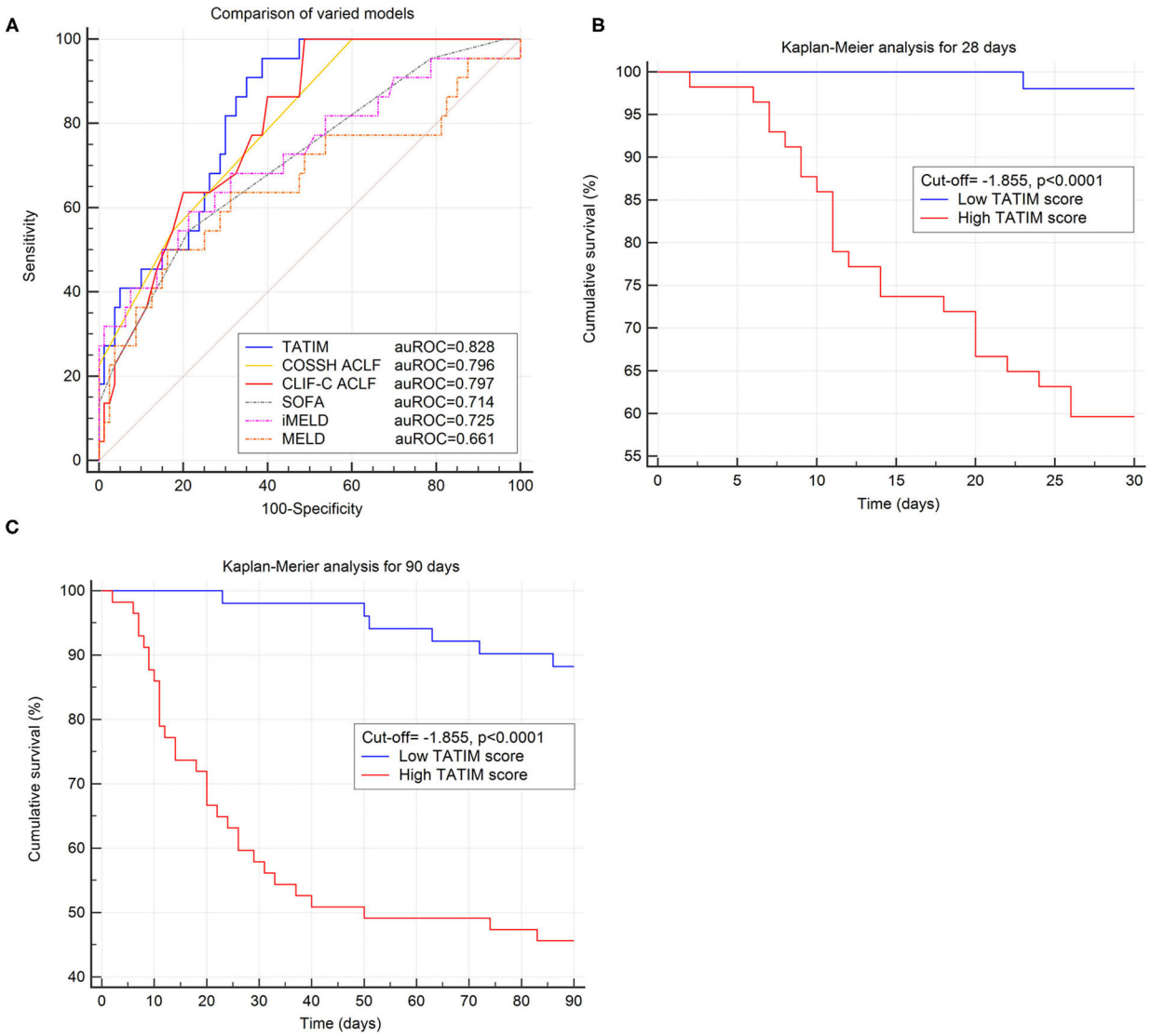


FIGURE 5 | Evaluation of TATIM model in predicting the short-term prognosis of ACLF. Comparison of predictive efficiency of varied prognostic models was conducted in the ACLF group (n = 108). **(A)** Compared the area under receiver operating characteristic cure (AUROC) of TATIM model and other prognostic scores for 28 day survival. The AUROC of TATIM was 0.828 ($p < 0.001$), which was higher than MELD, iMELD, SOFA, CLIF-SOFA and comparable with CLIF-C ACLF. **(B,C)** Demonstrated the differences of 28 day and 90 day cumulative survival rates according to the TATIM score (cut-off value = −1.855).

TABLE 3 | Comparison of predictive efficiency of varied prognostic models.

Models	28 day prediction						
	AUROC	**95%CI**	***Z* value**	***P* value**	**Sensitivity**	**Specificity**	**Cut-off**
TATIM	0.828	0.738–0.892	7.620	<0.001	95.5	60.1	−1.855
CLIF-C ACLF	0.797	0.706–0.870	6.441	<0.001	100.0	50.6	36
COSSH ACLF	0.796	0.705–0.869	7.293	<0.001	100.0	40.5	5
SOFA	0.729	0.635–0.811	4.091	<0.001	58.33	78.3	7
iMELD	0.725	0.628–0.809	4.113	<0.001	62.5	79.8	4
MELD	0.661	0.561–0.752	2.645	<0.001	54.2	84.2	25
Child-Pugh	0.547	0.446–0.646	1.280	0.201	66.7	53.6	10

CLIF-C ACLF, ACLF model of European Association for the Study of Chronic Liver Failure; SOFA, chronic liver failure-sequential organ failure assessment; MELD, Model for End-stage Liver Disease; SOFA, sequential organ failure assessment; COSSH ACLF, ACLF model of Chinese Group on the Study of Severe Hepatitis B.

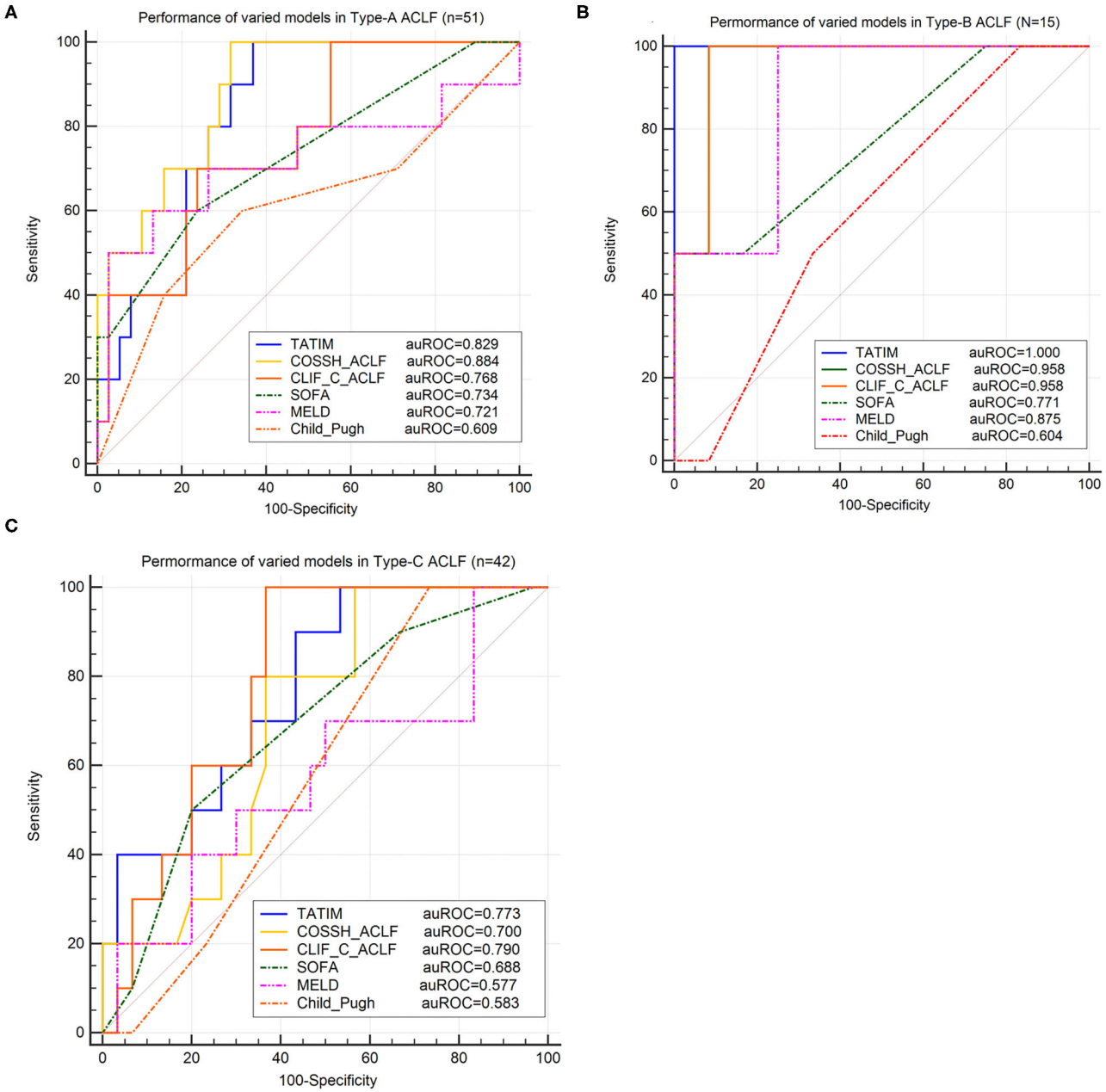


FIGURE 6 | Validation of TATIM model in ACLF-subtypes. ROC analysis was conducted in three ACLF-subtypes (Total $n = 108$, Type-A $n = 51$, Type-B $n = 15$ and Type-C $n = 42$). **(A–C)** Compared the area under receiver operating characteristic cure (AUROC) of TATIM model and other prognostic scores for predicting 28day survival in ACLF-subtype A, B and C, respectively. (AUROC of type-A, type-B and type-C were 0.829, 1.000 and 0.773, respectively; all $P < 0.001$).

outcome can be predicted with higher sensitivity and specificity. TATIM model developed in this study has a good performance and its AUROC in predicting 28d mortality is higher than that of Child-Pugh, MELD, iMELD and SOFA and is comparable or even higher than that of CLIF-C ACLF and COSSH ACLF scores. Secondly, it is simple and practical. It is best to maximize the prediction with the least number of parameters and operations. TATIM model is simple, consisting of age, gender, TBil, INR and testosterone. This is in sharp contrast with Child-Pugh, SOFA, CLIF-SOFA, CLIF-C ACLF, COSSH-ACLF and others, which is involved in complex multi-system scoring. Thirdly, it has a wide suitability. Child-Pugh, MELD, iMELD, SOFA, CLIF-SOFA and CLIF-C ACLF were developed in the western cohort where FLD and ALD were dominated. TATIM model was designed in HBV-ACLF cohort and had good predicting performance in ACLF subtype A, B and C. The

TATIM model fulfilled all the three requirements and had a promising prospect.

As for simple prognostic models, HINT model proposed in 2018 and P5 model proposed in 2020 are novel and efficient in predicting the short-term mortality of HBV-ACLF. HINT introduces thyroid-stimulating hormone (TSH) into the prognostic model for the first time. However, the reduction of TSH is a universal manifestation in patients with severe diseases (22) and the evidence of TSH's specific effect on ACLF is rare. Relevant studies suggested that the reduced thyrotropin -releasing hormone (TRH) may be a compensator response to the inflammatory cytokine induced by oxidative stress (26, 27), which is crucial in the pathogenesis of HBV-ACLF. The plasminogen mentioned in P5 score is another parameter involved in coagulation systems (25), which overlaps with INR to some extent. Likewise, it remains unclear how plasminogen interact with ACLF. The only evidence shows that plasminogen activation by the plasmin-α2-antiplasmin system plays a potential role in hepatocyte regeneration after damage (28) and decreased plasminogen levels would hamper liver's self-repairing capability (29). In spite of their high prediction accuracy in statistics, the mechanism underlying TSH and plasminogen remains further investigation.

As typical androgen and estrogen, testosterone and estradiol involving in this study have drawn extensive attention. Numerous studies show that gender disparity exist in many liver diseases such as NAFLD, alcoholic liver disease (ALD), CHB, autoimmune hepatitis (AIH) and HCC. In 1985, Nagasue observed serum levels of testosterone and estrogens in cirrhotic men with or without HCC and found the estrogen-testosterone ratio was significantly higher in HCC group (6), which raised the research trend of estrogen-carcinogenesis mechanism in liver. Grossman's research firstly showed the low testosterone levels were associated with poor prognosis in chronic liver diseases in 2012 (10) and 2016 (9) and Klair revealed that estrogen deficiency could increase fibrosis risk among postmenopausal women with NAFLD in 2016 (30). As for the underlying mechanism, Lee et al. and Kim et al. clarified that estrogen and estrogen-related receptor γ(ERRγ) affected the progression of ALD respectively via hepatic IκB (31) and CB1R-CYP2E1-mediated oxidative stress (15). Li et al. proved that Foxa1/a2 played essential roles in sexual dimorphism of HCC in 2012 (32) and Zhang et al. suggested that overexpression of nuclear androgen receptor driven by PI3K-AKT- mTOR pathway was associated with progression and prognosis of HCC in 2018 (33). For HBV-related HCC, additional evidence was found by Chiu et al. in 2007 and Yu et al. in 2014. Chiu et al. identified two conserved androgen response elements (ARE) with the enhancer I of HBV and suggested an AR-mediated stimulation of HBV transcription (13). Yu et al. found that cell cycle-related kinase (CCRK) mediated the HBx-AR signaling in viral-host oncogenic circuitry which induced hepatocellular proliferation and transformation (34). However, evidence of sex hormone's effects on HBV-related ACLF is rare and so is the research about mechanism.

It is the first time that the level of serum testosterone and estradiol were compared in patients with varied ACLF background, disease severity and cirrhosis conditions. Patients with severe clinical conditions tendered to have lower testosterone and higher estradiol levels irrespective of the history of liver cirrhosis or decompensation. Given the current understanding that sexual hormones are closely related with pro- and anti-inflammatory pathophysiological processes, further studies would spring up in the future.

This study had several limitations. Firstly, it lacks some longitudinal observations of sex hormone levels to evaluate the prognostic ability adequately. Secondly, multi-centered data are needed to make the new model more convincing. The specificity of TATIM model is 0.601, which is low and a verification data should be stronger and robust for the model. Thirdly, whether sex hormones can be prognostic predictor of ACLF caused by other etiologies still needs further investigations.

In conclusion, this study firstly highlighted the predictive ability of androgen and estrogen in the progression and prognosis of HBV-related ACLF. Moreover we developed a novel and simple prognostic model-TATIM, which had a high accuracy irrespective of the HBV-ACLF subtypes. We hope that the new findings will facilitate the clinical management of HBV-ACLF and enlighten researchers to explore the underlying mechanism.

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 Shanghai Jiaotong University School of Medicine, Renji Hospital Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GD and SS: study design. SS and BX: patient enrollment. SS, XX, WT, YZ, YD, and YG: data entry. SS and ZT: laboratory examination of samples. SS: statistical analysis and interpretation of data. SS and GD: drafting of the manuscript. GD: critical revision of the manuscript and study supervision. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported in part by the National Natural Science Foundation of China (81930061, 81900579), the Chinese State Key Project Specialized for Infectious Diseases (2018ZX10723203), the National Science and Technology Major Project (2018ZX10732202), and the

Chongqing Natural Science Foundation (CSTC2019jcyj-zdxmX0004).

ACKNOWLEDGMENTS

We thank all other members of our research group for their contributions to this study and assistance in daily life: Weiwei He, Juan Zhang, Haoliang Wang, Xing Wan, Fengming Sun, Xiuhua Wang, Rentao Yu, Wenying Wang, Jianmei Xiao and Ying Zhu.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.729030/full#supplementary-material

REFERENCES

1. Bernal W, Jalan R, Quaglia A, Simpson K, Wendon J, Burroughs A. Acute-on-chronic liver failure. *Lancet.* (2015) 386:1576–87. doi: 10.1016/S0140-6736(15)00309-8
2. Shi Y, Yang Y, Hu Y, Wu W, Yang Q, Zheng M, et al. Acute-on-chronic liver failure precipitated by hepatic injury is distinct from that precipitated by extrahepatic insults. *Hepatology.* (2015) 62:232–42. doi: 10.1002/hep.27795
3. Wu T, Li J, Shao L, Xin J, Jiang L, Zhou Q, et al. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure. *Gut.* (2018) 67:2181–91. doi: 10.1136/gutjnl-2017-314641
4. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int.* (2016) 10:1–98. doi: 10.1007/s12072-015-9675-4
5. Berry PA, Thomson SJ, Rahman TM, Ala A. Review article: towards a considered and ethical approach to organ support in critically-ill patients with cirrhosis. *Aliment Pharmacol Ther.* (2013) 37:174–82. doi: 10.1111/apt.12133
6. Nagasue N, Ogawa Y, Yukaya H, Ohta N, Ito A. Serum levels of estrogens and testosterone in cirrhotic men with and without hepatocellular carcinoma. *Gastroenterology.* (1985) 88:768–72. doi: 10.1016/0016-5085(85)90149-0
7. Porter LE, Elm MS, Van Thiel DH, Eagon PK. Hepatic estrogen receptor in human liver disease. *Gastroenterology.* (1987) 92:735–45. doi: 10.1016/0016-5085(87)90026-6
8. Tong S. Hepatitis B virus, a sex hormone-responsive virus. *Gastroenterology.* (2012) 142:696–9. doi: 10.1053/j.gastro.2012.02.036
9. Sinclair M, Grossmann M, Angus PW, Hoermann R, Hey P, Scodellaro T, et al. Low testosterone as a better predictor of mortality than sarcopenia in men with advanced liver disease. *J Gastroenterol Hepatol.* (2016) 31:661–7. doi: 10.1111/jgh.13182
10. Grossmann M, Hoermann R, Gani L, Chan I, Cheung A, Gow PJ Li A, et al. Low testosterone levels as an independent predictor of mortality in men with chronic liver disease. *Clin Endocrinol.* (2012) 77:323–8. doi: 10.1111/j.1365-2265.2012.04347.x
11. Zifroni A, Schiavi RC, Schaffner F. Sexual function and testosterone levels in men with nonalcoholic liver disease. *Hepatology (Baltimore, Md).* (1991) 14:479–82. doi: 10.1002/hep.1840140312
12. Ma WL, Hsu CL, Wu MH, Wu CT, Wu CC, Lai JJ, et al. Androgen receptor is a new potential therapeutic target for the treatment of hepatocellular carcinoma. *Gastroenterology.* (2008) 135:947–55, 955.e1-5. doi: 10.1053/j.gastro.2008.05.046
13. Chiu CM, Yeh SH, Chen PJ, Kuo TJ, Chang CJ, Chen PJ, et al. Hepatitis B virus X protein enhances androgen receptor-responsive gene expression depending on androgen level. *Proc Natl Acad Sci U S A.* (2007) 104:2571–8. doi: 10.1073/pnas.0609498104
14. Han SI, Komatsu Y, Murayama A, Steffensen KR, Nakagawa Y, Nakajima Y, et al. Estrogen receptor ligands ameliorate fatty liver through a nonclassical estrogen receptor/Liver X receptor pathway in mice. *Hepatology.* (2014) 59:1791–802. doi: 10.1002/hep.26951
15. Kim DK, Kim YH, Jang HH, Park J, Kim JR, Koh M, et al. Estrogen-related receptor γ controls hepatic CB1 receptor-mediated CYP2E1 expression and oxidative liver injury by alcohol. *Gut.* (2013) 62:1044–54. doi: 10.1136/gutjnl-2012-303347
16. Schwinge D, Carambia A, Quaas A, Krech T, Wegscheid C, Tiegs G, et al. Testosterone suppresses hepatic inflammation by the downregulation of IL-17, CXCL-9, and CXCL-10 in a mouse model of experimental acute cholangitis. *J Immunol.* (2015) 194:2522–30. doi: 10.4049/jimmunol.1400076
17. Kovats S. Estrogen receptors regulate innate immune cells and signaling pathways. *Cell Immunol.* (2015) 294:63–9. doi: 10.1016/j.cellimm.2015.01.018
18. Cholongitas E, Papatheodoridis GV, Vangeli M, Terreni N, Patch D, Burroughs AK. Systematic review: The model for end-stage liver disease–should it replace Child-Pugh's classification for assessing prognosis in cirrhosis? *Aliment Pharmacol Ther.* (2005) 22:1079–89. doi: 10.1111/j.1365-2036.2005.02691.x
19. Kamath PS, Kim WR. The model for end-stage liver disease (MELD). *Hepatology.* (2007) 45:797–805. doi: 10.1002/hep.21563
20. Jalan R, Saliba F, Pavesi M, Amoros A, Moreau R, Ginès P, et al. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. *J Hepatol.* (2014) 61:1038–47. doi: 10.1016/j.jhep.2014.06.012
21. Jalan R, Yurdaydin C, Bajaj JS, Acharya SK, Arroyo V, Lin HC, et al. Toward an improved definition of acute-on-chronic liver failure. *Gastroenterology.* (2014) 147:4–10. doi: 10.1053/j.gastro.2014.05.005
22. Wu D, Sun Z, Liu X, Rao Q, Chen W, Wang J, et al. HINT: a novel prognostic model for patients with hepatitis B virus-related acute-on-chronic liver failure. *Aliment Pharmacol Ther.* (2018) 48:750–60. doi: 10.1111/apt.14927
23. Gu WY, Xu BY, Zheng X, Chen J, Wang XB, Huang Y, et al. Acute-on-Chronic Liver Failure in China: Rationale for Developing a Patient Registry and Baseline Characteristics. *Am J Epidemiol.* (2018) 187:1829–39. doi: 10.1093/aje/kwy083
24. Li B, Gao Y, Wang X, Qian Z, Meng Z, Huang Y, et al. Clinical features and outcomes of bacterascites in cirrhotic patients: a retrospective, multicentre study. *Liver Int.* (2020) 40:1447–56. doi: 10.1111/liv.14418
25. Wu D, Zhang S, Xie Z, Chen E, Rao Q, Liu X, et al. Plasminogen as a prognostic biomarker for HBV-related acute-on-chronic liver failure. *J Clin Invest.* (2020) 130:2069–80. doi: 10.1172/JCI130197
26. Van den Berghe G. Non-thyroidal illness in the ICU: a syndrome with different faces. *Thyroid.* (2014) 24:1456–65. doi: 10.1089/thy.2014.0201
27. Warner MH, Beckett GJ. Mechanisms behind the non-thyroidal illness syndrome: an update. *J Endocrinol.* (2010) 205:1–13. doi: 10.1677/JOE-09-0412
28. Okada K, Ueshima S, Imano M, Kataoka K, Matsuo O. The regulation of liver regeneration by the plasmin/alpha 2-antiplasmin system. *J Hepatol.* (2004) 40:110–6. doi: 10.1016/j.jhep.2003.09.016
29. Shimizu M, Hara A, Okuno M, Matsuno H, Okada K, Ueshima S, et al. Mechanism of retarded liver regeneration in plasminogen activator-deficient mice: impaired activation of hepatocyte growth factor after Fas-mediated massive hepatic apoptosis. *Hepatology (Baltimore, Md).* (2001) 33:569–76. doi: 10.1053/jhep.2001.22650
30. Klair JS, Yang JD, Abdelmalek MF, Guy CD, Gill RM, Yates K, et al. A longer duration of estrogen deficiency increases fibrosis risk among postmenopausal women with nonalcoholic fatty liver disease. *Hepatology (Baltimore, Md).* (2016) 64:85–91. doi: 10.1002/hep.28514
31. Lee EG, Mickle-Kawar BM, Reinke LA, Gallucci RM. Estrogen suppresses heptatic IκB expression during short-term alcohol exposure. *Inflamm Res.* (2012) 61:1053–61. doi: 10.1007/s00011-012-0497-8
32. Li Z, Tuteja G, Schug J, Kaestner KH. Foxa1 and Foxa2 are essential for sexual dimorphism in liver cancer. *Cell.* (2012) 148:72–83. doi: 10.1016/j.cell.2011.11.026

33. Zhang H, Li XX, Yang Y, Zhang Y, Wang HY, Zheng XFS. Significance and mechanism of androgen receptor overexpression and androgen receptor/mechanistic target of rapamycin cross-talk in hepatocellular carcinoma. *Hepatology (Baltimore, Md).* (2018) 67:2271–86. doi: 10.1002/hep.29715
34. Yu Z, Gao YQ, Feng H, Lee YY Li MS, Tian Y, et al. Cell cycle-related kinase mediates viral-host signalling to promote hepatitis B virus-associated hepatocarcinogenesis. *Gut.* (2014) 63:1793–804. doi: 10.1136/gutjnl-2013-305584

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 15 October 2021
doi: 10.3389/fmed.2021.750061

Acute-On-Chronic Liver Failure Defined by Asian Pacific Association for the Study of the Liver Should Include Decompensated Cirrhosis

Manman Xu[1,2†], Ming Kong[1,2†], Pengfei Yu[1,2], Yingying Cao[3], Fang Liu[3], Bing Zhu[4], Yizhi Zhang[1,2], Wang Lu[1,2], Huaibin Zou[1,2], Shaoli You[4], Shaojie Xin[4], Zhongping Duan[1,2], Tao Han[3] and Yu Chen[1,2*]*

[1] Fourth Department of Liver Disease (Difficult & Complicated Liver Diseases and Artificial Liver Center), Beijing You'an Hospital Affiliated to Capital Medical University, Beijing, China, [2] Beijing Municipal Key Laboratory of Liver Failure and Artificial Liver Treatment Research, Beijing, China, [3] Department of Hepatology, Third Central Hospital of Tianjin, Tianjin, China, [4] Liver Failure Treatment and Research Center, The Fifth Medical Center of Chinese People's Liberation Army (PLA) General Hospital, Beijing, China

OPEN ACCESS

Edited by:
Tao Chen,
Huazhong University of Science and Technology, China

Reviewed by:
Nazri Mustaffa,
Universiti Sains Malaysia (USM), Malaysia
Yu Shi,
Zhejiang University, China

***Correspondence:**
Yu Chen
chybeyond1071@ccmu.edu.cn
Tao Han
hantaomd@126.com

[†] These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 30 July 2021
Accepted: 16 September 2021
Published: 15 October 2021

Citation:
Xu M, Kong M, Yu P, Cao Y, Liu F, Zhu B, Zhang Y, Lu W, Zou H, You S, Xin S, Duan Z, Han T and Chen Y (2021) Acute-On-Chronic Liver Failure Defined by Asian Pacific Association for the Study of the Liver Should Include Decompensated Cirrhosis. Front. Med. 8:750061. doi: 10.3389/fmed.2021.750061

Background and Aims: Acute-on-chronic liver failure (ACLF) is an acute deterioration of chronic liver disease with high short-term mortality. The inclusion or exclusion of previously decompensated cirrhosis (DC) in the diagnostic criteria of ACLF defined by the Asian Pacific Association for the Study of the Liver (APASL-ACLF) has not been conclusive. We aimed to evaluate the prognostic impact of decompensated cirrhosis in ACLF.

Methods: We retrospectively collected a cohort of patients with a diagnosis of APASL-ACLF (with or without DC) hospitalized from 2012 to 2020 at three liver units in tertiary hospitals. Baseline characteristics and survival data at 28, 90, 180, 360, 540, and 720 days were collected.

Results: Of the patients assessed using APASL-ACLF criteria without the diagnostic indicator of chronic liver disease, 689 patients were diagnosed with ACLF, of whom 435 had no decompensated cirrhosis (non-DC-ACLF) and 254 had previously decompensated cirrhosis (DC-ACLF). The 28-, 90-, 180-, 360-, 540-, and 720-day mortality were 24.8, 42.9, 48.7, 57.3, 63.4, and 68.1%, respectively, in DC-ACLF patients, which were significantly higher than in non-DC-ACLF patients ($p < 0.05$). DC was independently associated with long-term (180/360/540/720 days) but not short-term (28/90 days) mortality in patients with ACLF. Age, total bilirubin, international normalized ratio, and hepatic encephalopathy were independent risk factors for short- and long-term mortality risk in ACLF patients ($p < 0.05$).

Conclusions: Patients with DC-ACLF have a higher mortality rate, especially long-term mortality, compared to non-DC-ACLF patients. Therefore, DC should be included in the diagnostic criteria of APASL-ACLF and treated according to the ACLF management process.

Keywords: Asian Pacific Association for the Study of the Liver (APASL), acute-on-chronic liver failure (ACLF), diagnostic indicator, mortality, decompensated cirrhosis

INTRODUCTION

Acute-on-chronic liver failure (ACLF) is an acute deterioration of chronic liver disease (CLD) with high short-term mortality (1). The current widely accepted diagnostic criteria for ACLF is derived from the Asia-Pacific Association for the Study of the Liver (APASL) (2) and the European Association for the Study of the Liver (EASL) (3), which have different definitions of chronic liver disease in addition to differences in the indicators of organ dysfunction or failure. APASL-ACLF includes non-cirrhotic chronic liver disease but not decompensated cirrhosis as representing "chronic" whereas EASL-ACLF includes only cirrhosis, either compensated or decompensated to define the chronic liver disease.

Since the etiology of chronic liver disease in the Chronic Liver Failure (CLIF) ACLF in Cirrhosis (CANONIC) (3) study defining EASL-ACLF was mostly non Hepatitis B virus (HBV) infection, Chinese scholars (4) conducted a prospective multicenter study in which they proposed that patients with high short-term mortality in the non-cirrhotic HBV population should be diagnosed with ACLF in addition to developed diagnostic criteria for HBV-related ACLF. To further improve the definition of ACLF, the World Gastroenterology Organization (WGO) (5) suggests that ACLF may be divided into 3 categories: type-A for patients without cirrhosis, type-B for well-compensated cirrhosis, and type-C for previous decompensated cirrhosis (DC). The application value of WGO type in HBV-related ACLF patients diagnosed by EASL-ACLF diagnostic criteria has been preliminarily verified, which can distinguish the precipitating events, organ failure, and short-term prognosis (6). However, whether DC (type C) should be included in the APASL-ACLF diagnostic criteria has not been conclusive. It was proposed in the consensus recommendations of the APASL for ACLF that CLD should not include decompensated cirrhosis, but the evidence grade for this recommendation is C (Low or very low quality), stating that the opinion requires confirmation by further studies (7).

With this large, retrospective, and multi-center cohort, we aimed to compare the clinical characteristics and prognosis of patients with DC and non-DC-ACLF defined by APASL, to provide an evidence-based basis for whether DC should be included in the diagnostic criteria of APASL-ACLF.

METHODS

Study Design

We performed a retrospective cohort study using data from liver units in three university hospitals. Each liver unit had a regular ward, an intensive care unit, and a liver transplantation center. The same liver transplantation allocation policy was used at all study hospitals (8). Patients were screened from November 2012 to June 2019 in the Tianjin Third Central Hospital and the Fifth Medical Center of PLA General Hospital, and from January 2015 to June 2020 in Beijing You'an Hospital Affiliated with Capital Medical University. The study protocol was approved by the Clinical Research Ethics Committee of Beijing You'an Hospital Affiliated with Capital Medical University, the Tianjin Third Central Hospital, and the Fifth Medical Center of PLA General Hospital. Informed consent was waived due to the retrospective nature of this study.

Patients

We screened patients hospitalized for at least 1 day with an acute hepatic insult (2) that occurs in patients with chronic liver disease (CLD) of all etiologies, manifested by jaundice [serum total bilirubin (TB) $\geq$ 5 mg/dl] and coagulation dysfunction [international normalized ratio (INR) $\geq$ 1.5], and complicated within 4 weeks by ascites and/or encephalopathy. The enrolment criteria were the fulfillment of the above indications, and the CLD included non-cirrhotic chronic liver disease, well-compensated cirrhosis, and previous decompensated cirrhosis (5). Cirrhosis was diagnosed based on previous liver biopsy results, or findings provided by laboratory test results, endoscopy, and radiologic imaging. The previous decompensation of cirrhosis was defined by the acute development of large ascites, hepatic encephalopathy, gastrointestinal hemorrhage, bacterial infection, or any combination of these, and presented at 6 months before the present episode.

The exclusion criteria were as follows: liver cancer or other malignant tumors, severe chronic extra-hepatic disease, pregnancy or lactation, and coinfection with HIV. All patient data were retrieved from electronic medical records. All treatments, mainly including etiological and comprehensive treatment, that were performed comply with the guidelines for ACLF which is accredited by the Chinese Medical Association (9).

Data Collection

We collected data from all enrolled patients on medical history, detailed demographics, laboratory measurements, radiology results, and precipitating events of ACLF at the time of enrollment. Potential precipitating events included hepatitis B viral infection (HBV) reactivation (10), active alcoholism (3) (more than 14 drinks per week in women and more than 21 drinks per week in men within the previous 3 months), bacterial infection, and drug-induced liver injury. Information on liver transplantation and death were also collected from all enrolled patients and transplant-free survival/mortality was estimated for all enrolled patients at 28-, 90-, 180-, 360-, 540-, and 720-day after enrollment or ACLF diagnosis.

Procedures

The APASL-ACLF criteria (2), with the exception of the diagnostic indicator of chronic liver disease, were used to assess and identify two groups: patients with ACLF with decompensated cirrhosis (DC-ACLF) and patients with ACLF with non-cirrhotic chronic liver disease or compensated cirrhosis (non-DC-ACLF). The clinical and laboratory characteristics of patients with ACLF were compared among the above two groups. The 28/90/180/360/540/720-day mortality (patients who received a liver transplant were considered dead) was profiled in two groups. Then, multivariate Cox PH regression analysis, including decompensation cirrhosis (DC) and other clinical measurements as candidate risk factors, was conducted to select

the factors associated with short-term (28/90-day) and long-term (180/360/540/720-day) mortality.

Statistical Analysis

Continuous variables were presented as Mean ± SD and median [interquartile range (IQR)], and categorical variables as *n* (%). Wilcoxon rank-sum and chi-squared tests were performed for continuous and categorical variables, respectively. Kaplan-Meier survival curves were plotted and compared with log-rank tests. Multivariate Cox proportional hazards (PH) models were fitted with a stepwise method using significant baseline factors (candidate variables included DC, complications, and laboratory measurements, $p < 0.05$) that had been pre-filtered in univariate PH models to identify the independent relationship between DC and mortality of patients with ACLF. Transplant patients were included in the mortality analysis. A two-sided $p < 0.05$ was considered statistically significant. All statistical analyses were performed with R × 64 4.0.3 (http://www.r-project.org/).

RESULTS

Patients

A total of 689 patients were enrolled in the study. **Table 1** shows the characteristics of the enrollment of the whole group. In total, 407 patients (59.1%) had an HBV etiology with a diagnosed history of CHB with the most frequent complications was bacterial infection (83.9%), followed by ascites (72.3%), hepatic encephalopathy (19.2%), and gastrointestinal hemorrhage (8.3%). Of the patients assessed using APASL-ACLF criteria without the diagnostic indicator of chronic liver disease, 435 (63.1%) patients without decompensation cirrhosis were diagnosed with ACLF (non-DC-ACLF), and 254 (36.9%) had the previous decompensation of cirrhosis (DC-ACLF). Overall, the 28-, 90-, 180-, 360-, 540-, and 720-day transplant-free survival rate in this study was 78.8, 61.3, 55.7, 48.9, 42.7, and 38.2%, respectively.

Clinical Characteristics of ACLF Patients Stratified by Chronic Liver Disease

Demographics, laboratory data, scores (MELD scores and MELD-Na scores), and complications were compared between patients with non-DC-ACLF and DC-ACLF (**Table 2**). Compared with patients with non-DC-ACLF, those with DC-ACLF were more likely to be complicated with ascites ($p < 0.05$), gastrointestinal hemorrhage ($p < 0.05$). They had more severe disease as indicated by their significantly higher MELD scores [24.2 (IQR 19.5–27.2) vs. 22.7 (IQR 18.1–26.3)] and MELD-Na scores [26.9 (IQR 22.4–34.4) vs. 24.3 (IQR 20.0–29.5)] (both $p < 0.05$), and more severe kidney dysfunction as indicated by their significantly higher serum creatinine (mg/dl) [0.97 (IQR 0.73–1.15) vs. 0.76 (IQR 0.59–0.99)] and lower serum sodium [134 (IQR 129–137) vs. 135.3 (IQR 132.2–137.9)] (both $p < 0.05$). Coagulopathy (INR), liver (TB) dysfunction and precipitating events were not statistically different between the two groups ($p > 0.05$).

TABLE 1 | Baseline characteristics of the study patients.

Variables	*N* = 689
Age (y), median (IQR)	49 (41–57)
Male sex, *n* (%)	545 (79.1)
Underlying chronic liver disease, *n* (%)	
Compensated cirrhosis and non-cirrhosis	435 (63.1)
Decompensated cirrhosis	254 (36.9)
Etiology of liver disease, *n* (%)	
HBV	407 (59.1)
HCV	10 (1.5)
Alcohol	136 (19.7)
HBV + Alcohol	63 (9.1)
AIH	33 (4.8)
Others	40 (5.8)
Precipitating events, *n* (%)	
HBV reactivation	70 (10.1)
Bacterial infection	89 (12.9)
Alcohol	11 (1.6)
Drug	31 (4.5)
Unknown	488 (70.8)
Laboratory data at admission, median (IQR)	
ALT (U/L)	134 (47.2–440.0)
AST (U/L)	156.5 (85.2–398.8)
ALB (g/L)	28.9 (25.2–32.2)
TB (mg/dL)	16.4 (11.3–23.4)
INR	2.1 (1.8–2.6)
CR (mg/dL)	0.83 (0.64–1.04)
Na (mmol/L)	134.9 (131.2–137.6)
WBC ($\times 10^9$/L)	6.8 (5.0–9.4)
PLT ($\times 10^9$/L)	92 (62.5–132)
HGB (g/L)	119 (103–136)
Scores at admission, median (IQR)	
MELD score	23.1 (18.7–26.8)
MELD-Na score	25.4 (20.5–31.2)
Complications, *n* (%)	
Ascites	498 (72.3)
Bacterial infection	578 (83.9)
Gastrointestinal hemorrhage	57 (8.3)
Hepatic encephalopathy	132 (19.2)
Transplant-free survival, *n* (%)	
28-day	543 (78.8)
90-day	402 (61.3)
180-day	341 (55.7)
360-day	280 (48.9)
540-day	229 (42.7)
720-day	194 (38.2)

Clinical Outcomes

In DC-ACLF group, 1.6, 3.7, 4.2, 4.9, 5.1, and 5.3% patients who received a liver transplant by 28-, 90-, 180-, 360-, 540-, and 720-day, respectively, whereas a total of 24.8, 42.9, 48.7, 57.3, 63.4, and 68.1% patients died without receiving a liver transplant for the same corresponding periods (**Figure 1**). The transplant-free

TABLE 2 | Characteristics of acute-on-chronic liver failure (ACLF) patients with and without decompensated cirrhosis (DC).

Variables	DC-ACLF (*N* = 254)	Non-DC-ACLF (*N* = 435)	*P*-value
Age (y), median (IQR)	49 (44–57)	48 (40–57)	0.065
Male sex, *n* (%)	206 (81.1)	339 (77.9)	0.323
Precipitating events, *n* (%)			0.203
Intrahepatic insults	35 (13.8)	79 (18.2)	
Extrahepatic insults	38 (15.0)	51 (11.7)	
Unknown	181 (71.3)	305 (70.1)	
Laboratory data, median (IQR)			
ALT (U/L)	111 (62–233.2)	176.7 (61.8–550.3)	0.000
AST (U/L)	58.5 (53.3–64.4)	186 (99.9–482)	0.000
ALB (g/L)	27.4 (23.6–30.8)	29.5 (25.7–32.7)	0.000
TB (mg/dL)	16.02 (10.4–22.9)	16.6 (11.6–23.7)	0.201
INR	2.14 (1.86–2.53)	2.17 (1.83–2.62)	0.686
CR (mg/dL)	0.97 (0.73–1.15)	0.76 (0.59–0.99)	0.000
Na (mmol/L)	134 (129–137)	135.3 (132.2–137.9)	0.000
WBC ($\times 10^9$/L)	6.9 (5.1–9.9)	6.7 (4.9–8.9)	0.259
PLT ($\times 10^9$/L)	73 (47.5–112.5)	103 (71.3–141.8)	0.000
HGB (g/L)	113 (95–131)	121 (106.1–139.0)	0.000
Scores, median (IQR)			
MELD score	24.2 (19.5–27.2)	22.7 (18.1–26.3)	0.032
MELD-Na score	26.9 (22.4–34.4)	24.3 (20.0–29.5)	0.000
Complications, *n* (%)			
Ascites	203 (79.9)	295 (67.8)	0.001
Bacterial infection	220 (86.6)	358 (82.3)	0.137
Gastrointestinal hemorrhage	30 (11.8)	27 (6.2)	0.010
Hepatic encephalopathy	55 (21.7)	77 (17.7)	0.203

Intrahepatic insults include hepatitis B virus (HBV) reactivation, alcohol, hepatotoxic drugs.
Extrahepatic insults include bacterial infection.

survival was significantly lower in patients with DC-ACLF than in those without DC (non-DC-ACLF), irrespective of the diagnostic criteria (**Figure 2**), especially in terms of long-term transplant free survival, which was significantly different between the two groups with longer follow-up (720-day).

Kaplan-Meier curves showing the transplant-free survival of ACLF patients with or without DC according to the APASL-ACLF are provided in **Figure 3**. At up to 720 days of follow-up, transplant free survival at all time periods (28, 90, 180, 360, 540, and 720 days) was significantly lower for patients in the DC group than for patients in the non-DC group ($p < 0.05$ by log-rank test).

Decompensated Cirrhosis and Short- and Long-Term Mortality in Patients With ACLF

To clarify the independent relationship between DC and short and long-term outcomes in patients with ACLF, we performed univariate and multivariate Cox regression analyses of patients at various time periods of follow-up (**Table 3**). After adjusting for the factors that were statistically significant in the univariate regression analysis ($p < 0.05$), the results of multivariate analysis showed that patients without DC had a similar risk of death as those with DC on 28 days [aHR (95% CI) 0.717 (0.505–1.019), $p > 0.05$], and 90 days [aHR (95% CI) 0.753 (0.553–1.025), $p > 0.05$], implying that DC was not an independent risk factor for short-term mortality in ACLF patients.

However, at time periods beyond 180 days of follow-up, patients in the non-DC group had a lower risk of death compared with those in the DC group ($p < 0.05$), with an adjusted HR (95%CI) of 0.712 (0.524–0.967), 0.686 (0.512–0.920), 0.701 (0.526–0.935), and 0.694 (0.523–0.922) by 180, 360, 540, and 720 days of follow-up, respectively. From this, DC increases the long-term risk of death in ACLF patients.

The results of univariate and multivariate Cox regression analyses of factors associated with mortality risk in ACLF at each follow-up time are detailed in **Supplementary Tables 1–6**, and it is important to note that age, TB, INR, and hepatic encephalopathy were independent risk factors associated with both short and long-term mortality risk in patients with ACLF ($p < 0.05$).

We separately analyzed the independent risk factors for short-term outcome in DC-ACLF and non-DC-ACLF patients and found that age, TB, and INR were the common short-term mortality risk factors in both groups (**Supplementary Table 7**). In addition, hepatic encephalopathy was a risk factor for short-term mortality in DC-ACLF patients.

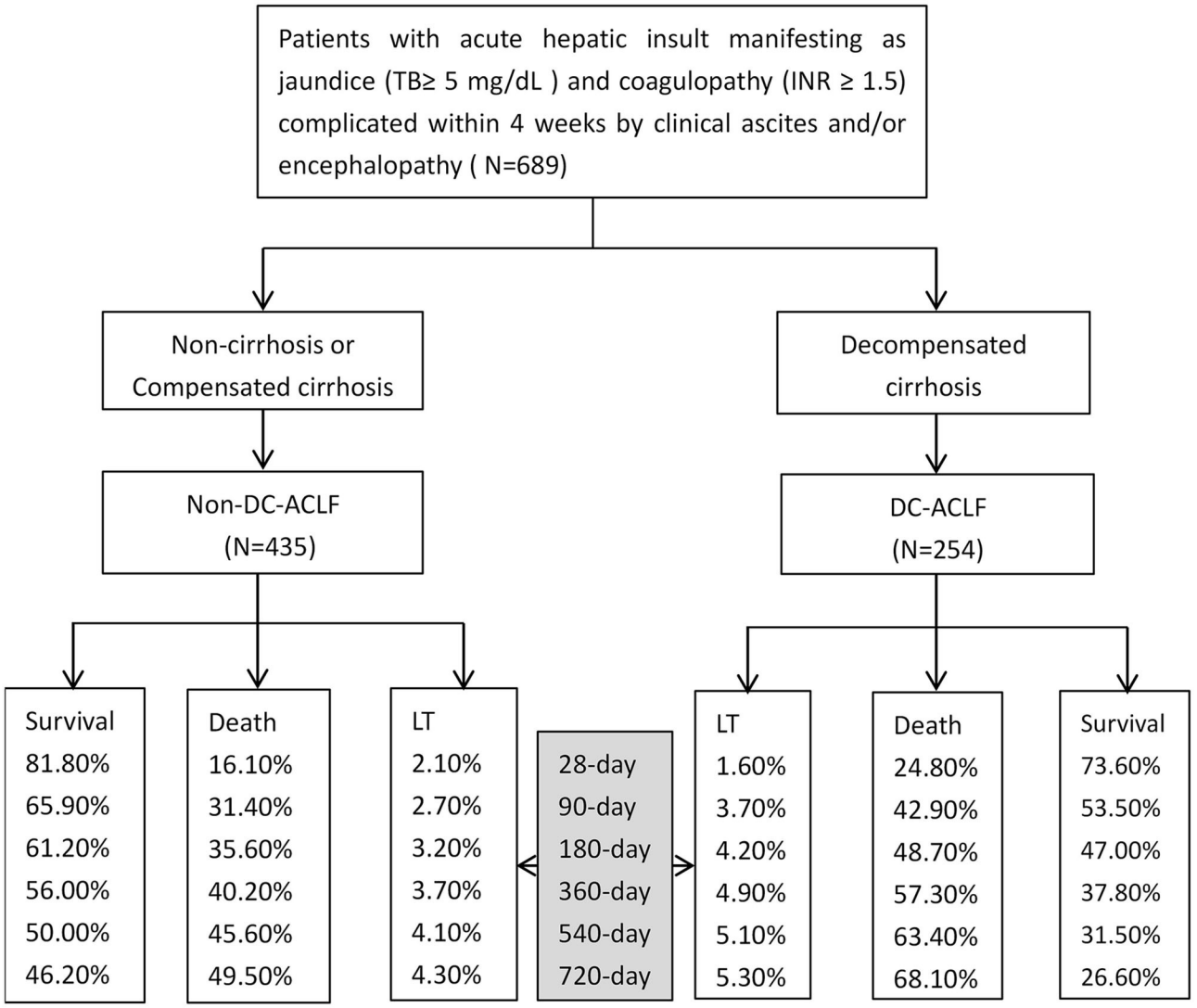


FIGURE 1 | Acute-on-chronic liver failure (ACLF) with different underlying chronic liver diseases and associated outcomes since admission.

DISCUSSION

When patients were diagnosed with ACLF according to EASL, the definition of CLD should include non-cirrhotic, compensated cirrhosis, and decompensated cirrhosis, an idea that has been confirmed in patients with HBV-related ACLF (4). Nevertheless, according to APASL, patients with a history of decompensated cirrhosis cannot be diagnosed with ACLF (2). In this retrospective study based on the enrolment standards of the APASL consortium definition of ACLF, most patients had HBV-related chronic liver disease (59.1%). When the APASL-ACLF criteria without non-DC were used, 245 additional patients with DC were diagnosed with ACLF. Baseline characteristics and short and long-term mortality were observed in populations with DC-ACLF. Compared with patients with non-DC-ACLF (fulfilling the APASL-ACLF criteria), those with DC-ACLF were more likely to be complicated with ascites, gastrointestinal hemorrhage, and had the more severe disease as indicated by their significantly higher MELD scores and MELD-Na scores, and more severe kidney dysfunction. The transplant free mortality rates of patients with DC-ACLF at 28, 90, 180, 360, 540, and 720 days were 24.8, 42.9, 48.7, 57.3, 63.4, and 68.1%, respectively, which were significantly higher than those of patients with non-DC-ACLF. Obviously, the 28 day mortality rate for patients with DC-ACLF is higher than the 15% used to develop the diagnostic criteria for ACLF (3, 4), and their 90 day mortality rate is higher than that of acute decompensation (23–29%) reported by APASL (2), indicating that patients with high short-term mortality in DC populations should be diagnosed with ACLF. The APASL guidelines emphasize that ACLF is a reversible syndrome, classifying acute liver injury occurring in patients with decompensated cirrhosis as chronic liver failure

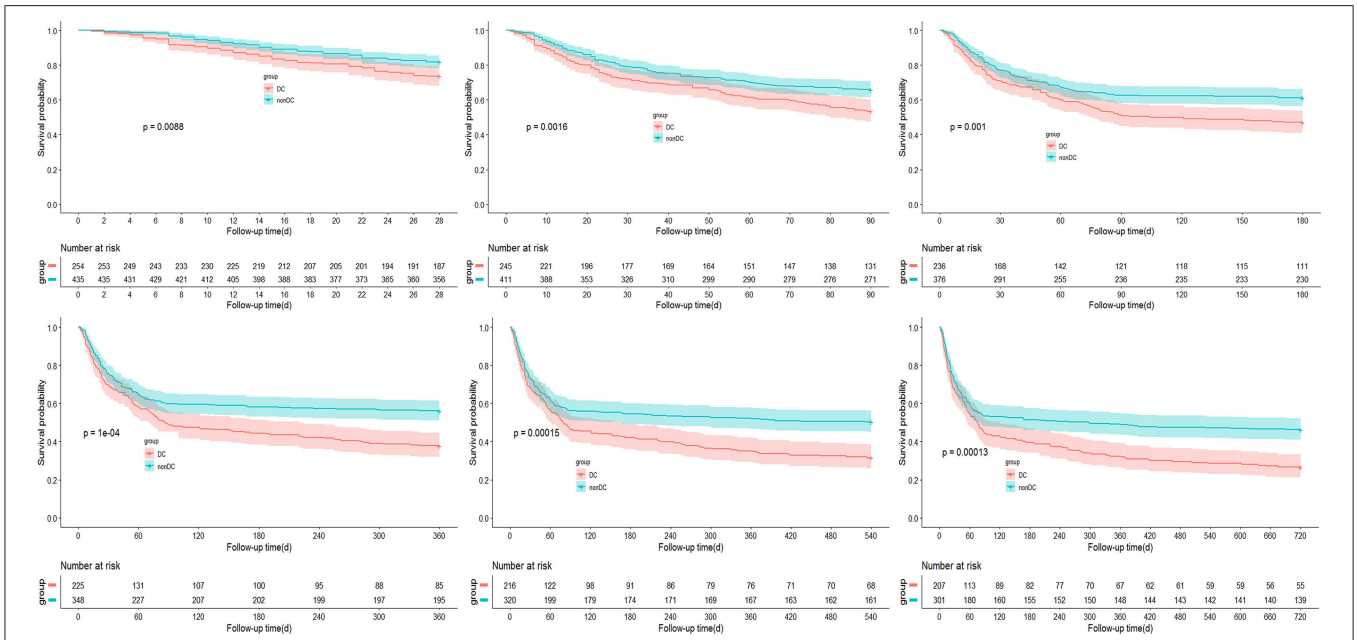


FIGURE 2 | Incidence of transplant-free survival in ACLF patients with and without decompensated cirrhosis (DC).

(CLIF), which also implies entry on the liver transplant waiting list. But it is noteworthy that the 1-year survival rate of patients with DC-ACLF in this study was 37.8% and this subset of patients is potentially reversible and does not urgently require liver transplantation. Therefore, managing DC-ACLF patients according to the ACLF management process facilitates organ allocation. Furthermore, the inclusion of DC in the APASL-ACLF definition expanded the application of ACLF definition (11) and was consistent with the WGO consensus (5).

Previous studies (12, 13) suggest that patients with previous DC had higher 90-day mortality than those without previous DC, and the prognostic model including DC showed excellent predictive value for 90-day mortality. This result was quite different from the results of the CANONIC study (3), in which the 90-day mortality of ACLF was higher in patients without previous acute decompensation (AD) than in patients with previous DC. Moreau (14) proposed that this can be explained by an inappropriate inflammatory response and a lack of tolerance to inflammation in patients without previous AD. The results presented in this study show that DC is not an independent risk factor for short-term mortality (28/90 days) but rather, a risk factor affecting the long-term outcome of patients (beyond 180 days). The controversial impact of decompensated cirrhosis on short-term outcomes may be explained by the different diagnostic criteria and etiologies of ACLF in different studies, but the results of DC on long-term mortality in ACLF are consistent. Some scholars (15, 16) prospectively followed up the long-term outcomes of ACLF patients and found that a prior history of AD is the most important factor affecting long-term mortality following an ACLF episode regardless of Model for End-stage Liver Disease score, considering that decreased hepatic reserve would be the predominant factor over inappropriate inflammatory response or ACLF severity with regard to the long-term outcome of patients who have survived ACLF.

In addition, our study found that age, TB, INR, and hepatic encephalopathy were independent risk factors associated with both short and long-term mortality in patients with ACLF. Among them, total bilirubin, INR, and hepatic encephalopathy were included in the APASL ACLF Research Consortium (AARC) score (17) used to manage APASL-ACLF, confirming again the prognostic importance of the above indicators.

This retrospective study with insufficient information does have its limitations in the nature of the study design. First, we did not observe changes in the dynamic clinical indicators of the patients, and it is possible that some potential influencing factors were ignored when analyzing the influencing factors on the long-term prognosis of ACLF patients. Second, a bacterial infection is judged according to the use of antibiotics, which is often related to the diagnosis and treatment experience of clinicians, leading to an overestimation of the bacterial infection rate in our patients. Finally, indeterminate precipitating events in our study denoted the absence of all previously described precipitating events. Since this study is a multicenter retrospective study, it is difficult to determine the precipitating events, especially as one of the centers did not give information about predisposing factors, which is also another limitation of our study. But this is a deletion from a single center and for the overall study population, it is not considered to be subject to deletion bias.

Future prospective studies of multicenter design are needed to verify the necessity of including DC in the diagnostic criteria of APASL. Additionally, it has been shown (18) that submassive hepatic necrosis could differentiate ACLF from AD. Thus, it is expected that DC-ACLF may be clarified by liver pathology in future studies.

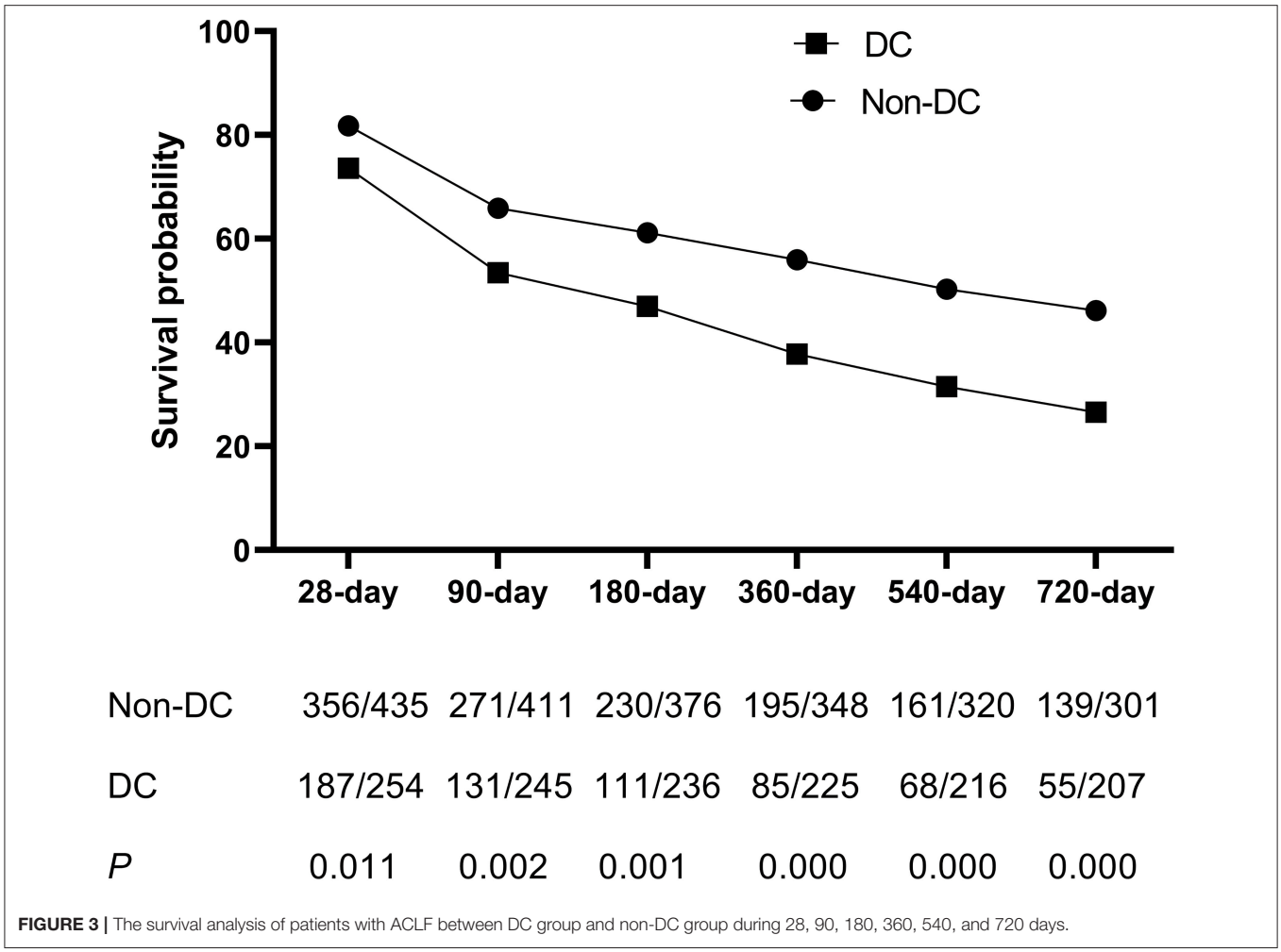


FIGURE 3 | The survival analysis of patients with ACLF between DC group and non-DC group during 28, 90, 180, 360, 540, and 720 days.

TABLE 3 | Adjusted HRs and 95% CI of decompensated cirrhosis (DC) for acute-on-chronic liver failure (ACLF) mortality by Cox proportional hazards model.

	Univariate analysis		Multivariate analysis	
Non-DC vs. DC	**HR (95% CI)**	***P*-value**	**aHR (95% CI)**	***P*-value**
28-day	0.649 (0.469–0.899)	0.009	0.717 (0.505–1.019)[a]	0.064
90-day	0.673 (0.525–0.862)	0.002	0.753 (0.553–1.025)[b]	0.052
180-day	0.672 (0.530–0.854)	0.001	0.712 (0.524–0.967)[c]	0.029
360-day	0.638 (0.507–0.803)	0.000	0.686 (0.512–0.920)[c]	0.011
540-day	0.651 (0.520–0.814)	0.000	0.701 (0.526–0.935)[d]	0.016
720-day	0.651 (0.522–0.813)	0.000	0.694 (0.523–0.922)[d]	0.012

The multivariate logistic regression model was fitted with a stepwise selection method using clinically and statistically baseline factors that had been screened in univariate analysis.
[a]*Adjusted for age, TB, INR, Na, Cr, MELD, MELD-Na, Bacteria, and HE.*
[b]*Adjusted for age, TB, INR, Na, Cr, PLT, MELD, MELD-Na, Bacteria, GIB, HE, and ascites.*
[c]*Adjusted for age, TB, INR, Na, Cr, ALT, PLT, HGB, MELD, MELD-Na, Bacteria, GIB, HE, and ascites.*
[d]*Adjusted for age, TB, INR, Na, Cr, ALT, PLT, WBC, HGB, MELD, MELD-Na, Bacteria, HE, and ascites.*

CONCLUSIONS

In conclusion, our study shows that patients with DC-ACLF have a higher mortality rate, especially long-term mortality, compared to non-DC-ACLF patients. Therefore, DC should be included in the diagnostic criteria of APASL-ACLF and treated according to the ACLF management process, which is clinically helpful for early diagnosis, management, and prognosis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Clinical Research Ethics Committee of Beijing You'an Hospital Affiliated to Capital Medical University. The Ethics Committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

MX and MK contributed to the conception and design of the study, drafted the initial manuscript, and reviewed and revised the manuscript. MX, MK, PY, FL, and YCa performed the initial data analysis and interpreted the data. SY, YZ, HZ, BZ, and WL coordinated and supervised the data collection and management. ZD and SX revised the manuscript critically for important intellectual content. YCh and TH contributed to the conception and design of the study and reviewed and revised the manuscript critically. All authors have read and approved the final manuscript.

FUNDING

This work was supported by the National 13th 5-Year Plan for Hepatitis Research (2017ZX10203201-005 and 2017ZX10203201-007), National Key R&D Program of China (2017YFA0103000), Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support (ZYLX201806), and the National Natural Science Foundation of China (81870429).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.750061/full#supplementary-material

REFERENCES

1. Arroyo V, Moreau R, Jalan R. Acute-on-Chronic liver failure. *N Engl J Med.* (2020) 382:2137–45. doi: 10.1056/NEJMra1914900
2. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. *Hepatol Int.* (2019) 13:353–90. doi: 10.1007/s12072-019-09946-3
3. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology.* (2013) 144:1426–37:37.e1–9. doi: 10.1053/j.gastro.2013.02.042
4. Wu T, Li J, Shao L, Xin J, Jiang L, Zhou Q, et al. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure. *Gut.* (2018) 67:2181–91. doi: 10.1136/gutjnl-2017-314641
5. Jalan R, Yurdaydin C, Bajaj JS, Acharya SK, Arroyo V, Lin HC, et al. Toward an improved definition of acute-on-chronic liver failure. *Gastroenterology.* (2014) 147:4–10. doi: 10.1053/j.gastro.2014.05.005
6. Tang X, Qi T, Li B, Li H, Huang Z, Zhu Z, et al. Tri-typing of hepatitis B-related acute-on-chronic liver failure defined by the world gastroenterology organization. *J Gastroenterol Hepatol.* (2021) 36:208–16. doi: 10.1111/jgh.15113
7. Atkins D, Best D, Briss PA, Eccles M, Falck-Ytter Y, Flottorp S, et al. Grading quality of evidence and strength of recommendations. *BMJ.* (2004) 328:1490. doi: 10.1136/bmj.328.7454.1490
8. Huang J, Mao Y, Millis JM. Government policy and organ transplantation in China. *Lancet.* (2008) 372:1937–8. doi: 10.1016/S0140-6736(08)61359-8
9. Liver F, Artificial Liver Group CSoIDCMA, Severe Liver D, et al. [Diagnostic and treatment guidelines for liver failure (2012 version)]. *Zhonghua Gan Zang Bing Za Zhi.* (2013) 21:177–83. doi: 10.3760/cma.j.issn.1007-3418.2013.03.006
10. Cao Z, Liu Y, Wang S, Lu X, Yin S, Jiang S, et al. The impact of HBV flare on the outcome of HBV-related decompensated cirrhosis patients with bacterial infection. *Liver Int.* (2019) 39:1943–53. doi: 10.1111/liv.14176
11. Wang SJ, Yin S, Gu WY, Zhang Y, Li H. Acute-on-chronic liver failure exists in patients with hepatitis B virus-related decompensated cirrhosis. *J Dig Dis.* (2018) 19:614–25. doi: 10.1111/1751-2980.12671
12. Mu X, Tong J, Xu X, Chen J, Su H, Liu X, et al. World gastroenterology organisation classification and a new type-based prognostic model for hepatitis B virus-related acute-on-chronic liver failure. *Clin Res Hepatol Gastroenterol.* (2020) 45:101548. doi: 10.1016/j.clinre.2020.09.009
13. Kim TY, Song DS, Kim HY, Sinn DH, Yoon EL, Kim CW, et al. Characteristics and discrepancies in acute-on-chronic liver failure: need for a unified definition. *PLoS ONE.* (2016) 11:e0146745. doi: 10.1371/journal.pone.0146745
14. Moreau R. Acute-on-chronic liver failure: a new syndrome in cirrhosis. *Clin Mol Hepatol.* (2016) 22:1–6. doi: 10.3350/cmh.2016.22.1.1
15. Yoon EL, Kim TY, Lee CH, Kim TH, Cho HC, Lee SS, et al. Long-Term prognosis of acute-on-chronic liver failure survivors. *J Clin Gastroenterol.* (2019) 53:134–41. doi: 10.1097/MCG.0000000000000987
16. Shi Y, Zheng MH, Yang Y, Wei W, Yang Q, Hu A, et al. Increased delayed mortality in patients with acute-on-chronic liver failure who have prior decompensation. *J Gastroenterol Hepatol.* (2015) 30:712–8. doi: 10.1111/jgh.12787
17. Choudhury A, Jindal A, Maiwall R, Sharma MK, Sharma BC, Pamecha V, et al. Liver failure determines the outcome in patients of acute-on-chronic liver failure (ACLF): comparison of APASL ACLF research consortium (AARC) and CLIF-SOFA models. *Hepatol Int.* (2017) 11:461–71. doi: 10.1007/s12072-017-9816-z
18. Li H, Xia Q, Zeng B, Li ST, Liu H, Li Q, et al. Submassive hepatic necrosis distinguishes HBV-associated acute on chronic liver failure from cirrhotic patients with acute decompensation. *J Hepatol.* (2015) 63:50–9. doi: 10.1016/j.jhep.2015.01.029

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 01 November 2021
doi: 10.3389/fmed.2021.748915

Development and Validation of a Novel Risk Prediction Model Using Recursive Feature Elimination Algorithm for Acute-on-Chronic Liver Failure in Chronic Hepatitis B Patients With Severe Acute Exacerbation

Mingxue Yu[1†], Xiangyong Li[1†], Yaxin Lu[2], Yusheng Jie[1], Xinhua Li[1], Xietong Shi[3], Shaolong Zhong[3], Yuankai Wu[1], Wenli Xu[1], Zifeng Liu[2‡] and Yutian Chong[1*‡]*

[1] The Department of Infectious Disease, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, [2] The Department of Clinical Data Center, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, [3] The Department of Infectious Disease, Jieyang People's Hospital (Jieyang Affiliated Hospital of Sun Yat-sen University), Jieyang, China

Background: Patients with chronic hepatitis B (CHB) with severe acute exacerbation (SAE) are at a progression stage of acute-on-chronic liver failure (ACLF) but uniform models for predicting ACLF occurrence are lacking. We aimed to present a risk prediction model to early identify the patients at a high risk of ACLF and predict the survival of the patient.

Methods: We selected the best variable combination using a novel recursive feature elimination algorithm to develop and validate a classification regression model and also an online application on a cloud server from the training cohort with a total of 342 patients with CHB with SAE and two external cohorts with a sample size of 96 and 65 patients, respectively.

Findings: An excellent prediction model called the PATA model including four predictors, prothrombin time (PT), age, total bilirubin (Tbil), and alanine aminotransferase (ALT) could achieve an area under the receiver operating characteristic curve (AUC) of 0.959 (95% CI 0.941–0.977) in the development set, and AUC of 0.932 (95% CI 0.876–0.987) and 0.905 (95% CI 0.826–0.984) in the two external validation cohorts, respectively. The calibration curve for risk prediction probability of ACLF showed optimal agreement between prediction by PATA model and actual observation. After predictive stratification into different risk groups, the C-index of predictive 90-days mortality was 0.720 (0.675–0.765) for the PATA model, 0.549 (0.506–0.592) for the end-stage liver disease score model, and 0.648 (0.581–0.715) for Child–Turcotte–Pugh scoring system.

OPEN ACCESS

Edited by:
Yu-Chen Fan,
Shandong University, China

Reviewed by:
Tianlu Chen,
Shanghai Jiao Tong University, China
Nan Lin,
Trinity Partners, United States

***Correspondence:**
Yutian Chong
chongyt@mail.sysu.edu.cn
Zifeng Liu
liuzf@mail.sysu.edu.cn

†These authors have contributed equally to this work and share first authorship

‡These authors have contributed equally to this work and share last authorship

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *28 July 2021*
Accepted: *21 September 2021*
Published: *01 November 2021*

Citation:
Yu M, Li X, Lu Y, Jie Y, Li X, Shi X, Zhong S, Wu Y, Xu W, Liu Z and Chong Y (2021) Development and Validation of a Novel Risk Prediction Model Using Recursive Feature Elimination Algorithm for Acute-on-Chronic Liver Failure in Chronic Hepatitis B Patients With Severe Acute Exacerbation. Front. Med. 8:748915. doi: 10.3389/fmed.2021.748915

Interpretation: The highly predictive risk model and easy-to-use online application can accurately predict the risk of ACLF with a poor prognosis. They may facilitate risk communication and guide therapeutic options.

Keywords: prediction model, machine learning, recursive feature elimination algorithm, chronic hepatitis B, acute exacerbation, acute-on-chronic liver failure (ACLF)

INTRODUCTION

Chronic hepatitis B virus (HBV) infection poses a global health challenge (1). Hepatitis activity with alanine aminotransferase (ALT) elevation, also called acute exacerbation or hepatitis flare, may occur spontaneously either over the natural course of the disease or following therapy among chronic HBV infection (2, 3). Up to 30% of patients with chronic hepatitis B (CHB) experience hepatitis reactivation every year (4), and some patients will experience severe acute exacerbation (SAE), accompanied by jaundice and hepatic decompensation (5–7). Indeed, compelling evidence shows that SAE has been proposed following the prewarning signs of HBV-related acute-on-chronic liver failure (ACLF) and has been considered a progressive stage in the development of ACLF (5–9). ACLF is a common acute deterioration of hepatic function syndrome, and the short-term in-hospital mortality rate is over 70% if emergency liver transplantation is not available (9). Although liver transplantation is the only effective treatment for ACLF, due to the high cost and shortage of liver source, only a small number of patients undergo liver transplantation (10). In this situation, it is believed that early identification of the high risk of ACLF is of vital importance so that physicians can focus and intervene in advance to slow down or stop the progression of SAE to ACLF (9, 11, 12) and improve the prognosis of the patient.

However, uniform criteria for predicting ACLF occurrence are lacking and patients who are truly at the risk of ACLF are still ill-defined. There are currently several models to evaluate the severity and prognosis of patients with severe liver disease, including the Child–Turcotte–Pugh (CTP) scoring system, the model for end-stage liver disease (MELD), the sequential organ failure assessment score (SOFA), and other predictive models, but none of them have been universally accepted for predict accurate incidence of ACLF (13–15). First, majority scoring systems were originally applied for the evaluation of liver disease severity to predict the outcome of patients. Second, most of these existing models were established among European and American populations. The etiology of ACLF varies with geographic location (16). The leading cause of ACLF among European and American patients is alcohol consumption, whereas among Asian patients is the infection of HBV (12, 16). Risk equations and risk functions are widely applied in patient management, clinical diagnosis, risk stratification, treatment selection, and prognosis prediction (17, 18). However, for new mathematical prediction models for ACLF, there is no model for external multicenter validation. These questions reflect high-priority areas for an accurate prediction model of HBV-ACLF.

Recursive feature elimination (RFE) algorithm (19) is an innovative machine learning algorithm and a backward selection procedure to determine if predictors would be advantageous and select the best predictors (according to the coefficient) to establish the model. To date, this method had not been used in the risk assessment of ACLF patients. In this study, in order to help physicians early identify high-risk patients with CHB of HBV-ACLF, we developed and validated a simple model in three independent cohorts by utilizing RFE analysis. Furthermore, for assessing the prognosis of patients, we compared the performance of the model in predicting 90-days mortality with MELD score and CTP score. The results of this study may further guide and optimize therapeutic strategies for SAE patients with CHB. To the best of our knowledge, our study is the first report of a polycentric risk prediction model in patients with CHB with SAE.

MATERIALS AND METHODS

The methods described in this article are in accordance with the Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD) statement (20).

Study Design and Population

We performed a multicenter retrospective cohort study. Data were collected in three independent hospitals. A total of 342 SAE patients with CHB were enrolled in the study as the development cohort from the Third Affiliated Hospital of Sun Yat-sen University in Guangzhou, China between 2011 and 2019. The preliminary screening identified 550 CHB hospitalized patients with ALT levels elevated. Patients who did not meet the research standards were excluded ($n = 208$). The flow chart of the training group selection process is presented in **Figure 1**. Patients in the validation cohorts were from two different geographic hospitals, namely Yuedong Hospital of the Third Affiliated Hospital of Sun Yat-sen University in Meizhou, China, between 2016 and 2020 and Jieyang People's Hospital in Jieyang, China, between 2014 and 2019, with a sample size of 96 and 65 patients, respectively, using the same inclusion and exclusion criteria as the development cohort.

Ethics Statement

This study was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University

Abbreviations: HBV, hepatitis B virus; CHB, chronic hepatitis B; ACLF, acute-on-chronic liver failure; SAE, severe acute exacerbation; ALT, alanine aminotransferase; AST, aspartate transaminase; Tbil, total bilirubin; PT, prothrombin time; Fib, fibrinogen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; INR, international normalized ratio; CI, confidence interval; ULN, upper limit of normal.

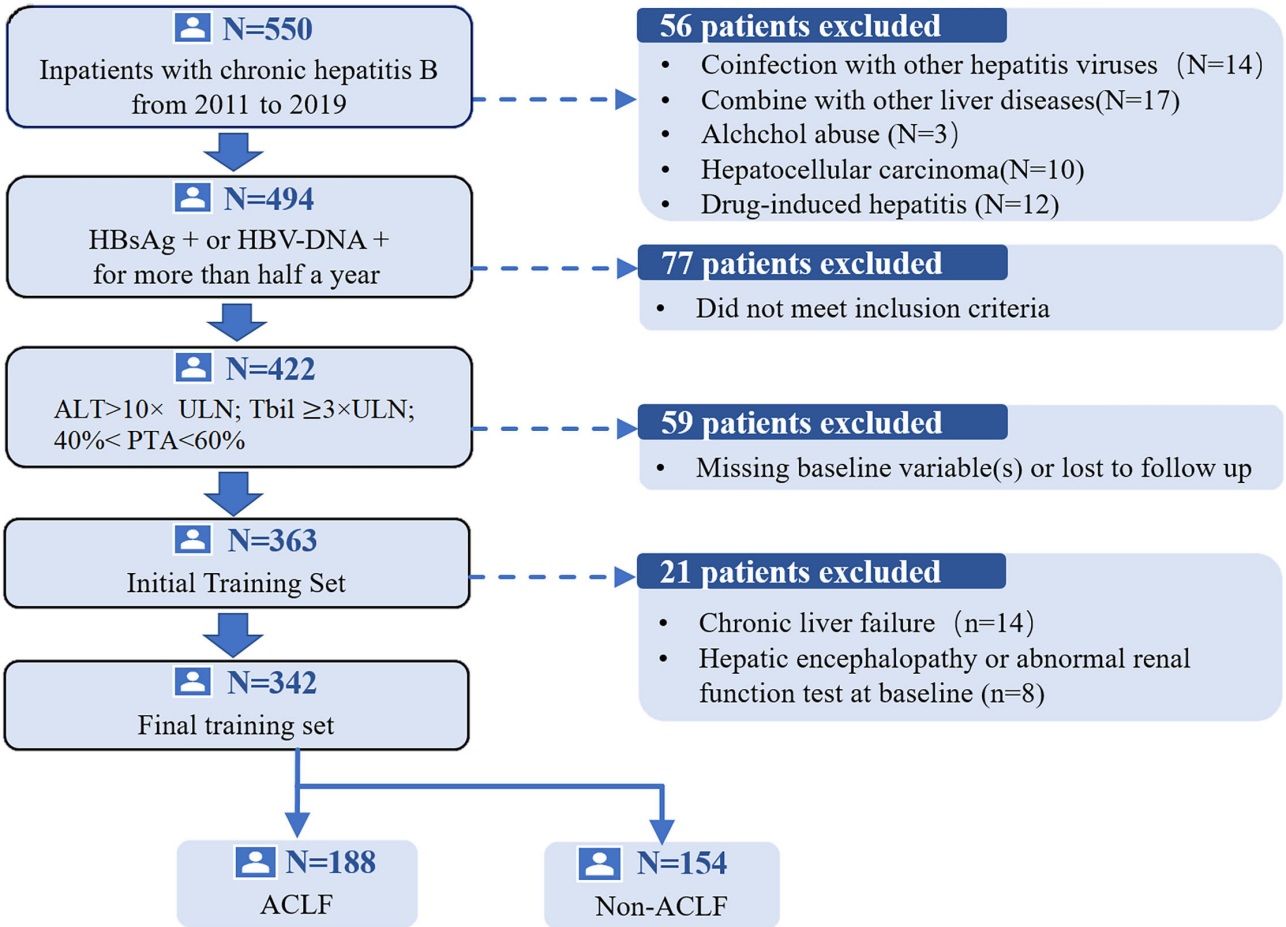


FIGURE 1 | The flow chart of the study group selection process.HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; ACLF, acute-on-chronic liver failure; PTA, prothrombin activity; TBil, total bilirubin; ULN, upper limit of normal.

[(2018)02-384-01]. This study was conducted according to the Declaration of Helsinki. All adult participants provided written informed consent.

Diagnostic Criteria

The inclusion criteria for SAE of CHB in both the development cohort and validation cohorts were based on those proposed by Tsubota et al. (21) and Wong et al. (22). The inclusion criteria were as follows: (1) presence of hepatitis B surface antigen and HBV DNA for >6 months before hospitalization; (2) ALT >10× the upper limit of normal (ULN); (3) total bilirubin (Tbil) ≥3× ULN; (4) 40%< prothrombin time activity (PTA) <60%. The exclusion criteria were coinfections with hepatitis A, C, D, or E viruses, or other viruses including HIV, cytomegalovirus, and Epstein-Barr virus; coexistence of other liver diseases, such as autoimmune liver disease, alcoholic hepatitis, drug-induced liver injury (DILI), and non-alcoholic steatohepatitis (NASH); concurrent diseases resulting in bilirubin elevation, such as hemolytic jaundice, non-hemolytic jaundice, and obstructive jaundice; metabolic liver diseases, including Wilson's disease and hemochromatosis; malignant tumors; and serious extrahepatic diseases. Those patients who had hepatic encephalopathy, ascites, abnormal renal function test at baseline, or did not have follow-up data were also excluded.

Acute-on-chronic liver failure was defined as jaundice (serum bilirubin >5 mg/dL) and coagulopathy (INR >1.5 or prothrombin activity<40%), complicated with ascites and/or encephalopathy as determined by a physical examination in 4 weeks based on the consensus recommendations of the Asian Pacific Association for the study of the liver (APASL 2019) (23). The MELD score was calculated according to the following formula: MELD score = 3.78 × ln[TBil (mg/dL)] +11.2 × ln [INR]+9.57 × ln [Cr(mg/dL)] + 6.43 × (constant for liver disease etiology = 0 if cholestatic or alcoholic, otherwise = 1). The modified CTP score included five parameters: TBil level, albumin level, PT, and the presence and severity of ascites and encephalopathy (24). Liver cirrhosis was defined as coarse liver echotexture with nodularity and small liver size and the presence of features of

portal hypertension (e.g., ascites, splenomegaly, and varices) by ultrasound (22).

Patient Management

All patients were managed by the attending physicians according to international and local guidelines and received conservative therapy. The therapy included bed rest, antiviral therapy, liver-protective treatment, nutritional and energy supplements, intravenous plasma and albumin infusions, water-electrolyte and acid-base equilibrium maintenance, and the prevention and treatment of complications.

Patients in the development cohort were all monitored regularly and followed until death, liver transplantation, or at least for 90 days. The patients who received liver transplantation within 90 days were considered dead and more than 90 days as survival (25, 26). Unfortunately, survival records in validation cohorts were not obtained.

Predictors

Predictors were collected using an online electronic case report form, and their integrity was systematically checked before being entered into the model. We selected the predictors from the electronic health records based on published literature (27–29) and our clinical experience. Baseline data were the data obtained at the first diagnosis of SAE of CHB from the computerized and paper medical records. We collected the data from patients with complete clinical, laboratory, and follow-up data. Data collection included demographics, basic diseases, precipitating factors, viral tests, liver function, etc. Laboratory variables included aspartate transaminase (AST), ALT, Tbil, albumin (ALB), prothrombin time (PT), fibrinogen (Fib), white blood cell count (WBC), hemoglobin (HGB), platelet (PLT), creatinine (Cr), quantitative determination of hepatitis Bsantigen (HBsAg), hepatitis B e antigen (HBeAg), and HBV DNA levels.

Data Preprocessing

Missing data were imputed with the mean of continuous variables and the mode of categorical variables. Missing data fill rates in the development set and the two external validation queues are 7, 6, and 5%, respectively (**Supplementary Figure 1**). Prior to modeling, the Yeo-Johnson (30) transformation was first applied to the raw data, followed by centralization and normalization (**Supplementary Table 1**).

Variable Selection

To explore the predictive power of individual variables, we first developed a univariate logistic model for each variable. Since the receiver operator characteristic (ROC) curves are not affected by monotonic transformations of predictors, the area under the receiver operating characteristic curve (AUC) is used as the measure of the strength of the association between predictors and outcomes. A better model was indicated with a higher

TABLE 1 | Clinical characteristics of study participants.

Characteristics	Development Cohort (*n* = 342)		Validation Cohort 1 (*n* = 96)		Validation Cohort 2 (*n* = 65)	
	Liver failure	Non-liver failure	Liver failure	Non-liver failure	Liver failure	Non-liver failure
No. of Patient, *N* (%)	188 (55.0%)	154 (45.0%)	33 (34.4%)	63 (65.6%)	13 (20.0%)	52 (80.0%)
Age (year), median [Q1, Q3]	46.0 [38.0–53.0]	38.0 [33.0–45.8]	45.0 [37.0–56.0]	39.0 [33.0–48.0]	58.0 [51.0–65.0]	37.5 [27.8–49.0]
Gender, N (%)						
Male	170 (90.4%)	139 (90.3%)	28 (84.8%)	53 (84.1%)	13 (100%)	43 (82.7%)
Female	18 (9.57%)	15 (9.74%)	5 (15.2%)	10 (15.9%)	0 (0.00%)	9 (17.3%)
BMI, median [Q1, Q3]	23.1 [21.5–25.4]	22.2 [20.0–24.8]	25.2 [21.7–27.6]	23.1 [20.0–24.7]	–	–
ALB (g/L), median [Q1, Q3]	34.1 [30.6–36.9]	38.3 [34.5–40.8]	34.7 [30.5–37.6]	38.5 [35.3–42.0]	32.0 [30.3–37.6]	38.7 [35.9–41.0]
ALT (U/L), median [Q1, Q3]	688 [251–1440]	874 [483–1560]	1,401 [997–2268]	1,223 [969–1,714]	1,181 [847–1,517]	1,340 [997–1,886]
AST (U/L), median [Q1, Q3]	496 [164–1,004]	511 [258–929]	569 [343–1,110]	497 [285–858]	859 [591–1,398]	706 [473–1,333]
PT (s), median [Q1, Q3]	21.4 [19.4–27.1]	15.5 [14.4–17.4]	20.4 [18.0–26.7]	13.2 [11.9–14.9]	19.0 [17.8–19.6]	14.2 [12.6–16.5]
TB (μmol/L), median [Q1, Q3]	304 [215–388]	106 [49.8–180]	139 [79.9–235]	67.8 [38.1–154]	237 [154–348]	100 [51.9–195]
Fibrinogen (g/L), median [Q1, Q3]	1.79 [1.48–2.24]	2.26 [1.98–2.61]	1.56 [1.27–1.85]	1.98 [1.62–2.38]	1.54 [1.40–1.69]	1.92 [1.58–2.23]
HGB (g/L), median [Q1, Q3]	130 [114–142]	141 [128–151]	130 [123–151]	141 [134–152]	143 [121–150]	140 [126–150]
PLT (10^9/L), median [Q1, Q3]	124 [95.0–168]	170 [138–211]	136 [124–177]	174 [144–208]	135 [89.0–189]	140 [122–186]
WBC (10^9/L), median [Q1, Q3]	6.94 [5.56–9.18]	6.06 [4.84–7.51]	6.72 [5.72–9.00]	6.00 [5.28–8.62]	6.87 [5.97–9.81]	6.17 [5.38–8.52]
Cr (μmol/L), median [Q1, Q3]	70.0 [62.2–81.0]	73.0 [66.0–81.6]	61.8 [56.4–75.6]	66.7 [58.9–75.0]	68.0 [52.0–79.0]	66.0 [60.8–73.5]
HBsAg (IU/mL), median [Q1, Q3]	7.65 [5.76–9.06]	8.35 [7.16–8.99]	5.99 [5.52–5.99]	5.99 [5.58–5.99]	7.82 [7.82–7.82]	7.82 [6.42–7.82]
HBeAg positive, N (%)	59 (31.4%)	84 (54.5%)	10 (30.3%)	25 (39.7%)	4 (30.8%)	14 (26.9%)
HBeAg negative, N (%)	129 (68.6%)	70(45.5%)	23(69.7%)	38 (60.3%)	9 (69.2%)	38 (73.1%)
Log (HBV-DNA), median [Q1, Q3]	13.4 [9.94–17.0]	16.0 [12.9–17.7]	12.4 [8.77–17.2]	14.5 [11.2–16.3]	12.1 [11.9–12.3]	13.4 [10.2–16.4]

AUC value, and a perfect model was indicated with an AUC value of 1. The AUC for each model was compared with the null model. Each variable with a *p*-value below 0.05 in the univariate analysis was entered into the model. Next, a quadratic term was applied to continuous variables to evaluate the non-linearity assumption. Subsequently, we used RFE algorithms described in **Supplementary Methods** for model variable and interaction selection (19). To reduce the risk of overfitting, a resampling algorithm with five repeats of 10-fold cross-validation were performed.

Model Development, Evaluation, and External Validation

We developed a logistic regression model for the optimal combination of variables ultimately selected by the RFE algorithm and evaluated model performance using AUC, precision-recall (PR) curves, sensitivity, specificity, accuracy, positive predictive values (PPV), negative predictive values (NPV), and brier scores (31). Hosmer-Lemeshow (32) tests were used to assess goodness-of-fit. We plotted the calibration curves by calculating the predicted and true probabilities. The closer the calibration curve is to the 45° diagonal, the better the model performs. We selected a threshold for the balance of sensitivity and specificity on the development set and used this threshold for the geographical external validation at two different hospitals. In addition, the methodology of the model updating to external validation is exhibited in **Supplementary Methods**.

Online Application

Nomograms are a graphical representation of predictive statistical models for individual patients (33). Nomogram scoring system based on the results of optimal combination using the RMS package in R version 3.6.2 (34) and web page calculator by using package Shiny for R statistical software (35) were created. Then, we developed an online app to facilitate the use of the data and results from the study. It consists of a website interface to make the results flexible and easily accessible. This application can be accessed and used by physicians.

TABLE 2 | Performance of prediction model.

Variable	Development cohort	Validation cohort 1	Validation cohort 2
AUC (95% CI)	0.959 (0.941, 0.977)	0.932 (0.876, 0.987)	0.905 (0.826, 0.984)
Cutoff	0.614*	0.614	0.614
Sensitivity	0.894	0.909	0.923
Specificity	0.896	0.762	0.596
Accuracy	0.895	0.813	0.662
Positive predictive value	0.913	0.667	0.364
Negative predictive value	0.873	0.941	0.969

**We chose cutoff based on a balance of sensitivity and specificity.*

Statistical Analysis

For continuous variables, data were described as mean (SD) or median (interquartile spacing), whereas categorical variables were presented as frequencies. Data preprocessing were constructed by R package recipes (36). RFE algorithm was performed using the RFE function in the caret (37) package. The brier score (38) was calculated using the Brier score function in the DescTools (39). We used the survminer (40) package to draw the Kaplan–Meier survival curves (41), calculated the log-rank *p*-value, and used the concordance index (C-index) (41), and also 95% CI as the evaluation index of comparing survival probability. All statistical analyses were performed using the R version 3.6.2 software (Institute for Statistics and Mathematics, Vienna, Austria; http://www.r-project.org) (42). All results were considered statistically significant at $P < 0.05$.

RESULTS

Characteristics of Patients

The clinicopathological baseline characteristics of the patients in each cohort are listed in **Table 1**. A total of 503 patients were enrolled in the study, including 446 (88.7%) men and 57 (11.3%) women. There were 196 (39.0%) patients with HBeAg positive and 130 (38.0%), 25 (26.0%), 12 (18.5%) patients had liver cirrhosis in the development cohort and two validation cohorts, respectively. In the development cohorts, precipitating event of 242 (67.2%) cases was a spontaneous hepatitis B flare-up. The other precipitating events of HBV reactivation were infection in 19.5% of the patients, inappropriate withdrawal of nucleos(t)ide analogs in 5.2% of cases, history of alcohol intake before hospitalization in 5.8% of cases, and the use of hepatotoxic herbal medications in 2.3% of cases. There were 482 patients who received antiviral therapy including entecavir, lamivudine, or telbivudine within 3 days of admission according to their HBV replication levels and willingness, while 21 patients refused to receive antiviral therapy, and 12 patients received artificial liver support system therapy. The mean number of days between hospital admission and the development of ACLF was 7.9 days (range, 2–28days). We observed 188 (55%), 33 (34.4%), 13 (20%) ACLF events within 4 weeks in the development cohort and two in the validation cohorts, respectively. The mean follow-up time was 118.1 weeks (range 12–196 weeks) for the primary cohort. The survival rate of all patients at 90 days was 292 (85.38%). There were 16 patients in the training cohort who received liver transplantation, and 34 patients died within 3 months.

Variable Selection

Univariate analysis showed that AST, creatinine, and Gender with $p > 0.05$ were eliminated (**Supplementary Table 2**). There was no evidence for non-linear relationships for any continuous predictors and no significant interaction effects for the model. To ensure the stability of the model, we also removed Fib that had the absolute correlation coefficient value >0.6 with PT. Finally, according to the results of the RFE algorithm, we selected the best combination from the remaining 12 candidate variables which were PT, age, TBil, and ALT (**Supplementary Table 3** and **Supplementary Figure 3**).

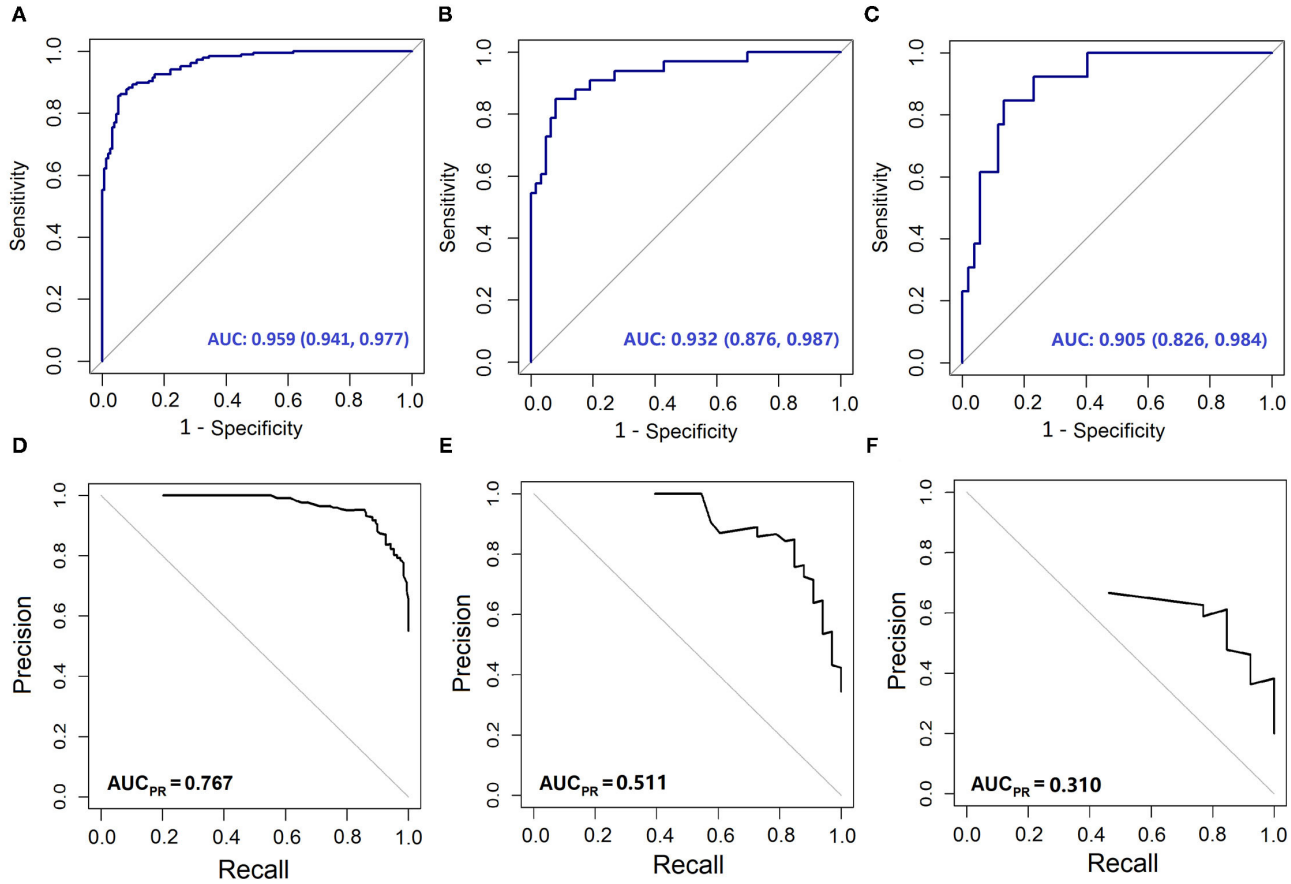


FIGURE 2 | The Area Under the receiver operating characteristic Curve (AUC) and Precision-Recall (PR) curves of different models. The AUC curves of **(A)** development set; **(B)** validation cohort 1; **(C)** validation cohort 2. The PR curve of **(D)** development set; **(E)** validation cohort 1; **(F)** validation cohort 2.

Model Performance and Validation for Predicting ACLF Development

The variables PT, age, TBil, and ALT were used to construct the logistic regression model called the PATA model. The prediction risk probability of ACLF can be calculated by the following model: linear predictor = 0.341 + 3.111*PT + 0.595*age + 0.626*TBil + (-0.295) *ALT. Predicted risk probability = 1 / (1 + e ^ linear predictor). The cut-off value for the high-risk and low-risk groups was 0.614 based on a balance of sensitivity and specificity (**Table 2**). The AUC of the model on the development set was 0.959 (0.941, 0.977). For two external validation cohorts, the AUC achieved 0.932 (0.876–0.987) and 0.905 (0.826–0.984) (**Table 2** and **Figure 2**), which means that the PATA model has a high predictive effect for liver failure. The calibration curves have good linearity with the brier scores of 0.083, 0.159, and 0.279, respectively (**Figure 3**). The calibration of the model was assessed *via* the Hosmer–Lemeshow goodness-of-fit test (P = 0.147). We also performed a model update on the external validation set using a closed likelihood ratio test in **Supplementary Methods** and **Supplementary Figure 4**. The related results after updating were shown in **Supplementary Tables 4**, **5**, **Supplementary Figure 5,** and **Figures 3C,E**.

Nomogram and Online Tools

We developed a nomogram scoring system and also a web page calculator to help physicians with quantitative scoring (**Figure 4**). The web interface created for clinicians allows the visualization of key information for risk prediction on cloud sever. This online application can be accessed by phone or computer but requires an internet connection for both private and public use (https://mia9510.shinyapps.io/MIA_LF/).

Predictive Power for 90 Days Mortality Compared With MELD Score and CTP Score

To explore the prognostic differences in the model risk stratified population, we compare our mortality prediction to the MELD score and CTP score in the development set. Patients were divided into a low-risk group (MELD

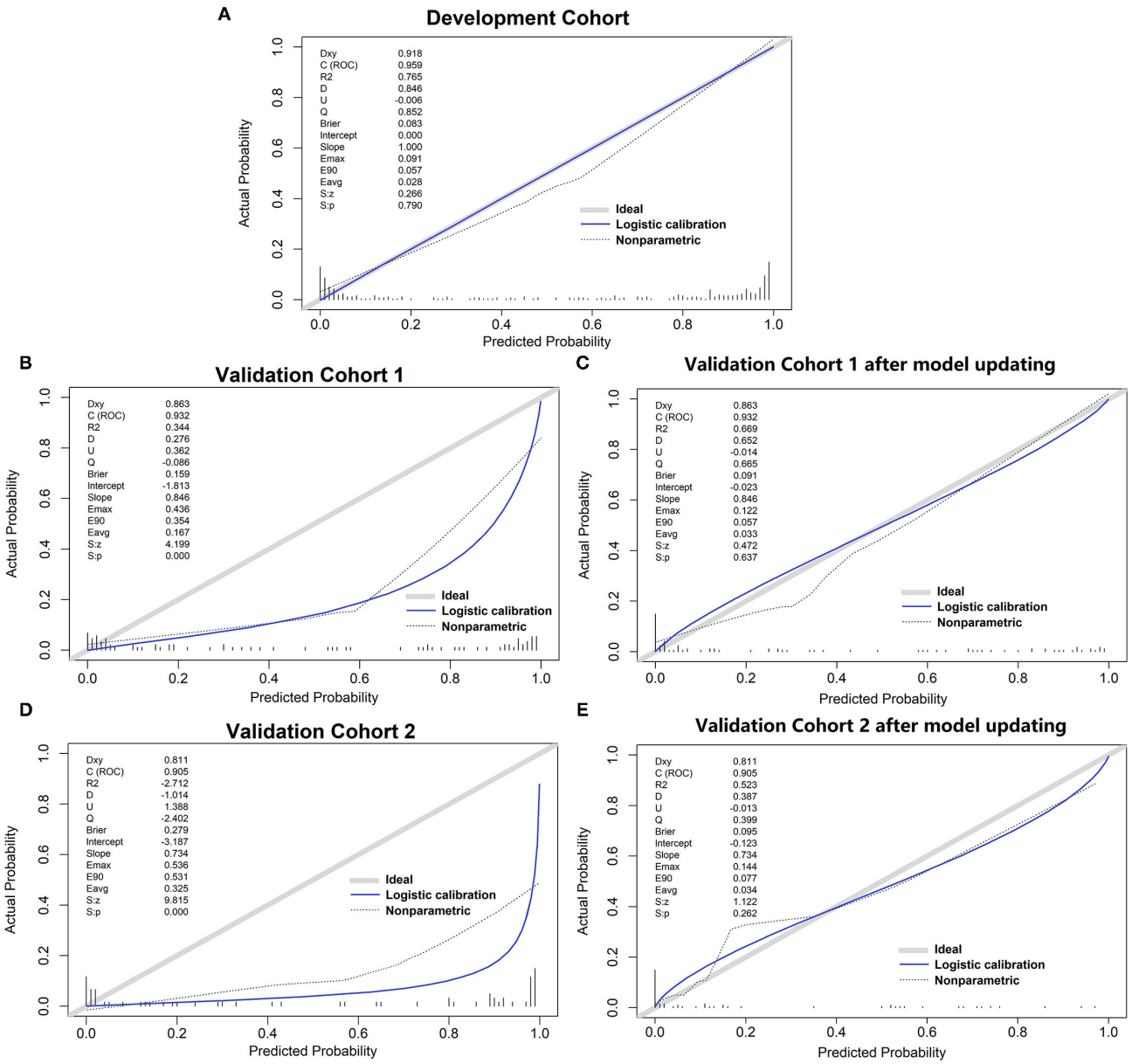


FIGURE 3 | The calibration curve of different models. **(A)** development set; **(B)** validation cohort 1; **(C)** validation cohort 1 after model updating; **(D)** validation cohort 2; **(E)** validation cohort 2 after model updating.

score ≤30) and a high-risk group (MELD score > 30) for further analysis. Accordingly, patients were also divided into three groups based on CTPstage as follows: low-risk group (CTP-A), medium-risk group (CTP-B), and high-risk group (CTP-C). The stratification into different risk subgroups allowed significant distinction between Kaplan–Meier curves for survival outcomes (log $p < 0.05$). As shown in **Figure 5**, the PATA model outperformed the other models in 90 days of prognostic stratification for patients.The C-index was 0.720 (0.675–0.765) for our PATA model, 0.549 (0.506–0.592) for MELD score, and 0.648 (0.581–0.715) for CTP score.

DISCUSSION

Owing to the unpredictable outcome of rapidly progressing liver failure, early identification of ACLF is fundamental to implement appropriate preventive strategies in SAE patients with CHB. In recent years, several prognostic models have been developed for risk stratification of liver failure, but no predictive model has been widely accepted. In the current study of 503 patients with CHB with SAE from three medical centers, by using the RFE algorithm, we developed and validated a novel risk prediction model for ACLF using PT, age, TBil, and ALT. The predictive model demonstrated reasonably good discrimination and calibration.

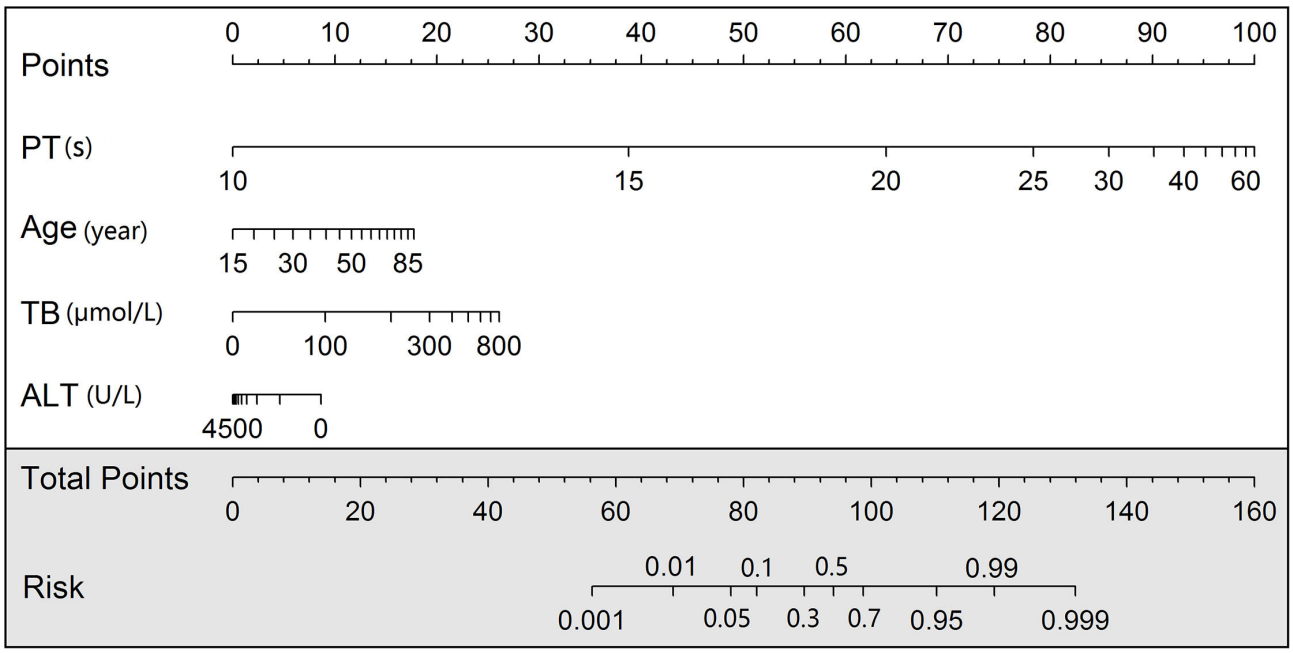


FIGURE 4 | Nomogram for development set.prothrombin time (PT), Tbil, alanine aminotransferase (ALT).

The AUC values of our PATA model were 95.9, 93.2, and 90.5% in the development cohort and two validation cohorts, respectively. The use of the PATA model may provide improved ability to early identify patients who are truly at increased risk of ACLF. We also compared the mortality predictive performance of the PATA model to that of the MELD score and CTP score. The results indicated that the PATA model showed better discrimination. The stratification of risk by the PATA model significantly improved on prediction and prognosis of ACLF for patients with CHB with SAE. It means that after screening high-risk groups by using the model, these patients not only have a higher incidence of liver failure but also a poor prognosis. Through this accurate prediction, the scoring system may be effective for guiding and optimizing therapeutic strategy. On the one hand, it can reduce the incidence of liver failure by early drug or artificial liver treatment, and on the other hand, it can make clear the prognosis of patients and prepare for liver transplantation.

Our PATA model with the best combination filtered by the RFE algorithm, including PT, age, TBil, and ALT. Older age has been identified as a risk factor in some studies (43, 44). So far, how the liver is affected by increasing age has not been fully elucidated. PT and TBil are commonly recognized as reliable markers of liver dysfunction (45–47). In this study and as well as previous studies (27, 29, 48), PT and TBil were significant independent risk factors for ACLF. Interestingly, low ALT level was an independent risk factor for progression to ACLF which was consistent with Yuan et al. (28) ALT level reflects the degree of hepatocyte necrosis resulting from acute injury and high levels of ALT persisted for several weeks after control of HBV and clearance of HBsAg from the circulation (49). Since a large number of liver cell necrosis occurs during liver failure, ALT in the blood decreases gradually, but bilirubin increases gradually, by the bilirubin-enzyme separation phenomenon (50), which is often the risk factor for the prognosis of patients with HBV-ACLF. Therefore, we assumed that early bilirubin-enzyme separation indicated a poor prognosis of SAE.

Our study has several strengths. First, we would like to emphasize that all variables were simple, readily available laboratory indices, and can be measured in the real-world clinical setting. When our model was applied to a new cohort, the cutoff recommended was the same as obtained from this study. The model can be updated as shown in **Supplementary Methods** (50–54) for improving transportability to other individuals if the new center has an expanded set of variables. Second, the prediction model does not require clinicians to perform complex calculations but simple, practical, and feasible calculations to be applied. Our online system supports mobile access, allowing physicians to assess in real-time and assist in decision making based on the results of the assessment. The model enables clinicians to more easily engage with the patient with CHB in a discussion of risk and thus enhance risk communication. Having a substantially high risk of ACLF could serve as a trigger to initiate more frequent clinical visits and more aggressive treatment. Third, by having two external validation sets, a fixed model was generated in the development set and then validated in another two hospitals.

We also acknowledge limitations to this work. First, more than a dozen definitions have emerged to describe ACLF. Our model was based on APASL 2019 using a population that was all Asian. We have not yet validated the performance of the model from other populations. Second, since the diversity existed between the training cohort and two external validation cohorts in this study, the performance of the model may be affected, especially

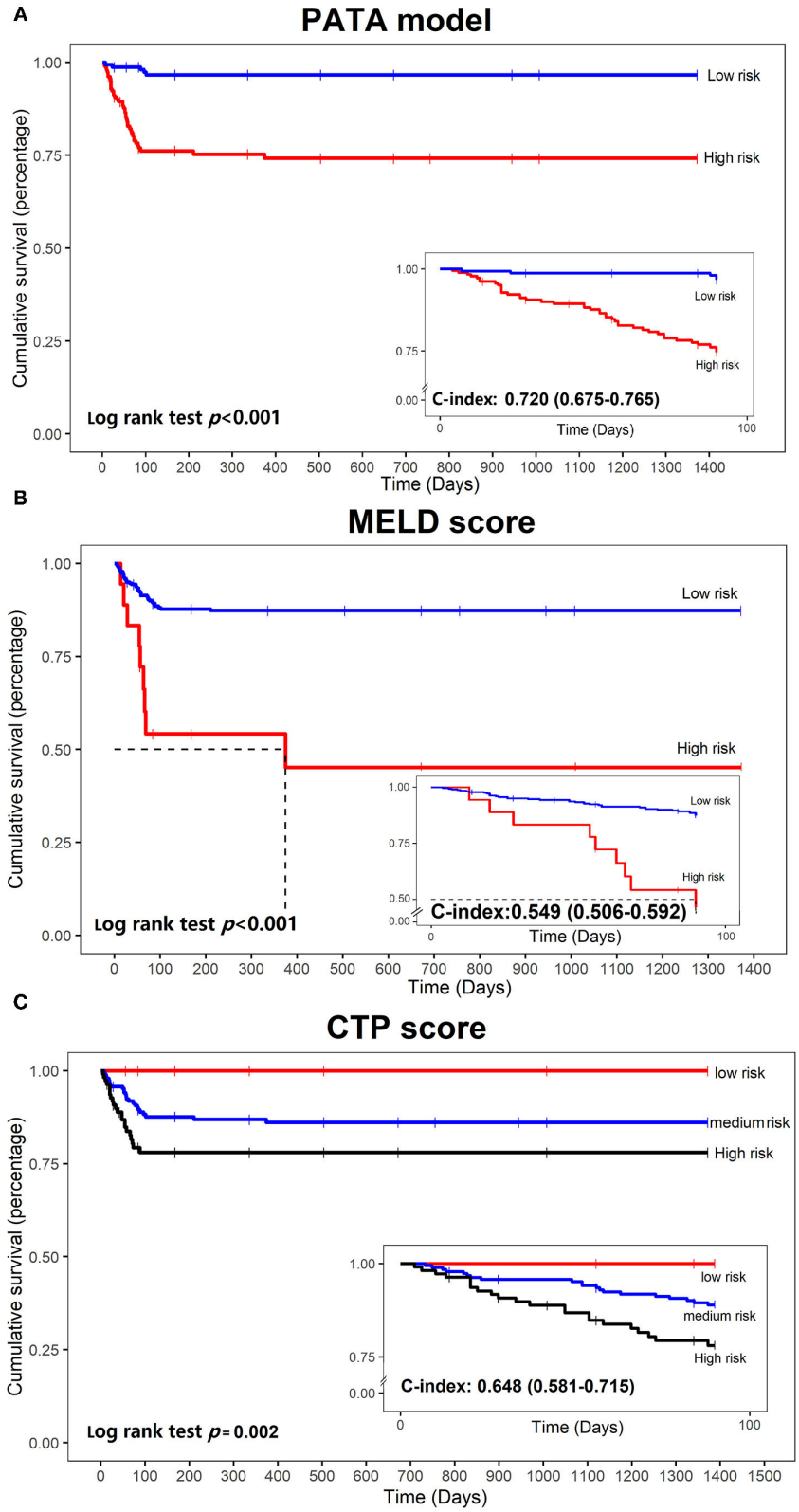


FIGURE 5 | Kaplan–Meier curves of mortality curves according to the different risks of liver failure. **(A)** PATA model; **(B)** model for end-stage liver disease (MELD) score; **(C)** Child–Turcotte–Pugh (CTP) scoring system.

PPV. We found that the incidence of liver failure in the training set was significantly higher indicating that liver injury severity in our patients was more aggressive. The Third Affiliated Hospital of Sun Yat-sen University hospital is a tertiary fixed-point hospital for hepatitis, almost all severe patients with liver diseases in Guangdong Province will come to see a doctor, so selection bias might exist in the recruitment of participants. Third, the at-risk patient populations may differ at baseline and several months after follow-up. Present models were generated using baseline data. Whether the model is suitable to use after situations of patients are changed during follow-up is questionable and needs further perspective experiment validation.

In summary, using data derived from a multicenter cohort, we constructed a novel prediction model that uses simple, readily available variables to predict ACLF and patient survival in patients with CHB with SAE. This model will empower clinicians and patients with more accurate, patient-specific information regarding the risk of ACLF. Identification of high-risk individuals may facilitate appropriate preventative options to reduce the occurrence of ACLF. Future studies are needed to confirm the applicability of our model in the clinical setting and to determine the effect of our model.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University [(2018)02-384-01]. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MY and XiaL conceived the study, drafted the manuscript, and was involved in the interpretation of the data. YL and ZL performed data analysis and interpretation. YJ, XinL, XS, SZ, YW, and WX assisted with the provision of study materials or patients, collection, and assembly of data. YC and ZL involved in revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

FUNDING

This work was supported by a grant for National Key Science Research and Development Program of China (2018YFC1315400); Key Science Research and Development Program of Guangdong province (2019B020228001); Guangdong Basic and Applied Basic Research Foundation (2019A1515110166); Science and Technology Program of Guangzhou (201804010474); the 5,010 Project of Clinical Research in Sun Yat-sen University (2016009); and Natural Science Foundation of Guangdong (2018A030310272).

ACKNOWLEDGMENTS

We would like to thank the Yuedong Hospital of the Third Affiliated Hospital of Sun Yat-sen University for their participation in the study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.748915/full#supplementary-material

REFERENCES

1. Liaw YF, Chu CM. Hepatitis B virus infection. *Lancet.* (2009) 373:582–92. doi: 10.1016/S0140-6736(09)60207-5
2. Chang ML, Liaw YF. Hepatitis B flares in chronic hepatitis B: pathogenesis, natural course, and management. *J Hepatol.* (2014) 61:1407–17.
3. Trépo C, Chan HL, Lok A. Hepatitis B virus infection. *Lancet.* (2014) 384:2053–63. doi: 10.1016/S0140-6736(14)60220-8
4. Seeff LB, Koff RS. Evolving concepts of the clinical and serologic consequences of hepatitis B virus infection. *Semin Liver Dis.* (1986) 6:11–22. doi: 10.1055/s-2008-1040788
5. Shouval D. The pros and cons of lamivudine vs. entecavir in decompensated or severe acute exacerbation of chronic hepatitis. *B J Hepatol.* (2014) 60:1108–9.
6. Kumar M, Chauhan R, Gupta N, Hissar S, Sakhuja P, Sarin SK. Spontaneous increases in alanine aminotransferase levels in asymptomatic chronic hepatitis B virus-infected patients. *Gastroenterology.* (2009) 136:1272–80. doi: 10.1053/j.gastro.2009.01.011
7. Wong VW, Chan HL. Severe acute exacerbation of chronic hepatitis B: a unique presentation of a common disease. *J Gastroenterol Hepatol.* (2009) 24:1179–86. doi: 10.1111/j.1440-1746.2009.05924.x
8. Liaw YF. Acute exacerbation and superinfection in patients with chronic viral hepatitis. *J Formos Med Assoc.* (1995) 94:521–8.
9. Chen EQ, Zeng F, Zhou LY, Tang H. Early warning and clinical outcome prediction of acute-on-chronic hepatitis B liver failure. *World J Gastroenterol.* (2015) 21:11964–73. doi: 10.3748/wjg.v21.i42.11964
10. Wlodzimirow KA, Eslami S, Abu-Hanna A, Nieuwoudt M, Chamuleau RA. A systematic review on prognostic indicators of acute on chronic liver failure and their predictive value for mortality. *Liver Int.* (2013) 33:40–52. doi: 10.1111/j.1478-3231.2012.02790.x
11. Wu T, Li J, Shao L, Xin J, Jiang L, Zhou Q, et al. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure. *Gut.* (2018) 67:2181–91. doi: 10.1136/gutjnl-2017-314641
12. Bernal W, Jalan R, Quaglia A, Simpson K, Wendon J, Burroughs A. Acute-on-chronic liver failure. *Lancet.* (2015) 386:1576–87. doi: 10.1016/S0140-6736(15)00309-8
13. Qiao CY Li F, Teng Y, Zhao J, Hu N, Fan YC, et al. Aberrant GSTP1 promoter methylation predicts poor prognosis of acute-on-chronic hepatitis B pre-liver failure. *Clin Exp Med.* (2018) 18:51–62. doi: 10.1007/s10238-017-0466-1
14. Botta F, Giannini E, Romagnoli P, Fasoli A, Malfatti F, Chiarbonello B, et al. MELD scoring system is useful for predicting prognosis in patients with liver cirrhosis and is correlated with residual liver function: a European study. *Gut.* (2003) 52:134–9. doi: 10.1136/gut.52.1.134
15. Sarmast N, Ogola GO, Kouznetsova M, Leise MD, Bahirwani R, Maiwall R, et al. Model for end-stage liver disease-lactate and prediction of inpatient

mortality in patients with chronic liver disease. *Hepatology.* (2020) 72:1747–57. doi: 10.1002/hep.31199
16. Xia Q, Dai X, Zhang Y, Guo Y, Xu X, Yang Q, et al. A modified MELD model for Chinese pre-ACLF and ACLF patients and it reveals poor prognosis in pre-ACLF patients. *PLoS ONE.* (2013) 8:e64379. doi: 10.1371/journal.pone.0064379
17. Yang H-I, Sherman M, Su J, Chen P-J, Liaw Y-F, Iloeje UH, et al. Nomograms for risk of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. *J Clin Oncol.* (2010) 28:2437–44. doi: 10.1200/JCO.2009.27.4456
18. Pankratz VS, Degnim AC, Frank RD, Frost MH, Visscher DW, Vierkant RA, et al. Model for individualized prediction of breast cancer risk after a benign breast biopsy. *J Clin Oncol.* (2015) 33:923–9. doi: 10.1200/JCO.2014.55.4865
19. Johnson MKK. *Feature Engineering and Selection. 1st Ed.* New York, NY: Chapman and Hall/CRC (2019) doi: 10.1201/9781315108230
20. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. *BMC Med.* (2015) 13:1. doi: 10.1186/s12916-014-0241-z
21. Tsubota A, Arase Y, Suzuki Y, Suzuki F, Sezaki H, Hosaka T, et al. Lamivudine monotherapy for spontaneous severe acute exacerbation of chronic hepatitis B. *J Gastroenterol Hepatol.* (2005) 20:426–32. doi: 10.1111/j.1440-1746.2004.03534.x
22. Wong VW, Wong GL, Yiu KK, Chim AM, Chu SH, Chan HY, et al. Entecavir treatment in patients with severe acute exacerbation of chronic hepatitis B. *J Hepatol.* (2011) 54:236–42. doi: 10.1016/j.jhep.2010.06.043
23. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, et al. Correction to: acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. *Hepatol Int.* (2019) 13:826–8. doi: 10.1007/s12072-019-09980-1
24. Botero RC, Lucey MR. Organ allocation: model for end-stage liver disease, child-turcotte-pugh, mayo risk score, or something else. *Clin Liver Dis.* (2003) 7:715–27. doi: 10.1016/s1089-3261(03)00052-7
25. Shi KQ, Zhou YY, Yan HD Li H, Wu FL, Xie YY, et al. Classification and regression tree analysis of acute-on-chronic hepatitis B liver failure: seeing the forest for the trees. *J Viral Hepat.* (2017) 24:132–40. doi: 10.1111/jvh.12617
26. Zaccherini G, Baldassarre M, Bartoletti M, Tufoni M, Berardi S, Tamè M, et al. Prediction of nosocomial acute-on-chronic liver failure in patients with cirrhosis admitted to hospital with acute decompensation. *JHEP Rep.* (2019) 1:270–7. doi: 10.1016/j.jhepr.2019.07.005
27. Gao FY, Liu Y, Li XS, Ye XQ, Sun L, Geng MF, et al. Score model for predicting acute-on-chronic liver failure risk in chronic hepatitis B. *World J Gastroenterol.* (2015) 21:8373–81. doi: 10.3748/wjg.v21.i27.8373
28. Yuan L, Zeng BM, Liu LL, Ren Y, Yang YQ, Chu J, et al. Risk factors for progression to acute-on-chronic liver failure during severe acute exacerbation of chronic hepatitis B virus infection. *World J Gastroenterol.* (2019) 25:2327–37. doi: 10.3748/wjg.v25.i19.2327
29. Li C, Zhu B, Lv S, You S, Xin S. Prediction model of the progression of patients with acute deterioration of hepatitis B virus-related chronic liver disease to acute-on-chronic liver failure. *Medicine.* (2018) 97:e11915. doi: 10.1097/MD.0000000000011915
30. Weisberg S. *Yeo-Johnson Power Transformations* (2001). Available online at: http://www.stat.umn.edu/arc/yjpower.pdf
31. Brier GW. Verification of Forecasts Expressed in Terms of Probability. *Mon Weather Rev*. (1950) 78:1–3.
32. Pan J, Zhu W, Li S, Xu J, He T. Extension of the hosmer-lemeshow goodness of fit statistic to linear models with repeated measurements. *European J Org Chem.* (2010) 2006:986–1001. doi: 10.1002/ejoc.200500642
33. Wang Y, Li J, Xia Y, Gong R, Wang K, Yan Z, et al. Prognostic nomogram for intrahepatic cholangiocarcinoma after partial hepatectomy. *J Clin Oncol.* (2013) 31:1188–95. doi: 10.1200/JCO.2012.41.5984
34. Frank E. *RMS: Regression Modeling Strategies*. R package version 6.0-1. 6.0-1 ed. Available online at: https://cran.r-project.org/web/packages/rms/
35. Winston C, Joe C, JJ A, Carson S, Barret S, Yihui X, Jeff A, et al. *Shiny: Web Application Framework for R: Web Application Framework.* (2017). Available online at: https://cran.r-project.org/web/packages/shiny/
36. Kuhn M, Wickham H. *RStudio Recipes: Preprocessing Tools to Create Design Matrices.* (2018). Available online at: https://cran.r-project.org/web/packages/recipes/index.html
37. Kuhn M. Caret: Classification and Regression Training (2013). Available online at: https://cran.r-project.org/web/packages/caret/
38. Gerds TA, Schumacher M. Consistent estimation of the expected Brier score in general survival models with right-censored event times. *Biom J.* (2010) 48:1029–40. doi: 10.1002/bimj.200610301
39. Signorell A, Aho K, Alfons A, Anderegg N, Zeileis A. *DescTools.* (2014). https://cran.microsoft.com/snapshot/2020-08-01/web/packages/DescTools/index.html
40. Kassambara A, Kosinski M, Biecek P, Fabian S. *survminer: Drawing Survival Curves using 'ggplot2' 0.4.9 ed2021.* (2021). Available online at: https://cran.r-project.org/web/packages/survminer/
41. Andersen P, Gill R. Cox's Regression Model for Counting Processes: A Large Sample Study. *Ann Stat*. (1982) 10:1100–20. doi: 10.1214/aos/1176345976
42. Team RDC. *A Language and Environment for Statistical Computing*. Vienna, Austria (2011). p. R Foundation for Statistical Computing.
43. Schiødt FV, Chung RT, Schilsky ML, Hay JE, Christensen E, Lee WM. Outcome of acute liver failure in the elderly. *Liver Transpl.* (2009) 15:1481–7. doi: 10.1002/lt.21865
44. O'Grady JG, Alexander GJ, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. *Gastroenterology.* (1989) 97:439–45. doi: 10.1016/0016-5085(89)90081-4
45. Zhang Q, Han T, Li Y, Nie C, Liu H. Predictors of progression into acute-on-chronic liver failure from acute deterioration of pre-existing chronic liver disease. *Hepatol Res.* (2016) 46:320–8. doi: 10.1111/hepr.12567
46. Huang K, Ji F, Xie Z, Wu D, Xu X, Gao H, et al. Artificial liver support system therapy in acute-on-chronic hepatitis B liver failure: Classification and regression tree analysis. *Sci Rep.* (2019) 9:16462. doi: 10.1038/s41598-019-53029-0
47. Furuyama T, Kudo A, Matsumura S, Mitsunori Y, Aihara A, Ban D, et al. Preoperative direct bilirubin to prothrombin time ratio index to prevent liver failure after minor hepatectomy. *J Hepatobiliary Pancreat Sci.* (2016) 23:763–70. doi: 10.1002/jhbp.400
48. Tiribelli C, Ostrow JD. New concepts in bilirubin and jaundice: report of the third international bilirubin workshop, April 6-8, 1995, Trieste, Italy. *Hepatology.* (1996) 24:1296–311. doi: 10.1002/hep.510240551
49. Webster GJ, Reignat S, Maini MK, Whalley SA, Ogg GS, King A, et al. Incubation phase of acute hepatitis B in man: dynamic of cellular immune mechanisms. *Hepatology.* (2000) 32:1117–24. doi: 10.1053/jhep.2000.19324
50. Ying L, Jing Z, Zhongfeng W. Prognostic factors for patients with hepatitis B virus-related acute-on-chronic liver failure. *J Clin Exp Hepatol.* (2017) 33:497–501.
51. Kuhn M, Johnson K. *Feature Engineering and Selection.* 1st ed. New York, NY: Chapman and Hall/CRC (2020).
52. Moons KG, Kengne AP, Grobbee DE, Royston P, Vergouwe Y, Altman DG, et al. Risk prediction models: II. External validation, model updating, and impact assessment. *Heart.* (2012) 98:691–8. doi: 10.1136/heartjnl-2011-301247
53. Pavlou M, Ambler G, Seaman SR, Guttmann O, Elliott P, King M, et al. How to develop a more accurate risk prediction model when there are few events. *BMJ.* (2015) 351:h3868. doi: 10.1136/bmj.h3868
54. Vergouwe Y, Nieboer D, Oostenbrink R, Debray TPA, Murray GD, Kattan MW, et al. A closed testing procedure to select an appropriate method for updating prediction models. *Stat Med.* (2017) 36:4529–39. doi: 10.1002/sim.7179

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.

frontiers in Medicine

ORIGINAL RESEARCH
published: 02 November 2021
doi: 10.3389/fmed.2021.751807

A Prospective Multicenter Study of the Chinese Scoring System for Hepatitis B Liver Failure

Wu Zhe-bin[1†], Lin Bing-liang[1†], Peng Liang[1†], Chen Zhi[2], Zhang Xin-xin[3], Tan De-ming[4], Ren Wan-hua[5], Wang Kai[6], Yan Xue-bin[7], Ke Wei-min[1], Zheng Yu-bao[1] and Gao Zhi-liang[1*]*

[1] Department of Infectious Diseases, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, [2] The First Affiliated Hospital of Zhejiang University, Hangzhou, China, [3] Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital, Shanghai, China, [4] Xiangya Hospital, Central South University, Changsha, China, [5] Department of Infectious Diseases, Shandong Provincial Hospital, Jinan, China, [6] Department of Infectious Diseases, Shandong University Qilu Hospital, Jinan, China, [7] The Affiliated Hospital of Xuzhou Medical University, Xuzhou, China

OPEN ACCESS

Edited by:
Tao Chen,
Huazhong University of Science and Technology, China

Reviewed by:
Jarosław Jerzy Sak,
Medical University of Lublin, Poland
Yuzheng Zhuge,
Nanjing Drum Tower Hospital, China

****Correspondence:***
Gao Zhi-liang
gaozl@mail.sysu.edu.cn
Zheng Yu-bao
zhybao@mail.sysu.edu.cn

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *02 August 2021*
Accepted: *22 September 2021*
Published: *02 November 2021*

Citation:
Zhe-bin W, Bing-liang L, Liang P, Zhi C, Xin-xin Z, De-ming T, Wan-hua R, Kai W, Xue-bin Y, Wei-min K, Yu-bao Z and Zhi-liang G (2021) A Prospective Multicenter Study of the Chinese Scoring System for Hepatitis B Liver Failure. Front. Med. 8:751807. doi: 10.3389/fmed.2021.751807

Objective: To evaluate the clinical utility of a Chinese scoring system for hepatitis B liver failure in a prospective and multicenter study.

Methods: Clinical data for 1,143 patients with hepatitis B liver failure who had been followed up for a minimum of 6 months were collected from seven liver disease centers across China. The disease severity and prognosis for the patients were predicted using the Chinese scoring system and compared to those predicted with the model for end-stage liver disease (MELD) score, MELD-Na score, and Child-Turcotte-Pugh (CTP) score.

Results: The Chinese scoring system was more effective at predicting the outcomes of survival and mortality than the MELD score. In the peak disease stage, the area under the receiver operating characteristic curve for the Chinese scoring system was 0.954, significantly higher than that (0.896) for the MELD scoring system ($P < 0.001$). The positive prediction at 30, 90, and 180 days with the Chinese scoring system was 0.764 (95% CI: 0.714–0.808), 0.731 (95% CI: 0.694–0.769), and 0.724 (95% CI: 0.679–0.765), also significantly higher than that with the MELD, MELD-Na, and CTP scores ($P < 0.001$). In addition, the Chinese scoring system was superior to the MELD, MELD-Na, and CTP scores ($P < 0.001$) at predicting the prognosis of patients with hepatitis B liver failure at both 30 and 180 days.

Conclusion: The Chinese scoring system demonstrated superior performance to the three established scoring systems in assessing the severity and outcomes of hepatitis B liver failure in this cohort.

Keywords: hepatitis B, liver failure, scoring system, end-stage liver disease model, assessing

INTRODUCTION

Liver failure is a severe complication in which the liver fails to perform basic functions due to severe or massive liver injury, which also disrupts the vital functions of other organs and systems. It typically manifests as jaundice, hepatic encephalopathy, and coagulopathy (1, 2). The mortality rate of liver failure may be as high as 70% (3). Hepatologists must be able to assess and predict the

outcomes of liver failure accurately and early in patients, especially to determine if and when liver transplantation is required. Dynamic changes in the conditions of a patient should also be closely monitored and followed, as timely prevention and treatment of complications significantly impact the clinical outcomes. The models for end-stage liver disease (MELD), MELD-Na, and Child-Turcotte-Pugh (CTP) scores are three established systems used to numerally grade the severity of liver failure in patients, which can reveal the probability for favorable or non-favorable outcomes (4, 5). All three model systems were established by assessing patients with liver failure mainly caused by hepatitis C virus (HCV) infection and alcoholic liver disease. However, liver failure caused by hepatitis B virus (HBV) infection is characterized by acute onset, severe liver injury, and high mortality, with distinct pathogenesis and clinical characteristics from those caused by HCV and alcohol. The three model systems can be expanded or modified to better grade HBV liver failure. We previously established a new scoring system known as the Chinese scoring system and its performance and utility were evaluated for patients with hepatitis B liver failure in a retrospective study (6–9). To further investigate the utility of this new scoring system in grading the severity of hepatitis B liver failure and predicting outcomes, we conducted this prospective study at seven liver disease centers across China.

MATERIALS AND METHODS

Patients

Enrollment

We selected seven liver disease centers across China (infection department of the Third Affiliated Hospital of Sun Yat-sen University, infection department of the First Affiliated Hospital of Zhejiang University, Shanghai Ruijin Hospital, Xiangya Hospital of Central South University, Qilu Hospital of Shandong University, Shandong Provincial Hospital, and Xuzhou Medical College) with a high reputation in the country, high diagnosis and treatment capabilities in the field of liver disease, and more than 40 beds as the enrollment unit. The sample size was determined using a single population proportion formula by considering a 5% margin of error, 95% confidence level, 50% case fatality rate of liver failure in previous studies, 5% non-response rate, and 1.5 design effect. The resulting total sample size was 1,078. To account for potential dropouts, the total sample size was fixed to 1,200 and proportioned to the selected participating hospitals based on their clients' size: infection department of the Third Affiliated Hospital of Sun Yat-sen University ($n = 400$), infection department of the First Affiliated Hospital of Zhejiang University ($n = 200$), Shanghai Ruijin Hospital ($n = 200$), Xiangya Hospital of Central South University ($n = 100$), Qilu Hospital of Shandong University ($n = 100$), Shandong Provincial Hospital ($n = 100$), and Xuzhou Medical College ($n = 100$).

Between October 2013 and June 2016, a total of 1,143 chronic hepatitis B inpatients with liver failure (acute-on-chronic liver failure, ACLF) were enrolled from seven liver disease centers. Among them, 936 were men, 207 were women, and the average age was 43.4 ± 12.6 (ranged from 18 to 65). The patients were followed up for >6 months, during which 464 died and 680 survived. This study was registered in the Clinical Trials Registry (registration number: NCT01961440, https://clinicaltrials.gov/).

HBV-related ACLF diagnosis for all patients followed the criteria established by the 18th Asia-Pacific Association of Liver Research consensus on chronic-acute liver failure (10). The criteria include a history of chronic hepatitis B and an acute flare-up of liver injury with clinical manifestations of jaundice (total bilirubin ≥ 85 μmol/l) and coagulation disorder (prothrombin time international standardized ratio ≥ 1.5), ascites, and/or hepatic encephalopathy within 4 weeks.

Inclusion Criteria

Male or female patients aged 18–60; HBsAg-positive history > 6 months, HBV DNA-positive (≥20 IU/ml); HBeAg-positive or negative; persistent hepatitis symptoms of fatigue, anorexia, abdominal distention, or yellow urine; gradual aggravation of jaundice over a short period; total serum bilirubin ≥85 μmol/l or daily elevation ≥17.1 μmol/l; abnormal coagulation function; and the international standardized ratio of prothrombin time ≥1.5 were included.

Exclusion Criteria

Patients were excluded if they: (1) had other hepatitis virus infection; (2) had HIV infection, biliary, alcoholic liver, or autoimmune liver diseases; drug poisoning, liver tumors, or were undergoing liver transplantation, renal insufficiency, or long-term anticoagulant therapy related to renal diseases.

Observations and Follow-Up Endpoints

Clinical information and test findings for all patients at and after admission were collected weekly. The clinical information mainly consisted of the stages of hepatic encephalopathy. Laboratory findings included serum total bilirubin, albumin, creatinine, prothrombin time, prothrombin time international normalized ratio (INR), serum sodium ion concentration, liver size (B-mode ultrasound measurement), ascites, and pleural effusion (B-ultrasound measurement), and also infection (peripheral white blood cell count, neutrophil ratio, and chest inflammation images). The endpoint of 180 days of follow-up was used to determine the survival rate. The death count included patients that rejected rescue treatment and were discharged from the hospital and patients that died during the hospital stay or within 180 days of follow-up.

Score Calculation

MELD Score

Since all subjects had hepatitis B-related liver failure, the MELD score was calculated as 3.8 × loge (serum bilirubin μmol/l × 0.058) + 1.2 × loge (prothrombin time INR) + 9.6 × loge (serum creatinine μmol/l × 0.011) + 6.4 (6).

MELD-Na

This score was calculated as MELD + 1.59×(135-Na^+), wherein serum Na^+ levels ≥ 135 mmol/l were treated as 135, ≤ 120 as 120 mmol/l, and between 120 and 135 mmol/l as the specific value (7).

TABLE 1 | Assigned scores of the Chinese scoring system.

Scoring (score)	Hepatic encephalopathy (Stage)⑤	Total bilirubin (μmol/L)	The maximum depth of ascites (mm)②	Activity of PT (%)	Right hepatic oblique diameter/ thickness (mm)③	Creatinine (μmol/L)	Infection (depend on WBC 10^9/L)④
1	I	≥10∼20ULN①	>0∼40	30∼ <40	Oblique diameter ≥ 120 or Thickness ≥ 110	>1.0∼1.1ULN	WBC>10∼15 Or N>70%∼80%
2	II	>20∼30ULN	>40∼80	20∼ <30	Oblique diameter 110 ∼ <120 or Thickness 100∼ <110	>1.1∼1.2ULN	WBC>15∼20 Or N>80%∼90%
3	III	>30∼40ULN	>80	10∼ <20	Oblique diameter100∼ <110 or Thickness 90∼ <100	>1.2∼1.3ULN	WBC>20 Or N>90%
4	IV	>40ULN	>80 + one or both side pleural fluid	<10	Oblique diameter <100 or Thickness <90	>1.3ULN	Inflammation manifestation of lung

注: ① *ULN: Upper limit of normal.*

② *Score of ascites: Regardless of the amount of ascites, the presence of unilateral or bilateral pleural fluid is counted as 4 points.*

③ *Liver size scoring: If the right liver oblique diameter and thickness are measured at the same time on the ultrasound image, the high-scoring scoring results are taken.*

④ *Infection score: The standard of 4 points is the imaging changes of pulmonary inflammation, regardless of the white blood cells and neutrophil counted as 4 points.*

⑤ *HE grade 1: Trivial and mild clinical signs, such as mild cognitive impairment, decreased attention, sleep disorders (insomnia and sleep inversion), euphoria, or depression; Grade 2: Marked personality or behavioral changes, lethargy or apathy, slight orientation abnormality (time and orientation), decreased mathematical ability, dyskinesia, or unclear speech; Grade 3: Marked dysfunction (time and spatial orientation), abnormal behavior, semi-coma to coma, but responsive; Grade 4: Coma (no response to speech and external stimuli).*

CTP Score

This score was calculated using the scoring standards for five indexes, i.e., grade of hepatic encephalopathy, ascites, total bilirubin, albumin, and prolonged prothrombin time. A score of 1, 2, or 3 was assigned to each index to reflect the severity of each condition, and the CTP score was the sum of the five indexes (8).

Chinese Scoring System

This system consists of seven clinical indicators: prothrombin activity (PTA), serum creatinine, hepatic encephalopathy, serum total bilirubin, and liver size (B-ultrasonic measurement: oblique diameter of the right lobe of the liver: The standard measurement section is the oblique section of the right subcostal liver where the right hepatic vein and the middle hepatic vein merge into the inferior vena cava. The measurement points are placed at the liver envelope on the anterior and posterior edges of the right lobe to measure the maximum vertical distance; thickness of the right liver lobe: The largest section of the right lobe of the liver in the fifth or sixth intercostal space is the standard measurement section. The measurement points are placed at the liver envelope on the anterior and posterior edges of the right lobe to measure the maximum vertical distance), ascites/pleural fluid (B-ultrasonic measurement: When lying supine, measure the depth of the anterior liver, liver and kidney recesses, splenic fossa, right abdomen, and pelvic ascites, and take the largest value for score), volume, and infection (peripheral blood leukocyte count, neutrophil ratio, and chest inflammation image). A score of 1, 2, 3, or 4 was assigned to each indicator to reflect the severity, and the sum of the seven indicator scores was used to assess the severity of the overall condition (see **Table 1** for details). Unified operation method of ultrasound.

Statistical Analysis

(1) All numeral data were expressed as mean ± SD (±s) and the difference was computed using the f-test. (2) The count data were expressed as a percentage (%), and the difference was assessed with the χ^2 test. (3) Assessments of the short-term and long-term prognoses for patients with hepatitis B liver failure using the Chinese scoring system, MELD score, MELD-Na score, and CTP score were compared using receiver operating characteristic (ROC) curves and the ROC area under the curve (AUC). AUC > 0.7 was deemed to be of clinical utility and >0.8 to be of good prediction accuracy. The AUC values were compared using normal Z-tests and the ROC curve sensitivity and specificity were used to determine the best cut-off values for the score and the Youden's index. SPSS l8.0 statistical software (IBM SPSS Inc., Chicago, USA) was used for all analyses. $P < 0.05$ was considered statistically significant.

RESULTS

Demographic Features and Groups

A total of 1,143 patients with hepatitis B liver failure were enrolled in this study. Among them, 936 were men and 207 were women, and the mean age was 43.4 ± 12.6 years old (ranged between 18 and 65). A total of 463 patients died while the remaining 680 survived (**Table 2**).

The Cutoff Value and Distribution Range for the Chinese Scoring System and the MELD Score to Separate the Patients That Survived From Those That Did Not

The cutoff value of 12.14 with the Chinese scoring system separated 83.65% of patients in the survival and death groups, i.e., 83.65% of patients in the survival group scored between 3.68 and 12.14 points, while 83.65% of patients in the death group scored between 12.14 and 22.13 points. With the MELD score, the cutoff value was 31.78; 64.52% of patients in the survival group scored between 20.37 and 31.78 points, and 64.52% of patients in the death group scored between 31.78 and 49.88 points (see **Figures 1**, **2**, $P < 0.01$).

TABLE 2 | Baseline characteristics of included patients at admission.

Parameters	Death group (n = 463)	Survival group (n = 680)	t/x2 value	*P*
Age (year)	45.38 ± 11.73	40.06 ± 10.47	1.84	0.12
Males (%)	364/99	572/108	1.99	0.06
WBC (×109/L)	7.82 ± 3.54	6.98 ± 3.72	1.25	0.38
ALT (U/L)	568.47 ± 376.94	629.76 ± 504.57	1.56	0.28
Albumin (g/L)	31.64 ± 3.56	32.85 ± 4.02	0.98	0.47
TB (lmol/L)	428.75 ± 284.63	353.96 ± 249.66	3.45	<0.001
PTA (%)	29.86 ± 11.58	34.38 ± 10.85	4.78	<0.001
INR	3.41 ± 0.68	2.83 ± 0.62	4.27	<0.001
BUN/(mmol/L)	5.84 ± 3.68	5.22 ± 3.76	1.96	0.08
CR/(μmol/L)	82.37 ± 54.16	67.47 ± 44.85	1.92	0.09
HBV DNA, median (range), Log10 IU/ml	6.89 (4.30-8.86)	6.62(5.02-8.64)	1.89	0.16
Without cirrhosis, *n*(%)	296(63.93)	258(37.94)	8.94	<0.001
Encephalopathy (%)	16.58	9.73	10.76	<0.001
HRS (%)	8.23	5.13	2.95	<0.001
SBP (%)	54.32	31.06	6.83	<0.001
Ascites (%)	67.43	48.14	3.12	<0.001
MELD	40.52 ± 6.83	26.84 ± 6.18	16.83	<0.001
MELD-Na	44.76 ± 7.83	28.62 ± 5.93	19.05	<0.001
CTP	11.87 ± 1.68	10.56 ± 1.47	2.01	0.04
Chinese scoring system	17.53 ± 3.92	8.46 ± 3.28	22.18	<0.001

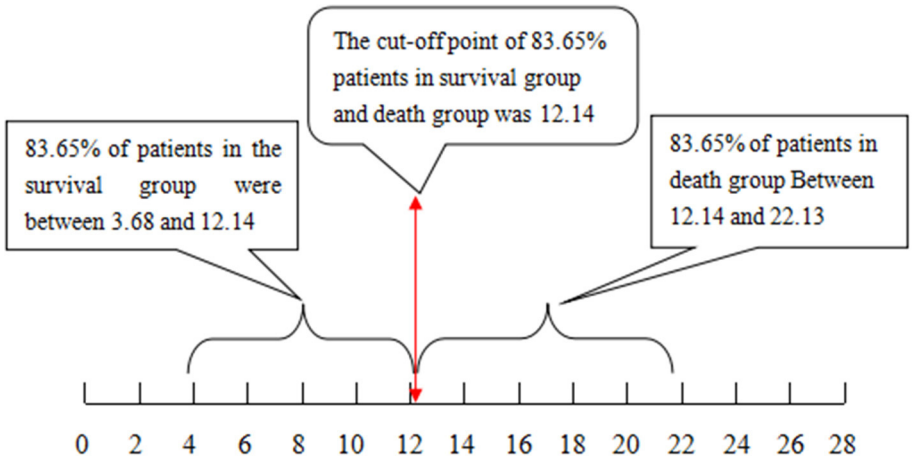


FIGURE 1 | The cutoff value and distribution range for the Chinese scoring system to separate the patients that survived from those that did not.

Comparison of the Performance to Grade the Severity at Peak Disease With the Chinese Scoring System and MELD Scoring System

The ROC curve was plotted using the total scores. The area under the ROC curve for the Chinese scoring system was 0.954 (95% CI: 0.943–0.971) and the standard error was 0.013, with $P < 0.001$, suggesting good prediction performance. The area under the ROC curve for the MELD score was 0.896 (95% CI: 0.881–0.924), indicating less effectiveness than the Chinese score system (see **Figure 3** and **Table 3**).

The Ability to Predict and Differentiate Disease Outcomes at Different Times With Different Scoring Systems

The predictive values for the outcomes on days 30, 90, and 180 after discharge using the Chinese scoring system were 0.764 (95% CI: 0.714–0.808), 0.731 (95% CI: 0.694–0.769), and 0.724 (95%

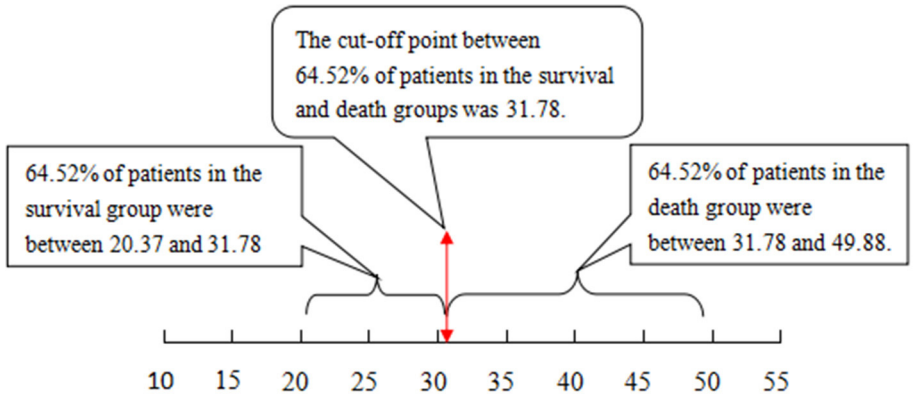


FIGURE 2 | The cutoff value and distribution range for the MELD score to separate the patients that survived from those that did not. MELD, model for end-stage liver disease.

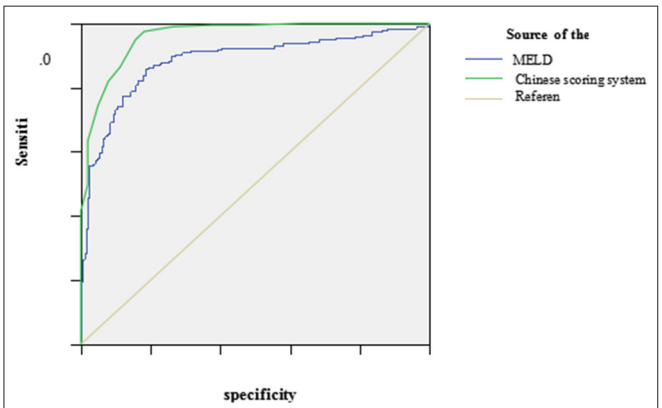


FIGURE 3 | The ROC curve of the Chinese scoring system and MELD scoring system at the peak disease. MELD, model for end-stage liver disease; ROC, receiver operating characteristic.

CI: 0.679–0.765), respectively, which were significantly higher than those for the other three scoring systems (see **Table 4**, $P < 0.001$).

Comparison of the Performance Predicting Short-Term (30 Days) Prognosis for Patients With Hepatitis B Liver Failure Using the Chinese Scoring System, MELD Score, MELD-Na Score, and CTP Score

The area under the ROC curve with the total score from the Chinese scoring system was 0.783 (95% CI: 0.732–0.865) and the standard error was 0.026, with $P < 0.001$, suggesting good predictive performance. The areas under the ROC curves for the MELD score, MELD-Na score, and CTP score were 0.706 (95% CI: 0.623–0.778), 0.716 (95% CI: 0.644–0.779), and 0.686 (95% CI: 0.623–0.767), respectively, which were significantly lower than that for the Chinese scoring system (see **Figure 4** and **Table 5**).

TABLE 3 | Comparison of the performance to grade the severity at the peak disease by Chinese scoring system and MELD scoring system.

	AUROC (95% CI)	*p*-value vs. CSS-LFB
CSS-LFB	0.954 (0.943–0.971)	
MELDs	0.896 (0.881–0.924)	<0.001

Comparison of the Performance Predicting Long-Term (180 Days) Prognosis for Patients With Hepatitis B Liver Failure Using the Chinese Scoring System, MELD Score, MELD-Na Score, and CTP Score

The area under the ROC curve with the total score from the Chinese scoring system was 0.748 (95% CI: 0.692–0.837) and the standard error was 0.022, with $P < 0.001$, suggesting good predictive performance. The areas under the ROC curves for the MELD score, MELD-Na score, and CTP score were 0.657 (95% CI: 0.588–0.725), 0.676 (95% CI: 0.605–0.743), and 0.682 (95% CI: 0.621–0.752), respectively, which were significantly lower than that for the Chinese scoring system (see **Figure 5** and **Table 6**).

DISCUSSION

MELD, MELD-Na, and CTP scores have been established to address the need to accurately identify patients with liver failure who require liver transplants. All three systems are widely accepted in the clinical setting. However, these models were constructed using clinical data largely from patients with liver failure in Europe and the United States where the major etiologies for liver failure are alcohol, drugs, hepatitis C, and cholestasis. In contrast, hepatitis B represents the dominant etiology for liver failure in China and is responsible for 85% of liver failure cases in the country (11). Therefore, these three models (12, 13) may not completely fit Chinese patients with liver failure. As noted by Lin Xianfeng et al. (14), the MELD score failed to accurately

TABLE 4 | The ability to predict and differentiate disease outcomes at different times with different scoring systems.

	CSS-LFB C-index (95% CI)	MELD C-index (95% CI)	MELD-Na C-index (95% CI)	Child-Pugh C-index (95% CI)
30-day mortality	0.764 (0.714–0.808)	0.684 (0.632–0.743)	0.685 (0.634–0.738)	0.664 (0.612–0.725)
p-value vs. CSS		<0.001	<0.001	<0.001
90-day mortality	0.731 (0.694–0.769)	0.657 (0.612–0.713)	0.662 (0.616–0.712)	0.656(0.607–0.704)
p-value vs. CSS		<0.001	<0.001	<0.001
180-day mortality	0.724 (0.679–0.765)	0.648 (0.604–0.698)	0.655 (0.607–0.699)	0.642 (0.593–0.691)
p-value vs. CSS		<0.001	<0.001	<0.001

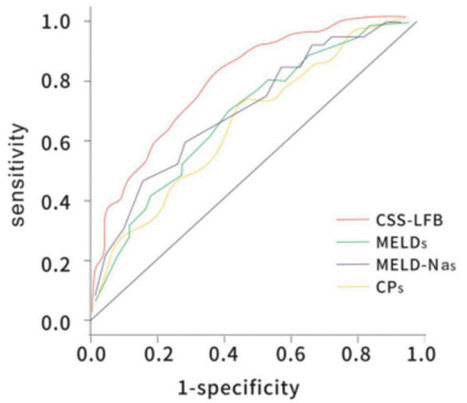


FIGURE 4 | The ROC curve of predicting short-term (30 days) prognosis of patients with hepatitis B liver failure among the Chinese scoring system, MELD score, MELD-Na scoring, and CTP scoring. CTP, Child-Turcotte-Pugh; MELD, model for end-stage liver disease; ROC, receiver operating characteristic.

TABLE 5 | Comparison of the area under the ROC curve of predicting short-term (30 days) prognosis of patients with hepatitis B liver failure among Chinese scoring system, MELD score, MELD-Na scoring, and CTP scoring.

	AUROC (95% CI)	*p*-value vs. CSS-LFB
CSS-LFB	0.783 (0.732–0.865)	
MELDs	0.706 (0.623–0.778)	0.0089
MELD-Nas	0.716 (0.644–0.779)	0.0097
CPs	0.686 (0.623–0.767)	0.0075

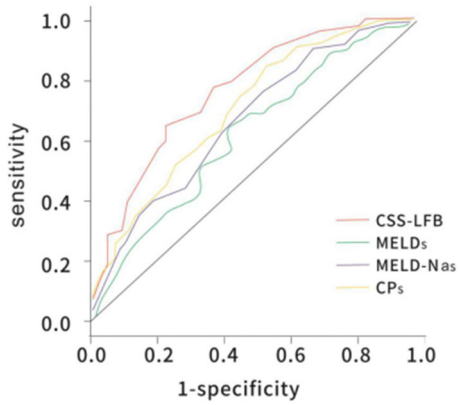


FIGURE 5 | The ROC curve of predicting long-term (180 days) prognosis of patients with hepatitis B liver failure among the Chinese scoring system, MELD score, MELD-Na scoring, and CTP scoring. CTP, Child-Turcotte-Pugh; MELD, model for end-stage liver disease; ROC, receiver operating characteristic.

TABLE 6 | Comparison of the area under the ROC curve of predicting long-term (180 days) prognosis of patients with hepatitis B liver failure among Chinese scoring system, MELD score, MELD-Na scoring, and CTP scoring.

	AUROC (95% CI)	*p*-value vs. CSS-LFB
CSS-LFB	0.748 (0.692–0.837)	
MELDs	0.657 (0.588–0.725)	0.0014
MELD-Nas	0.676 (0.605–0.743)	0.0082
CPs	0.682 (0.621–0.752)	0.0012

predict the prognosis of 327 Chinese patients with hepatitis B liver failure. One factor that may have affected this result is that patients with HBV liver failure do not respond well to comprehensive treatment and often need an artificial liver, liver transplant, or other treatment urgently. The other factor is that chronic HBV infection may exhaust the immunity of patients, leading to severe complications. A new system that considers these factors is required (15).

Child-Turcotte-Pugh (CTP) score has been widely employed in managing patients with liver diseases because of its simplicity and ease of calculation. However, it is often hindered by a lack of consistency as CTP is mainly based on albumin level and prothrombin time, both of which can be masked if exogenous albumin and plasma products are infused. In addition, hepatic encephalopathy and ascites measurement can be subjective and variable. CTP only consists of three grades with a narrow score range of 5–15, which limits the ability to differentiate. Patients with the same score may differ greatly in the severity of the liver disease.

Although the MELD and MELD-Na scoring systems are recognized as easy to use, reproducible, and accurate in clinical applications, they bear an inherent limitation because they only include bilirubin, coagulation function, renal function, and etiology, while excluding the complications of liver failure (infection, hemorrhage, hepatic encephalopathy, brain edema, or ascites). The Na concentration in the MELD-Na scoring system can be easily affected by insufficient intake, diuretics, or sodium

pump failure (12, 16–20). A study conducted in China (21) showed that the AUC of MELD and MELD-Na scores for the 12-week outcomes of patients with hepatitis B-related liver failure was only 0.731, 0.735, and 0.773, respectively, and the sensitivity was below 0.7.

This prospective study conducted at seven large liver disease centers across China showed that the Chinese scoring system better distinguished patients that survived from those who did not, in comparison to MELD ($P < 0.001$). The area under the ROC curve for the Chinese scoring system was 0.954 in the peak disease stage, suggesting a stronger ability to grade the severity of the liver failure. The AUC for the MELD scoring system was 0.896, which was relatively high, but significantly lower than that for the new scoring system ($P < 0.001$). We also found that the efficiency in predicting outcomes on the 30th, 90th, and 180th days after discharge with the Chinese scoring system was significantly higher than that with MELD, MELD-Na, and CTP ($P < 0.001$).

Massive or submassive liver injury following a flare-up of HBV replication or HBV reactivation compromises synthetic, metabolic, and detoxification functions in the liver, leading to reduced albumin levels and ascites, hyperbilirubinemia, decreased PTA, increased international standard ratio of prothrombin (INR), and hepatic encephalopathy. In addition, a severely injured liver may also hurt Kupffer cell function and reduce complement levels that may limit the anti-infective capacity. The accumulation of toxins and a decrease in renal blood flow may facilitate hyperbilirubinemia, renal function insufficiency, or even renal failure. In theory, the severity of liver failure among different individuals can be indicated by biomarkers that reflect critical alterations in the pathophysiology of liver failure. Thus, we formulated this new scoring system to improve severity grading and outcome prediction. A total of seven clinical indicators of hepatitis B liver failure are included in the Chinese scoring system, consisting of not only objective indicators such as Cr, total bilirubin, PTA, and liver size but also the complications of hepatic encephalopathy, ascites with pleural effusion, and pulmonary infection, which reflect the pathophysiological changes resulting from hepatitis B liver failure. The score boundary point was confirmed using the interactive chi-squared test, guided by the principle that emphasizes simplicity and clarity. Each index is graded between 0 and 4 points, allowing a high degree of differentiation that increases prediction accuracy. This new scoring system utilizes common clinical indicators, which can be routinely ordered to allow easy and wide applications.

This new scoring system demonstrated superior performance in grading the severity and predicting the outcomes of HBV liver failure in this cohort compared to the three established scoring systems, representing progress in improving the management of HBV liver failure. However, our findings will need further verification in larger cohorts that include not only patients with HBV liver failure but also those with liver failure with other etiologies.

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 Clinical Trials registry (registration number: NCT01961440, https://clinicaltrials.gov/). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

WZ-b performed the case summary and statistical analysis and wrote the manuscript. LB-l and PL were responsible for statistical analysis. CZ, ZX-x, TD-m, RW-h, WK, and YX-b were responsible for case enrollment and data collection in other subcenters. KW-m designed this scoring system. ZY-b was responsible for the whole quality of the study. GZ-l was responsible for research design and the whole quality of the study. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Science and Technology Major Project (2018ZX10302204-002) and the National Natural Science Foundation of China (81672701).

REFERENCES

1. Zou Z, Chen J, Xin S, Xing H, Li B, Li J, et al. Features of onset of chronic severe hepatitis in 520 cases. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi*. (2002) 16:322–5.
2. Hepatic Failure and Artificial Liver Group, Infectious Disease Branch, Chinese Medical Association, Severe Liver Disease and Artificial Liver Group, Hepatology Branch, Chinese Medical Association. Guidelines for diagnosis and treatment of liver failure. *Chin J Hepatol*. (2006) 14:643–6.doi: 10.3760/cma.j.issn.1007-3418.2019.01.006
3. Vizzutti F, Arena U, Laffi G, Marra F. Acute on chronic liver failure: from pathophysiology to clinical management. *Trends Anaesth Crit Care*. (2013) 122–9. doi: 10.1016/j.tacc.2013.01.006
4. Christensen E. Prognostic models including the Child-Pugh, MELD and Mayo risk scores: where are we and where should we go? *J Hepatol*. (2004) 41:344–50. doi: 10.1016/j.jhep.2004.06.005
5. Kamath PS, Wiesner RH, Malinchoc M, Kremers M, Therneau TM, Kosberg CL, et al. A model to predict survival in patients with end-stage liver disease. *Hepatology*. (2001) 33:464–70. doi: 10.1053/jhep.2001.22172
6. Zheng YB, Huang ZL, Wu ZB, Zhang M, Gu YR, Su YJ, et al. Dynamic changes of clinical features that predict the prognosis of acute-on-chronic hepatitis B liver failure: a retrospective cohort study. *Int J Med Sci*. (2013) 10:1658–64. doi: 10.7150/ijms.6415
7. Zheng Y, Xie D, Gu Y, Yan Y, Wu YB, Lei ZY, et al. Development of a sensitive prognostic scoring system for the evaluation of severity of acute-on-chronic

hepatitis B liver failure: a retrospective cohort study. *Clin Invest Med*. (2012) 35:E75–85. doi: 10.25011/cim.v35i2.16291
8. Zheng YB, Xie SB, Xie DY, Peng L, Lei ZY, Deng H, et al. A novel prognostic scoring system to predict 3-month mortality risk in patients with acute-on-chronic liver failure in hepatitis B: a retrospective cohort study. *Hepatol Int*. (2012) 6:727–34. doi: 10.1007/s12072-011-9335-2
9. Xinyan L, Shulin H, Liang F, Ruixiang L, Weimin K. Simple scoring system for the severity of chronic hepatitis A with chronic liver failure. *Chin Med J*. (2009) 89:3353–5.
10. Liver Failure and Artificial Liver Group, Chinese Society of Infectious Diseases, CMA; Severe Liver Disease and Artificial Liver Group, Chinese Society of Hepatology, CMA. Guideline for diagnosis and treatment of liver failure. *Chin J Infect Dis*. (2012) 5:321–7.
11. Huang K, Hu J-H, Wang H-F, He W-P, Chen J, Duan X-Z, et al. Survival and prognostic factors in hepatitis B virus-related acute-on-chronic liver failure. *World J Gastroenterol*. (2011) 17:3448–52. doi: 10.3748/wjg.v17.i29.3448
12. Ruf AE, Kremers WK, Chavez LL, Descalzi VI, Podesta LG, Villamil FG. Addition of serum sodium into the MELD score predicts waiting list mortality better than MELD alone. *Liver Transpl*. (2005) 11:336–43. doi: 10.1002/lt.20329
13. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg*. (1973) 60:646–9. doi: 10.1002/bjs.1800600817
14. Yang W-B, Chen E-Q, Bi H-X, Bai L, Chen X-B, Feng P, et al. Different models in predicting the short-term prognosis of patients with hepatitis B virus-related acute-on-chronic liver failure. *Ann Hepatol*. (2012) 11:311–9.
15. Duan XZ, Xin SJ. Artificial liver support as a therapy for liver failure. *Chin J Hepatol*. (2012) 20:411—3.
16. Malinchoc M, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunt. *Hepatology*. (2000) 31:864–71. doi: 10.1053/he.2000.5852
17. Kim JM, Kwon CH, Joh JW, Park JB, Lee JH, Kim GS, et al. Can the model for end-stage liver disease score replace the indocyanine green clearance test in the selection of right hemihepatectomy in Child-Pugh class A. *Ann Surg Treat Res*. (2014) 86:122–9. doi: 10.4174/astr.2014.86.3.122
18. Duan BW, Lu SC, Wang ML, Liu JN, Chi P, Lai W, et al. Liver transplantation inacute-on-chronic liver failure patients with high model for end-stage liver disease (MELD) scores:a single center experience of 100 consecutive cases. *J Surg Res*. (2013) 183:936–43. doi: 10.1016/j.jss.2013.03.008
19. Basile-Filho A, Nicolini EA, Auxiliadora-Martins M, Alkmim-Teixeira GC, Martinez EZ, Martins-Filho OA, et al. Comparison of acutephysiology and chronic health evaluation II death risk, Child-Pugh, Charlson and model for end-stage liver disease indexes to predict early mortality after liver transplantation. *Transplant Proc*. (2011) 43:1660–4. doi: 10.1016/j.transproceed.2010.11.029
20. Biselli M, Dall'Agata M, Gramenzi A, Gitto S, Liberati C, Brodosi L, et al. A new prognostic model to predict dropout from the waiting list in cirrhotic candidates for liver transplantation with MELD score<18. *Liver Int*. (2015) 35:18–191. doi: 10.1111/liv.12538
21. Garg H, Kumar A, Garg V, Sharma P, Sharma BC, Sarin SK. Clinical profile and pre-dictors of mortality in patients of acute-on-chronic liver failure. *Dig Liver Dis*. (2012) 44:166–71. doi: 10.1016/j.dld.2011.08.029

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.

frontiers in Medicine

REVIEW
published: 08 November 2021
doi: 10.3389/fmed.2021.752875

Acute-on-Chronic Liver Failure: Pathophysiological Mechanisms and Management

*Arshi Khanam and Shyam Kottilil**

Division of Clinical Care and Research, Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD, United States

Acute-on-chronic liver failure (ACLF) is a multifaceted condition with poor treatment options and high short-term mortality. ACLF can develop in patients with or without liver cirrhosis, where patients with decompensated cirrhosis display a higher risk of short-term mortality. Pathophysiological mechanisms include systemic inflammation due to bacterial and fungal infections and acute hepatic insult with drug, alcohol, and viral hepatitis. Cryptogenic factors also contribute to the development of ACLF. The clinical outcome of patients with ACLF gets further complicated by the occurrence of variceal hemorrhage, hepatorenal syndrome, hepatic encephalopathy, and systemic immune dysfunction. Regardless of the better understanding of pathophysiological mechanisms, no specific and definitive treatment is available except for liver transplantation. The recent approach of regenerative medicine using mesenchymal stem cells (MSCs) could be advantageous for the treatment of ACLF as these cells can downregulate inflammatory response by inducing antiinflammatory events and prevent hepatic damage and fibrosis by inhibiting hepatic stellate cell activation and collagen synthesis. Moreover, MSCs are involved in tissue repair by the process of liver regeneration. Considering the broad therapeutic potential of MSCs, it can serve as an alternative treatment to liver transplant in the near future, if promising results are achieved.

Keywords: acute-on-chronic liver failure, cirrhosis, immunopathology, liver transplantation, stem cell therapy

OPEN ACCESS

Edited by:
Yu Shi,
Zhejiang University, China

Reviewed by:
Chenxia Hu,
Zhejiang University, China
Sergio Rodríguez Tajes,
Hospital Clínic de Barcelona, Spain

***Correspondence:**
Shyam Kottilil
skottilil@ihv.umaryland.edu

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *03 August 2021*
Accepted: *07 October 2021*
Published: *08 November 2021*

Citation:
Khanam A and Kottilil S (2021) Acute-on-Chronic Liver Failure: Pathophysiological Mechanisms and Management. Front. Med. 8:752875. doi: 10.3389/fmed.2021.752875

INTRODUCTION

Acute-on-chronic liver failure (ACLF) is a serious condition which develops in patients with chronic liver disease (CLD) with compensated and decompensated cirrhosis. ACLF is defined as acute hepatic decompensation, development of multiorgan failure, and high risk of short-term mortality (1–3). Based on different diagnostic criteria, various international consortiums around the world projected distinct definitions for this syndrome. Until now more than 13 different definitions of ACLF have been proposed, but the definitions of the Asian Pacific Association for the Study of the Liver (APASL) ACLF Research Consortium and the European Association for the Study of the Liver-Chronic Liver Failure (EASL-CLIF) Consortium are widely acknowledged (1, 2). There is heterogeneity present between different definitions related to the underlying CLD, precipitating events, and multi-organ failure, though, different definitions provide their consensus over high short-term mortality. Later, it was suggested that the differences in definitions are associated with discrete epidemiology of liver diseases in the Eastern and Western Hemispheres. Recently, North America Consortium for the Study of End-Stage Liver Disease (NACSELD)

introduced another definition of ACLF, which defines ACLF as a condition that develops in CLD patients with or without cirrhosis. NACSELD definition also agreed that with high short-term mortality in these patients in the absence of the proper management of underlying liver disease, liver support, and liver transplantation (4) studies are focusing on the further validation of the current definition of ACLF. Management of the underlying cause of CLD with suitable therapies, including antivirals for hepatitis B (HBV)- and hepatitis C virus (HCV)-related liver disease, alcohol abstinence in alcoholic liver disease (ALD), and immunosuppressive therapies in autoimmune liver disease may avoid or reverse the development of cirrhosis (5–7). Contrarily, if the underlying cause is left untreated or it persists in patients with compensated cirrhosis, extended hepatic necrosis can destroy hepatic architecture, increase intrahepatic resistance, portal hypertension, damage liver parenchymal cells, and subsequently cause acute decompensation of the disease (8–10). Since, ACLF may establish at any phase of the disease from CLD to compensated to early or late decompensated cirrhosis, it is not considered as a terminal incidence of long-standing decompensated cirrhosis (11), although the risk of mortality is significantly higher in patients with compensated and decompensated cirrhosis in comparison with the general population (12) (**Figure 1**). In the present review, we will focus on the discrete pathophysiological mechanisms, complications, and management of patients with ACLF.

PATHOPHYSIOLOGICAL MECHANISMS IN ACLF

Systemic Inflammation

Current advancements in the understanding of the pathophysiological basis suggest that hyperreactive systemic inflammatory response is a critical driver of tissue damage and organ injury in patients with acutely decompensated cirrhosis leading to the development of ACLF (13). Extensive production of inflammatory mediators including cytokines, chemokines, growth factors, bioactive lipid mediators, and expression of chemokine receptors by different immune cells induce systemic inflammation, immune-mediated tissue damage, and subsequently liver failure (14–19). Activated immune cells release other mediators such as proteases, reactive oxygen species (ROS), prostaglandins, and leukotrienes that further aggravate tissue damage (16, 20).

Systemic inflammation may occur in the presence and absence of identifiable and non-identifiable triggers. Identifiable triggers may include bacterial infections, excessive alcohol consumption, and relapse of chronic viral hepatitis, whereas non-identifiable

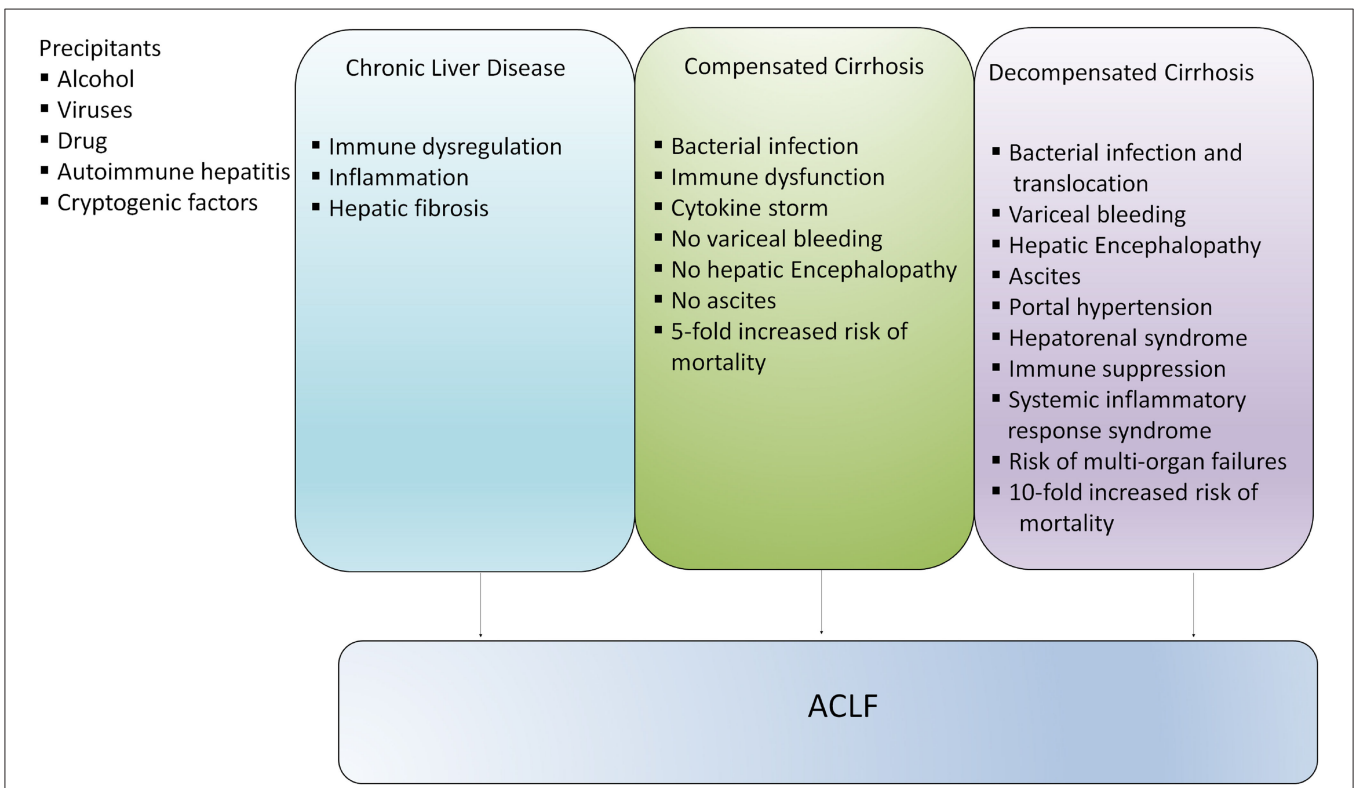


FIGURE 1 | Development of ACLF in different stages of liver disease. ACLF can develop directly in patients with chronic liver disease (CLD), compensated and decompensated cirrhosis or it may progress slowly from CLD to compensated and decompensated cirrhosis and eventually develop into ACLF. However, development of ACLF in patients with compensated and decompensated cirrhosis inflict higher risk of mortality.

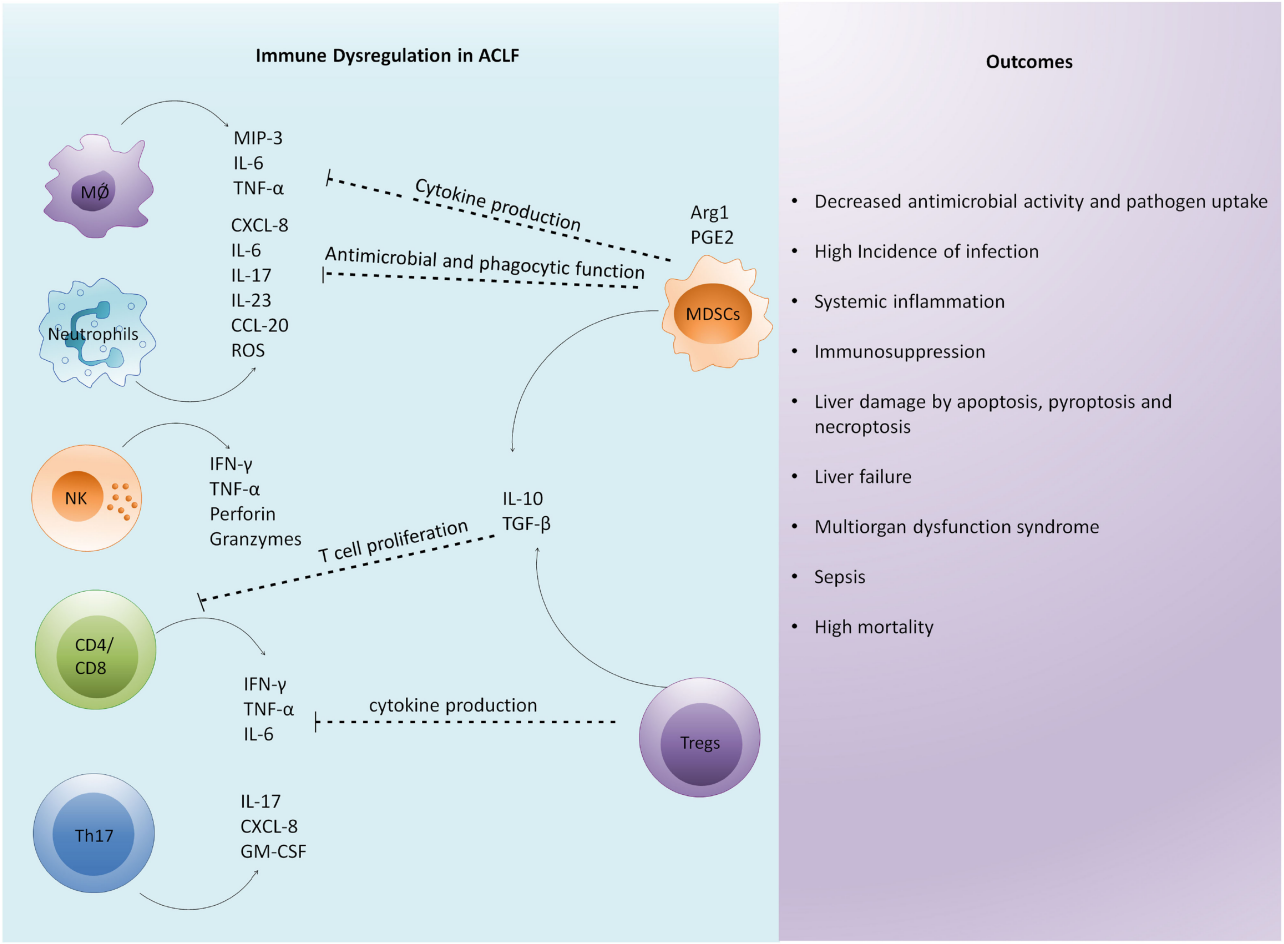


FIGURE 2 | Immune dysregulation is a critical factor in the pathophysiology of ACLF. Excessive immune activation drives systemic and intrahepatic cytokine storms in patients with ACLF leading to inflammation. Enormous cytokine secretion in the liver results in the infiltration of circulating immune cells that further induce hepatic damage. Subsequently, an antiinflammatory response is generated to control excessive inflammation. However, excessive antiinflammatory response by regulatory cells impairs the function of other immune cells by inhibiting their antimicrobial and phagocytic activities, cytokine secretion, and T cell proliferation. The overall immune dysregulation increases the risk of infection and sepsis development. In addition, it induces distinct cell death related pathways in the liver, causing multiorgan dysfunction leading to high mortality. MØ, macrophage; NK cells, natural killer cells; MIP-3, macrophage inflammatory protein; TNF-α, tumor necrosis factor- α; IFN-γ, interferon-γ; ROS, reactive oxygen species; GM-CSF, granulocyte macrophage colony stimulating factor; Arg1, arginase-1; PGE2, prostaglandin E2; MDSC, myeloid derived suppressor cells; Tregs, regulatory T cells; TGF-β, transforming growth factor-β.

triggers do not have any clinically identifiable cause (21–23). The mechanism of inflammation is not very well-characterized. It is believed that bacterial products and endogenous molecules are potential inducers of inflammation. Patients with acute decompensated cirrhosis and ACLF may develop systemic inflammation even in the absence of bacterial infections and their translocation through the release of damage-associated molecular patterns (DAMPs) from injured tissues and organs (24, 25). Various components of injured or dying cells including cytosol, mitochondria, and nucleus release DAMPs by the process of necrotic, pyroapoptotic, and necroapoptotic cell death which contribute to inflammation (26, 27). Cell death mechanisms including apoptotic pathways influence the recovery of HBV-related ACLF (28). Recently, it has been identified that caspase-cleaved keratin-18 (ck18) can predict the progression of acute decompensation to ACLF (29). Biomarkers including caspase-cleaved neoepitope of cytokeratin-18 and intact cytokeratin-18 variant recognized as M30 and M65, respectively have been investigated in ACLF. A higher ratio of M30:M65 in patients with ACLF can serve as a good indicator of apoptosis severity (30).

Host immune and genetic factors exaggerate systemic inflammation. Different immune cells including monocytes, macrophages, neutrophils, natural killer cells (NK cells), myeloid-derived suppressor cells (MDSCs), CD4, CD8, and Th17 cells immensely contribute to cytokine and chemokine production (16, 31–36) leading to cytokine storm, systemic inflammation, and cell death (**Figure 2**). In fact, various immune surface molecules comprising chemokine receptors and coinhibitory molecules derive inflammatory responses leading to necrotic and apoptotic cell death and further aggravate

hepatic damage (16, 37, 38). Similarly, host genetic factors, for instance, single nucleotide variants might modulate the release of inflammatory mediators by innate immune cells and can change the expression of pattern recognition receptors (PRRs). Genetic variations in genes coding for innate immune receptors including nucleotide-binding oligomerization domain (NOD)-2, mannan-binding lectin (MBL), and MBL-associated serine protease (MASP)-2 are associated with increased short-term mortality in ACLF and patients with acute decompensation (39). In addition, single nucleotide polymorphism with IL-1 gene clusters plays a protective role in patients with acute decompensated cirrhosis by controlling systemic inflammation and reducing the development of ACLF (40).

Immune Cell Paralysis and Immunosuppression

Immunosuppression also acts as a potential contributor to the pathogenesis of ACLF mainly through the amplification of immune paresis that further increases the risk of bacterial infections (41). MER receptor tyrosine kinase (MERTK) expression on monocytes and macrophages are known to suppress innate immune cells. MERTK expressing monocytes and macrophages were increased in the circulation, liver, and lymph nodes of patients with ACLF and correlated with severity of hepatic and extrahepatic systemic inflammatory response and disease (42). MERTK expressing monocytes exhibited decreased response toward lipopolysaccharide (LPS), and its blockade with UNC569 restored monocytes function (42). Patients with ACLF do not only have a hyperactive inflammatory response, but also hyper antiinflammatory response and dysfunctional immune response exist in parallel. Interestingly, hyperinflammatory and immunosuppressive conditions both coexist in the same individual. However, the prevalence of one or the other depends on sequential and longitudinal aspects. Circulating and intrahepatic immune cells may act differently. Circulating immune cells might display inflammatory phenotype whereas hepatic immune cells may exhibit antiinflammatory phenotype since the liver is an immunotolerogenic organ (38, 43).

An intense antiinflammatory response along with immune dysregulation and exhaustion are associated with immune cell paralysis (44). Inhibitory pathways also exist to maintain immune homeostasis and avoid the overactivation of immune cells; however, hyper-reactive inhibitory pathways cause immune exhaustion and paresis. High expression of programmed death-1 (PD-1) and T-cell immunoglobulin and mucin domain 3 (TIM3) play a crucial role in the immune paresis in patients with alcoholic hepatitis (45). Immunosuppression in alcoholic hepatitis is associated with the pathogenesis of ACLF. Patients with ACLF show a similar degree of cellular immune depression as in severe sepsis that contributes to increased infectious morbidity in these patients (46). Also, patients with ACLF display highly compromised tumor necrosis factor-α (TNF-α) production and HLA-DR expression under *ex vivo* conditions (46). Moreover, immune dysfunction in ACLF is independent of the underlying etiology of liver cirrhosis and is common in all patients. Increased level of prostaglandin E2 (PGE2), an immunosuppressive lipid mediator, inhibits TLR4 expression. PGE2 also inhibits macrophage proinflammatory cytokines in response to LPS; thus, it decreases macrophage bacterial killing in ACLF (47). Expansion of CD14+HLA-DR- MDSCs in the circulation of ACLF decreases T cell proliferation, TNF-α production following TLR stimulation, and has reduced phagocytic potential against *E. Coli* (48). Since MDSCs can impair both innate and adaptive responses to microbial products, it enhances the risk of infections and displays great pathological significance. Immunosuppression also contributes to acquire nosocomial infection in ACLF (49). Generally, it is speculated that immunosuppression is a regulatory mechanism to control the exaggerated inflammatory response; however, there is no proof of the concept, and future studies are required to determine whether the development of systemic inflammation and immunosuppression associate with each other during ACLF.

Extensive Alcoholism

A high percentage of ACLF cases develop due to excessive alcohol consumption leading to severe alcoholic hepatitis (sAH) and further development of ACLF (50). CANONIC study reported that 25% of ACLF cases occur due to sAH (1). Alcohol not only impairs immune responses but also enables gut bacterial translocation that initiates inflammation (51). One of the most compelling direct effects of alcohol is that it affects the structure and integrity of the gastrointestinal (GI) system as this is the first point of contact for alcohol. Alcohol alters the numbers and relative plethora of gut microbiome affecting the normal gut function, maturation, and function of the immune system, further disturbing the crosstalk between gut organisms and immune system (52). Moreover, alcohol ingestion destructs epithelial cells, activates neutrophils, and T cells, upsetting gut barrier function, resulting in the leakage of microbes into the circulation (50). Disturbance of gut barrier function has critical consequences beyond the intestinal system. Leakage of bacterial products such as LPS from the gut activates the innate immune system in the liver, prompting inflammation and eventually causing liver cirrhosis and cancer (53). Irrespective of whether the bacteria from leaky gut causes infection or not, they release pattern-associated molecular patterns (PAMPs) including LPS that reach the liver and get recognized by toll-like receptors (TLRs) present on hepatic Kupffer cells (KCs), and encourage the production of proinflammatory cytokines and chemokines that chemoattract neutrophils (54). It is well-documented that acetaldehyde metabolism induces ROS. Abundance of ROS causes mitochondrial DNA stress that generates inflammatory response and subsequently contributes to liver failure (55). Moreover, sAH hampers liver regeneration despite the presence of activated hepatic progenitor cells (HPCs) that fail to differentiate into hepatocytes and hence, no replacement of damaged hepatocytes occurs (56). In addition, cumulative effects of alcohol on both innate and adaptive immunity tremendously weaken the host defense, which in turn increases the susceptibility of chronic drinkers toward various infections that further exaggerate systemic inflammation. In fact, it has been reported that alcohol exposure restricts the development of the immune system in the fetus, shown by

in utero exposure of alcohol. This exposure escalates the risk of a newborn of getting an infection (57). Also, the harmful effects of alcohol on the development of the immune system last into adulthood. Collectively, these findings propose that sAH might be the result of both impaired hepatocyte regeneration and immunopathology.

Viral Infections

HBV Reactivation

Development of ACLF is attributed to both viral and host factors. HBV viral factors include its genotypes, hepatitis B e antigen (HBeAg) status, and mutations in the HBV precore and core promoter regions (58). High viral replication of certain variants has been associated with a more hostile disease course. A strong correlation between HBV DNA level and the development of cirrhosis and HCC has been reported in patients infected with chronic HBV (59).

Hepatitis B virus reactivation is one of the most common precipitating events associated with acute decompensation or ACLF in patients with HBV related cirrhosis (60). HBV reactivation is a well-characterized condition, marked by an abrupt reappearance or rise of HBV DNA in patients with previously inactive or resolved HBV infection. Reactivation is often spontaneous, but can also be triggered by immune suppression or alterations in immune function and after cancer chemotherapy (61). However, the main challenge in diagnosing reactivation of CHB is to differentiate it from acute hepatitis B due to the lack of pathological evidence. A low titer of anti-HBc IgM and high HBV DNA are useful in identifying severe acute reactivation of HBV from acute HBV (62). Also, the presence of basal core promoter mutation and precore stop codon mutations can differentiate severe acute exacerbations of chronic HBV from acute HBV infection (63). Moreover, submissive hepatic necrosis helps in distinguishing HBV-related ACLF from cirrhotic patients with acute decompensation (64). Patients with submissive hepatic necrosis display severely compromised hepatic function, high occurrence of multiorgan failure, and a smaller interval between acute decompensation and liver transplantation. Patients with ACLF with hepatic precipitants, such as HBV reactivation, have short-term mortality similar to patients with extrahepatic precipitant, suggesting that short-term mortality is not related to the presence and type of precipitating events (65). Rather it is the number of organ failures that are related to high mortality and not the etiology of cirrhosis or precipitating events.

Recently, plasminogen, an inactive precursor of plasmin, a potent serine protease that is involved in the dissolution of fibrin blood clots, served as a promising prognostic biomarker for HBV-related ACLF (66). The study reported that P5 is a high-performance prognostic score for HBV-related ACLF and it causes a decrease in plasminogen level at admission associated with mortality. Longitudinal analysis reveals a gradual increase in plasminogen in HBV-related ACLF survivors, but a steady decline in non-survivors. The changes in plasminogen levels imitated the course of improvement, fluctuation, and deterioration. Of note, plasminogen levels were negatively associated with the number of failed organs and were lower in patients with cerebral and coagulation failure, suggesting plasminogen as an independent prognostic factor.

Hepatitis E Virus Infection

Hepatitis E virus (HEV) infection is one of the commonest causes of acute viral hepatitis (AVH) around the globe, especially in Asia and Africa, where it causes the epidemic of AVH (67). HEV infection is mostly transmitted through the feco-oral route, and the clinical manifestations differ between patients ranging from asymptomatic infection to uncomplicated AVH and severe fulminant liver failure. Generally, AVH, owing to HEV infection, is an acute and self-limiting illness; however, when it occurs in CLD patients, it may progress rapidly to ACLF resulting in high mortality. Several studies reported HEV infection as one of the main causes for decompensation of cirrhosis in Asia and Africa, where HEV is an endemic. In almost 21% cases of ACLF, HEV infection was the precipitating cause for liver decompensation accounting for 0–67% mortality (67). Though it differs strikingly from the Western countries where HEV infection is hardly the main cause of acute decompensation in ACLF, a Chinese study reported that out of 188 patients with CHB, 136 encountered superinfections with HEV and only 52 patients had hepatitis A virus (HAV) superinfection. Also, complications, liver failure, and mortality were frequent in HEV infected groups, indicating HEV superinfection causes more severe liver disease and poor prognosis than those with HAV superinfection (68). Another study from India reported that 61% of ACLF cases had HEV infection as the main precipitant event, 33% had HAV, and 6% had both the infections (69). Since there is no recommended vaccine against HEV, appropriate precautions such as ingestion of boiled water and well-cooked food in the HEV-endemic regions are required to avoid HEV superinfection. Moreover, ribavirin can be used for the treatment of acute and chronic hepatitis E (70) and can decrease the severity of the disease in patients with acute and chronic liver failure. Though the optimization of the dose and duration is critical as treatment failure may occur, a course of 3 months is the optimal duration for the ribavirin monotherapy, and longer treatment periods are available for the patients with severely compromised immune function (71).

Autoimmune Hepatitis

Autoimmune hepatitis (AIH) in an infrequent condition but associated with liver disease-related morbidity and mortality. AIH is an immune-mediated, inflammatory condition of the liver, which occurs when the host immune system turns against the liver cells. It is characterized by the presence of circulating autoantibodies, hypergammaglobulinemia, and discrete features on liver biopsy (72) that mainly present necroinflammatory liver disease. It may also include discrete disease subtypes ranging from benign chronic hepatitis and indolent disease to fulminant hepatic failure. Nearly, 20% of patients with AIH present severe jaundice, coagulopathy, and encephalopathy with or without ascites coinciding with the features of ALF or ACLF (73). Clinically it is difficult to distinguish autoimmune-ALF from ACLF (74); though histological features are distinct (74, 75). In AIH-related ACLF, advanced fibrosis, ductular reactions, and huge parenchymal

collapse with lymphoplasmacytic inflammation are common, whereas lymphoid aggregates and perivenulitis are less frequent (74). Since AIH is considered uncommon in the Asian Pacific region, AIH flare as a cause of ACLF is frequently disregarded leading to disease severity, delay in treatment, and poor outcome. Treatment options for AIH-related ACLF includes the use of corticosteroids, which demonstrated survival benefits compared with those who did not receive it; although, patients with high MELD score >27 and HE in advanced fibrosis (>−F3) displayed poor corticosteroid response, serving them as predictors of an unfavorable response (74). As AIH is a rare disorder, data is quite limited in this field requiring further investigations.

COMPLICATIONS IN ACLF

Bleeding

Acute variceal bleeding is a serious complication of liver cirrhosis resulting from portal hypertension. Variceal bleeding is often associated with ACLF and accounts for 70% of all upper gastrointestinal bleeding episodes in cirrhosis (76). Patients with ACLF display an imbalance in systemic and hepatic hemodynamics with severe portal hypertension and worsening of systemic vasodilation (77). The increased portal pressure arises as a consequence of hepatic and systemic inflammation, reduced hepatic perfusion, and high intrahepatic resistance. As patients with ACLF have high baseline hepatic venous pressure gradient and lower hepatic blood flow, the chances of variceal bleeding are also high (78). Although a significant progress has been made in the treatment of acute variceal bleeding comprising transjugular intrahepatic portosystemic shunt (TIPS) (79), endoscopic treatment, and drug therapy, 10–20% of the patients experience treatment failure that associate with a high short-term risk of further liver decompensation and death (80). Recently, a study reported the prevalence of ACLF in patients with acute variceal bleeding and found its association with rebleeding and mortality (79). ACLF nearly doubled the risk of rebleeding and emerged as an independent risk factor for rebleeding and mortality in acute variceal bleeding patients. Patients with ACLF with variceal bleeding may benefit from the placement of TIPS. In fact, the insertion of TIPS improves the 42-day and the 1-year survival in patients with ACLF (79). Also, preemptive placement of TIPS is helpful in patients with ACLF with acute variceal rebleeding.

Hepatic Encephalopathy

Hepatic encephalopathy (HE) is another frequent manifestation of ACLF. Localized and systemic alterations on the background of cirrhosis are accountable for the pathogenesis of encephalopathy. However, the exact pathophysiological mechanism of HE is not well-defined. Patients with HE manifest a range of neuropsychiatric symptoms including sensory abnormalities, psychomotor dysfunction, and impaired memory (81). Hyperammonemia, systemic inflammation including sepsis, bacterial translocation, insulin resistance, and oxidative stress remain as key factors in the development of HE, driving cerebral edema and inflammation (82). Since, patients with chronic liver failure frequently undergo immunoparesis, an association between ammonia and inflammation has been anticipated. During liver failure, reduced usage of ammonia as a substrate in the ammonia detoxification pathway (urea cycle) and portosystemic shunting increases ammonia accumulation in the systemic circulation (83). Also, impaired hepatic metabolism leads to decreased elimination of nitrogen-based waste products such as ammonia which crosses the blood–brain barrier, where it combines with glutamate to form glutamine (84). Cerebral accumulation of glutamine employs an osmotic effect that leads to increased retention of water in the brain, resulting in swelling and cytotoxic edema. HE in a hospitalized cirrhotic patient is related to high mortality that further increases in case of patients with ACLF (82). The effect of the systemic inflammatory response on ammonia-induced neurological dysfunction has been defined in cirrhotic patients hospitalized with an infection. In fact, systemic inflammation in patients with progressive HE is associated with mortality (85). Existence of severe HE in cirrhotic patients requires management in the ICU, and patients frequently require tracheal intubation for airway protection (86). Careful sedation is also needed. For the precise management of HE, the early step is to identify and reverse any precipitating event such as infection or bleeding (87). Therapies lowering ammonia are commonly used. Moreover, lactulose, a non-absorbable disaccharide that converts into short-chain fatty acids by the colonic microbiome generates an acidic environment, leading to the inactivation of ammonia-producing colonic bacteria, and the conversion of ammonia to non-absorbable ammonium (88). Antibiotics are also recommended (89), generally in combination with lactulose that is helpful in reducing mortality and the length of hospital stay in comparison with lactulose alone (90). As ammonia is considered a key participant in the pathogenesis of HE, antibiotics that reduce the ammonia-producing enteric bacteria including vancomycin, neomycin, paromomycin, and metronidazole are used in combination with or without lactulose (89). These antibiotics serve as second-line agents; though, few of the antibiotics are not acclaimed for long-term use due to nephrotoxicity, ototoxicity, and neurotoxicity. For instance, neomycin is ototoxic and nephrotoxic, whereas metronidazole has neurotoxic effects. Another antibiotic Rifaximin, a nominally absorbed oral antimicrobial agent, is highly efficacious in treating HE through eliminating ammonia-producing colonic bacteria, resulting in reduced ammonia concentration. Rifaximin is poorly absorbed and has minimal systemic bioavailability which favors its long-term use than the other antibiotics (91).

Concomitant Infection

Bacterial Infection

Patients with ACLF are prone to develop infection. Bacterial infections play an essential role in the development and further progression of ACLF, and participate either as a key precipitating event or as a complication (92). At the time of ACLF diagnosis, approximately 37% of the patients exhibit bacterial infections, whereas 46% of the remaining patients with ACLF develop bacterial infections during the 4 weeks follow-up (93). Both Gram-positive and Gram-negative bacteria contribute to the infection (21). The incidence of Gram-positive bacterial infections mainly *Staphylococcus* are increasing than

the Gram-negative bacterial infections. The common Gram-positive bacteria includes *Staphylococcus aureus* and *Enterococcus* (94). *S. aureus* causes respiratory tract and also skin infection, whereas *Enterococcus* frequently causes urinary tract infection. Besides, due to the improper use of antibiotics, antimicrobial resistance including methicillin-resistant *S. aureus (MRSA) and Vancomycin-resistance Enterococcus (VRE)* is also increasing in patients with cirrhosis (95, 96). The common Gram-negative bacteria causing infections include *Escherichia coli and Klebsiella pneumoniae* (97). *E. coli* instigate spontaneous bacterial peritonitis (SBP), whereas *Klebsiella pneumoniae* is a common cause of pneumonia. Another Gram-negative bacteria Acinetobacter causes respiratory tract infection. In ACLF, urinary tract, and also skin infections, pneumonia, and SBP are predominant and complicate the condition of these patients (98). In fact, the severity of ACLF measured by the prevalence of organ failure and mortality was higher in patients where ACLF was caused by an infection in comparison to those with non-infectious etiologies (99).

Patients with bacterial infections exhibited a higher grade of systemic inflammation, worse clinical course, and lower probability of 90-day survival than those without infection (100). Cirrhotic patients, especially the decompensated ones, are extremely susceptible to develop bacterial infection due to impaired gastrointestinal barriers and increased gut permeability that allows bacterial translocation to the surrounding tissues and end up in the blood stream leading to systemic inflammation, sepsis, and ACLF development (101). Continuous translocation of bacteria and its products stimulate the immune cells after identification by pathogen-recognition receptors, typically TLRs, causing overwhelming inflammatory response *via* producing inflammatory cytokines (25). High levels of circulatory proinflammatory cytokines induce systemic inflammation and increase disease severity. Further, systemic inflammatory responses encourage organ damage through oxidative stress, endothelial dysfunction, and reduced organ perfusion. Also, pathogen and pathogen-derived endotoxins are efficient in promoting direct tissue damage (49). In general, hepatocytes are moderately protected against LPS-induced tissue damage *via* the induction of NF-κB pathway; however, this mechanism is impaired in cirrhotic patients causing direct tissue damage (102, 103). Moreover, translocation of bacteria or their PAMPs impair the contractility of mesenteric vessels that supply blood to the small and large intestines and increase portal hypertension in cirrhotic patients, which further affect the microbiota and increase bacterial translocation (104). Studies believe that liver, intestinal barrier and microbiota, and immune response preserve equilibrium through complex interactions, and perturbation in this balance leads to increased gut permeability, although the precise mechanism is not clear.

Early diagnosis and appropriate antibiotic use on time are critical factors to improve the prognosis of patients with bacterial infections. Also, biomarkers of infection may aid in the early diagnosis of infection. Acute-phase proteins including C-reactive protein (CRP) and procalcitonin (PCT) are early markers of infection that are frequently used to diagnose the infection (49). A study described that a value of CRP >12.15 mg/L is a good indicator of bacterial infection in patients with ACLF (105). Unfortunately, due to the increased use of antimicrobial agents, antimicrobial resistance has increased over the years. In fact, a study advocated against the use of antibiotics except in distinct conditions such as gastrointestinal bleeding, history of SBP, and ascites fluid protein concentration <1.5 g/dL (95). However, that cannot be considered in patients with septic shock, as each hour delay in antibiotic treatment following identified hypotension can decrease patient's survival up to 7.6% for the first 6 h (106). Due to the prolonged wait time in getting bacterial culture results, we lose vital time to treat the patient with antibiotics; however, antibiotic treatment without identifying the infection will put unwarranted stress on the liver; therefore, techniques that could identify the infections in short span of time are highly needed.

Fungal Infection

Persistent impaired immune response and hepatocyte damage reduce the efficiency of inhibiting and clearing the pathogen in ACLF. A study reported the occurrence of invasive fungal disease in 43% of patients with ACLF and observed higher mortality in these patients than those without the invasive fungal disease (107). *Candida* as well as *Aspergillus* species are the common infections in ACLF and primarily infect urinary and respiratory tracts (108, 109). Like a bacterial infection, a fungal infection could act as the main precipitating event in ACLF; however, the mechanism is not well-recognized. It is believed that the exacerbation of ACLF induces immune paralysis, which can lead to invasive fungal infection (108). In addition, the invasive fungal disease is also responsible for increased inflammatory cytokine response that further augments organ failure (107). To identify the fungal infection, specific tests including fungal culture, serologies, and fungal tissue staining are required (108). The invasive fungal infection is diagnosed by 1,3-β-D-glucan and galactomannan index (110). Recently, it has been shown that bacterial and fungal infections are associated with poor clinical course and high 28 and 90-day mortality (111). Fungal infections not only increase short-term (28 days) and medium term (90 days) mortality, but also enhance the risk of 1-year mortality. This finding allows identifying patients with ACLF who survive an infection but are intended for a poor long-term prognosis; hence, close monitoring and specific management is demanding in these patients. The identification of an infection at the initial stage is the most challenging. The current approach is to culture the patient's sample, which is a time-consuming process and inclined to crosscontamination. Therefore, it is critical to identify the infection based on the other clinical markers. Currently, the most common indicators of infection are systemic inflammatory response syndrome (SIRS), PCT, serum lactate, and few others (95, 112). Although few studies support the relation between SIRS and infection in liver disease patients without sepsis, others question the sensitivity of SIRS in critically ill and cirrhotic patients.

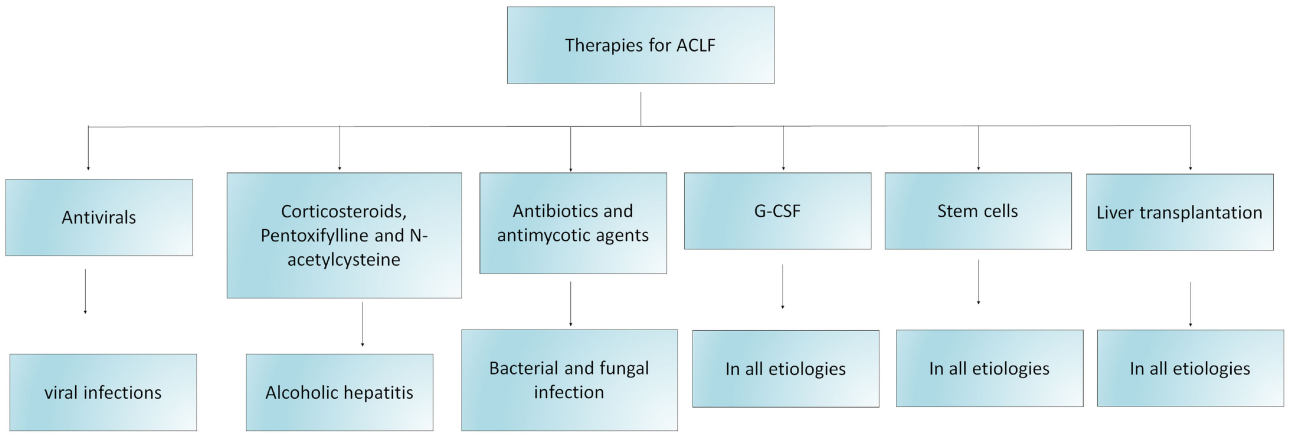


FIGURE 3 | Schematic diagram indicating therapeutic strategies for the management of ACLF. The figure demonstrates different treatment options for patients with ACLF based on their etiology, precipitating events, complications and the requirement of liver transplantation. Several clinical trials are investigating the efficacy of stem cell therapy as one of the potential therapeutic approach for the treatment of ACLF. G-CSF: granulocyte macrophage colony stimulating factor.

STRATEGIES FOR THE MANAGEMENT OF ACLF

At present, there is no specific treatment accessible for patients with ACLF. The management of ACLF includes etiology-based treatment, controlling and treating complications, providing artificial liver support system, and liver transplantation, summarized in **Figure 3** and further detailed below. Admission of patients with ACLF should be considered preferably in transplant centers. Organ functions need to be monitored closely and organ-specific treatment is required to restrict the development of multiorgan failures.

Antivirals for the Treatment of HBV-Related ACLF

The primary aim to use antiviral for the treatment of HBV-related ACLF is to substantially decrease the viral load, thereby inhibiting hepatocyte cell death and improving survival outcomes by constraining the decompensation-related multiorgan complications. Low HBV DNA level at baseline and a further reduction in viral load improve the outcomes in HBV-related ACLF, suggesting that early implementation of antiviral therapy could improve the prognosis of HBV-related patients with ACLF (113). A two log reduction in HBV DNA at 2 weeks improves survival benefits in HBV-related ACLF (114). Antiviral therapy also increases the chances of stabilization to liver transplant time and expands the transplant outcomes. Antivirals including Lamivudine and Entecavir (ETV) displayed short-term survival benefits in HBV-related patients with ACLF, despite the prevalence of drug resistance with Lamivudine (115). Recently, a study demonstrated the role of Tenofovir Disoproxil Fumarate (TDF) and ETV for the treatment of HBV-related ACLF and reported that the short-term efficacy of TDF is greater than ETV (115). Furthermore, TDF showed a higher reduction in HBV DNA level and undetected HBV-DNA in 2 weeks and lowered the model of end stage liver disease (MELD) as well as Child-Turcotte-Pugh (CTP) scores that are potential markers of disease severity. Importantly, the survival rate was higher in patients who received TDF than those who were treated with ETV. HBV-related patients with ACLF treated with Tenofovir Alafenamide (TAF), TDF, and ETV have comparable 48-weeks liver transplant-free survival (116). TAF have similar efficacy in HBV DNA reduction and liver biochemical responses as observed in TDF and ETV group. TAF, TDF, and ETV possess equal safety and efficacy in short as well as long-term treatment of HBV-related ACLF. Several studies demonstrated that ETV had comparable short-term efficacy with LAM; however, more promising in long-term (117). Due to the high incidence of drug resistance, clinical guidelines do not recommend the use LAM, whereas ETV has lower rates of resistance; therefore, widely used in clinical practice. In fact, antiviral treatment using ETV, TDF, and Telbivudine (LDT) is being given to pregnant females who encounter ACLF. These antivirals showed >2 log reduction in HBV DNA levels. A study by Yang et al. suggested that initial combinatorial use of antiviral is efficient in reducing short-term fatality in HBV-related ACLF (118).

The Asian Pacific Association for the Study of Liver Disease guidelines defines the importance of early administration of antiviral therapy in HBV-related ACLF and suggests that those patients with CHB, who need to undergo chemotherapy or immunosuppression procedures, require immediate antiviral treatment to prevent the consequences associated with HBV reactivation (119). While EASL and AASLD guidelines suggest that antiviral with a higher barrier to resistance are needed for patients in whom long-term antiviral prophylaxis is required, predominantly in patients who have higher HBV DNA levels before immunosuppressive therapy (120, 121).

Treatment for Alcohol-Related ACLF

Corticosteroids to Treat Alcohol-Related ACLF

Active alcoholism and sAH are the most common precipitating events in ACLF in the Western world that contribute to

alcohol-related ACLF (122). Unfortunately, alcoholic hepatitis often advances into multiorgan failure, leading to high mortality. To treat sAH, corticosteroids remain the first line of treatment; however, they might not be effective in all patients due to non-response to corticosteroids (123). Therefore, it is critically important to calculate the efficacy of steroids by Lille score after 4 or 7 days of treatment, which is based on age, total bilirubin levels, baseline creatinine, albumin levels, prothrombin time, and repeat total bilirubin levels (124, 125). Based on the Lille scores, patients can be categorized into a full responder, partial responder, and non-responder having a Lille score of $\leq$0.16, 0.16–0.56, and >0.56, respectively. Therefore, Lille score is vital to decide whether corticosteroid therapy needs to be continued or stopped. However, the prospect of response to corticosteroids confide in the existence and non-existence of ACLF. Undoubtedly, the corticosteroid response is lower in patients with ACLF than those without ACLF, and further decreases with ACLF grades (126), which could be due to the fact that corticosteroids are more effective in patients at the preliver failure stage. Patients who have the Lille score <0.45 designate poor response to corticosteroids and poor survival rate at 6 months (125). On the other hand, corticosteroid responders get survival benefits, but due to the risk of bacterial infection, careful evaluation considering the risk to benefit ratio should be investigated prior to the introduction of corticosteroids in ACLF and sAH patients. Incidence of bacterial infection is higher in corticosteroid non-responder than in the responder. Prednisolone, a corticosteroid with antiinflammatory action is widely recommended at a dose of 40 mg/day to treat sAH patients (127). Although short-term use of corticosteroids has promising results, showing a reduced risk of 1-month mortality, long-term use does not appear useful and does not improve survival beyond 1 month (128). Since, infections are common in alcoholic hepatitis patients and corticosteroids suppress the immune system, by reducing the proinflammatory cytokines such as tumor necrosis factor-α, and increases antiinflammatory cytokines, including IL-10 to reduce inflammation, immune suppression mediated by corticosteroids will further increase the incidence of bacterial infection in these patients; therefore, long-term use of corticosteroids should be avoided to lessen the risk of bacterial infection. Furthermore, application of corticosteroid therapy imposes the risk of sepsis development; therefore, selection of patients for corticosteroid treatment is critical.

Pentoxifylline for the Treatment of Alcohol-Related ACLF

In addition to corticosteroids, Pentoxifylline (PTX) is also considered for the treatment of alcoholic hepatitis (127). PTX is a non-phosphodiesterase inhibitor that possesses antiinflammatory properties, inhibits TNF-α production, and has anti-fibrogenic properties (129). The useful effects of PTX are also related to the downregulation of IL-1, IL-6, transforming growth factor-beta (TGF-β), interferon gamma (IFN-γ), inhibition of stellate cell activation, and procollagen I messenger ribonucleic acid expression in rats (130). Moreover, it can efficiently decrease the risk of Hepatorenal Syndrome (HRS) (131). Serum TNF-α levels are elevated in ALD, especially in alcoholic hepatitis, and the use of infliximab and etanercept, TNF-α inhibitors, increased mortality in these patients; therefore, PTX appeared useful in preventing HRS in sAH patients.

Both Prednisolone and PTX are beneficial in treating sAH; however, PTX is possibly superior to prednisolone in the cases where contradictions exist for the use of corticosteroids. Studies also investigated the use of combination therapy of Prednisolone and PTX to treat sAH patients; however, the results did not show any additional benefits in terms of morbidity and mortality (132). While treatment of patients with sAH with PTX provides promising results, it is not recommended as a first line of treatment due to the lack of evidence for its efficacy in comparison to the standard treatment with corticosteroids. However, the American Association for the Study of Liver Disease guidelines recommended the use of PTX for sAH, particularly, when contradictions exist for the use of corticosteroids (133). Also, the European Association for the Study of Liver guidelines recommended the use of PTX for the cases where the presence of sepsis inhibits the use of corticosteroids (134).

N-Acetylcysteine for the Treatment of sAH

The underlying molecular mechanisms of alcoholic liver disease pathogenesis are multifaceted and have not been completely elucidated. However, oxidative stress has been shown to play a critical role in mediating the inflammatory response and causing liver damage (55). Therefore, a therapeutic strategy that could control or prevent oxidative stress might be helpful in patients with ALD. N-acetylcysteine (NAC) is an antioxidant that neutralizes free radicals by increasing the intracellular glutathione and counteracting oxidative stress and inflammation protecting cells from damage (135). In case of the liver, NAC protects against liver injury by restoring hepatic glutathione supplies (136). Hence, NAC might serve as an option for the treatment of sAH patients. Several studies investigated the efficacy of NAC for the treatment of ALD and inconsistent findings were revealed. A study by Nguyen-Khac et al. discovered that intravenous administration of NAC in combination with prednisolone may be beneficial for the patients with alcoholic hepatitis. The study reported that the combination of NAC + prednisolone improved 1-month survival in comparison with those patients who only received prednisolone. However, the combination of NAC + prednisolone did not improve 6-month survival (137). Also, recent studies confirmed no 90-day survival benefit of the combination use NAC + prednisolone over prednisolone alone (138) and combination of G-CSF + NAC and G-CSF alone (139). In fact, a high dose of intravenous NAC for 14 days along with enough nutritional support neither delivered any survival benefits nor early biological improvement in sAH. MELD score and bilirubin level also did not improve at 7 and 30 days and the trials were terminated due to futility (140).

Antimicrobial Therapies for the Treatment of Bacterial and Fungal Infections

A high percentage of patients with ACLF encounter infections either as a main precipitating event or as one of the complications in ACLF. In fact, one-third of the ACLF cases are infected

with multidrug resistance (MDR) pathogens; however, that varies according to the region (100, 141). A broad spectrum of antibiotics is required if the infection is severe or MDR pathogens are present (49). Since a majority of the patients with ACLF reveal infection sooner or later, complete screening of infections is beneficial for early detection and quick antibiotic treatment. If CRP or PCT, predictors of infection, are positive, treatment should be given immediately. The selection of preliminary treatment is extremely important as it governs the patient's outcome. In the absence of suitable antibiotics, the risk of mortality increases to 74% (49). Also, therapeutic interventions primarily depend on the type, severity, and site of infection. Infections in ACLF could be community-acquired, healthcare-acquired, or nosocomial. If the infection is acquired through the community, third-generation cephalosporins are beneficial. In case of nosocomial infections, the selection of antibiotics with a broader antibacterial spectrum such as carbapenems is recommended. However, there is no standardized therapy for cirrhotic patients with nosocomial infection, as the local resistance spectrum needs to be considered. A randomized study for hospital-acquired SBP infection investigated the effect of Ceftazidime with Meropenem plus Daptomycin treatment and found that Meropenem plus Daptomycin enhanced the response to 86%, which was initially 25% (142). Further, it increased the probability of survival by 94% compared with 50% non-responders. Guidelines acclaim the use of Piperacillin/Compactum or Meropenem plus glycopeptides in nosocomial infections (143). Patients infected with SBP also require albumin replacement therapy to prevent HRS and improve outcomes (49, 144). Also, the presence of Gram-positive bacteria in cellulitis and soft tissue infections reveal the requirement of adding Oxacillin or glycopeptides. Regardless of suitable antibiotic treatment, bacterial infections display poor outcomes leading to worse clinical courses, high admissions in ICU, and short-term mortality in ACLF. Non-response to antibiotics might be due to either bacterial resistance or fungal infection requiring further investigations for advancement in the treatment strategies. Although the main cause of infection in cirrhotic patients remains bacterial infection, a fungal infection also accounts for 2–4% of the patients and causes serious complications and mortality increased by up to 70% (112). In the case of fungal infection, antimycotic therapy needs to be administered (145). Currently, there is no evidence suggesting the association of fungal infection with acute deterioration of previously compensated or decompensated cirrhosis. Patients with fungal infection are expected to have anemia, elevated bilirubin, and alkaline phosphatase in comparison with Gram positive and negative bacterial infections (112). Elevated MELD score closely associates with fungal infection. Patients with fungal infection require longer hospitalization, frequent readmissions, and are at higher risk of death.

Fungal infections particularly, invasive pulmonary aspergillosis (IPA), are detected in patients with sAH, decompensated cirrhosis, liver failure, and ACLF (146). IPA infection is prevalent in patients with ACLF and reported in 5–8.3% of HBV-related patients with ACLF and reaching ∼14% in patients with sAH (147, 148). The short-term mortality is extremely high 73.5–100% (149, 150), although, at present, there are no criteria available to identify the patients with poor prognosis. Prescription drugs for the treatment of IPA include voriconazole, liposomal amphotericin B, and echinocandins, of which voriconazole is recommended as the first-line option for primary treatment of IPA (151); though due to the hepatotoxic nature of voriconazole and absence of pharmacokinetics or pharmacodynamics its use is limited in patients with ACLF (152); therefore the optimization of the voriconazole treatment regimen is strongly needed. A study tried to establish an optimal voriconazole regimen in patients with ACLF using a therapeutic drug monitoring method (153). Based on plasma voriconazole concentration measurement, an optimal voriconazole regimen including loading doses: 0.2 g twice daily and maintenance doses 0.1 g once daily was established. It was found that the voriconazole regimen was able to maintain stable and rational therapeutic trough concentrations ranging from 1 to 5 μg/mL, and patients treated with optimal voriconazole regimen displayed good clinical outcomes and higher 90-day survival i.e., 75% who also correspond to early IPA diagnosis as designated by lower CLIF-SOFA lung score (<2) in all patients preceding optimal regimen prescription. CLIF-SOFA lung score >1 was able to identify patients with ACLF complicated with IPA, encountering a much higher 28-day mortality. Also, the optimal voriconazole regimen appeared to be safe and did not display any adverse events.

Stem-Cell-Based Therapies

Stem cell technology has provided hope to identify new expandable sources that can induce liver regeneration. Recently stem cell-based therapies are getting enough attention for the treatment of patients with ACLF (154–157). MSCs have massive expansion potential in the culture system and play a crucial role in tissue repair and regeneration by differentiating into several cell types and replacing the injured tissues (158). The homing potential of MSCs to the site of injury extended the spectrum of therapeutic application including the models of hepatic injury (159). After homing into the liver MSCs transdifferentiate into hepatocytes in the local microenvironment and improve hepatocyte damage and promote liver regeneration. There are ongoing phase II clinical trials (NCT04229901, NCT02946554) investigating the efficacy of HepaStem cells, a highly advanced cell therapy platform comprising human-liver-derived MSCs obtained from healthy donors and expanded in the lab. After intravenous administration, HepaStem cells migrate to the liver through circulation where they perform various functions including the downregulation of proinflammatory response, inhibition of hepatic stellate cell (HSC) activation, and reduction of collagen secretion, ultimately reducing fibrosis. An imbalanced extracellular matrix synthesis and degradation mediated by portal fibroblasts, bone marrow-derived fibroblasts, and activated hepatic stellate cells initiate hepatic fibrosis in ACLF. Immunomodulatory as well as antifibrotic function of HepaStem cell might be beneficial for treating patients with ACLF (156). MSCs mediate their antifibrotic function by downregulating myofibroblasts, which leads to antifibrotic activity (160). MSCs secrete several growth factors which

stimulate resident cells and induce matrix remodeling to promote the differentiation of native progenitor cells and initiate the recovery of injured cells (160). In addition, they also possess antioxidant properties and cytoprotective effects by inducing antioxidant response elements in CCL4 and thioacetamide-induced liver injury (161, 162). Umbilical-cord-derived MSCs and also allogenic ABCB5-positive MSCs that improve liver fibrosis enhance regeneration, suppress inflammation, and downregulate Notch and Stat1/Stat3 signaling in rats (163) are under clinical trials (NCT04822922, NCT03860155) for the treatment of patients with ACLF. Although the mechanisms of MSCs have been well-described in CLDs, the mechanistic approach of MSCs in the treatment of ACLF is not well-documented since it was recently introduced as a therapeutic intervention for ACLF and clinical trials are ongoing. It is believed that immunomodulatory and antiinflammatory function of MSCs relieve hepatic inflammation, improve liver function, decrease the incidence of infection, and enhance survival rate as shown in a prospective randomized controlled clinical trial which investigated the safety, efficacy, and outcome of MSCs in HBV-related ACLF after intravenous infusion (164). The study reported that there were no infusion-related side effects except more frequent fever than patients who received standard medical therapy. Clinical laboratory measurements including total bilirubin and model for end-stage liver disease scores were improved and the incidence of severe infections was decreased (164). In fact, multiorgan failure and severe infection-related mortality were significantly lower in the MSC group. Importantly, the 24-weeks survival rate of the MSC group was higher (73.2%) than the standard medical treatment patients' group (55.6%). Another study also examined the long-term efficacy of autologous bone marrow mononuclear cells (BM-MNCs) transplantation through the hepatic artery and checked the improvement in terms of hepatic functions and decreasing complications in patients with decompensated cirrhosis (165). The study reported that the efficacy of BM-MNCs transplantation persisted for 3–12 months in comparison with the control group. Serious complications including HE and SBP were declined significantly; however, these improvements vanished after 24 months of transplantation. Few other clinical trials also demonstrated the beneficial role of MSCs in cirrhosis and patients with ACLF; therefore, regenerative medicine using stem cell technology appears promising to treat patients with ACLF and may help in reducing the requirement of liver transplantation (**Figure 4**).

Granulocyte-Colony Stimulating Factor Therapy

Granulocyte colony stimulating factor (G-CSF) is a glycoprotein, which stimulates the bone marrow production of stem cells and granulocytes and releases them into the circulation. Several clinical trials studied the efficacy of G-CSF for the treatment of patients with ACLF. In fact, our previous studies identified the role of G-CSF therapy in patients with ACLF. One week of G-CSF treatment increased leukocyte as well as neutrophil count and reduced disease severity indices in patients with ACLF. G-CSF therapy also prevented the development of sepsis, HRS and HE, and improved survival of these patients (166). CD34 expressing cells are generally considered to be hematopoietic stem cells that differentiate into all hepatic cell types and recover hepatic damage by inducing liver regeneration. We observed an increase in the mobilization of CD34+ stem cells after G-CSF treatment. Moreover, G-CSF has immunomodulatory effects shown by an increase in myeloid dendritic cells and a decrease in IFN-γ producing T cells after G-CSF therapy in patients with ACLF, which is beneficial in terms of reducing IFN-γ mediated inflammation and hepatic damage in these patients (32). G-CSF therapy may also benefit patients with alcoholic hepatitis, considering that these patients are at increased risk of developing bacterial infections. Our group has previously shown that G-CSF in combination with erythropoietin can decrease the risk of septic shock in patients with decompensated cirrhosis (167). On the contrary, a recent European multicenter clinical trial reveals that G-CSF does not have any superior benefits than the standard medical treatment. The findings unveil that G-CSF is ineffective in improving patient survival and other clinical endpoints including MELD score, CLIF-C organ failure score, and the occurrence of infection, recommending G-SCF not be used as a standard treatment for ACLF (168).

Liver Transplantation

Liver transplantation remains the ultimate therapy for patients with ACLF who face unsuccessful medical treatment (169). However, it is not feasible in all patients because of its high cost and lack of liver donors. Since, liver transplantation may be a lifesaving treatment, critical evaluation of final indication of liver transplant such as high MELD score, complications due to cirrhosis such including ascites, variceal hemorrhage, and HE should be considered (170). Patients with high MELD score have quick access to liver transplant; though, the requirement of suitable organ donors impose a major limitation (171). Liver transplant in severely ill patients with cirrhosis and organ failures is fetching attention and performed more frequently. Patients with ACLF with grade 1 and 2 display a similar posttransplant survival rate as those without ACLF (172). One-year post transplant survival rate of patients with ACLF with grade 3 varies between 44 and 83%. Since ACLF patient has poor short-term prognosis, a liver transplant might be a suitable therapeutic strategy for these patients; though the prioritization for liver transplant in these patients is quite challenging. It is important to consider if the patient has enough reserve to survive the preoperative and operative period and will have significant survival and quality of life from the liver transplant. Another major issue with the liver transplant is to choose the timing. Some recommend prioritizing the liver transplant of patients with ACLF in the waiting list after an initial stabilization. However, liver transplant in alcoholic hepatitis remains controversial as these patients drink often until or right before their presentation (173). Alcohol abstinence for 6 months is the universal requirement for the evaluation of liver transplant in these patients (174). Moreover, the shortage of liver donors along with the concerns for relapse, as it is a self-indulgent disease, enforce constant challenges to consider liver transplant

as a therapeutic option for patients with sAH. The rule for 6-month alcohol abstinence is to allow the liver to get enough time to improve and regenerate. If this period does not improve liver function and/or decrease episodes of decompensation, a liver transplant can be considered. However, the concept of 6-month alcohol abstinence was challenged by a study where

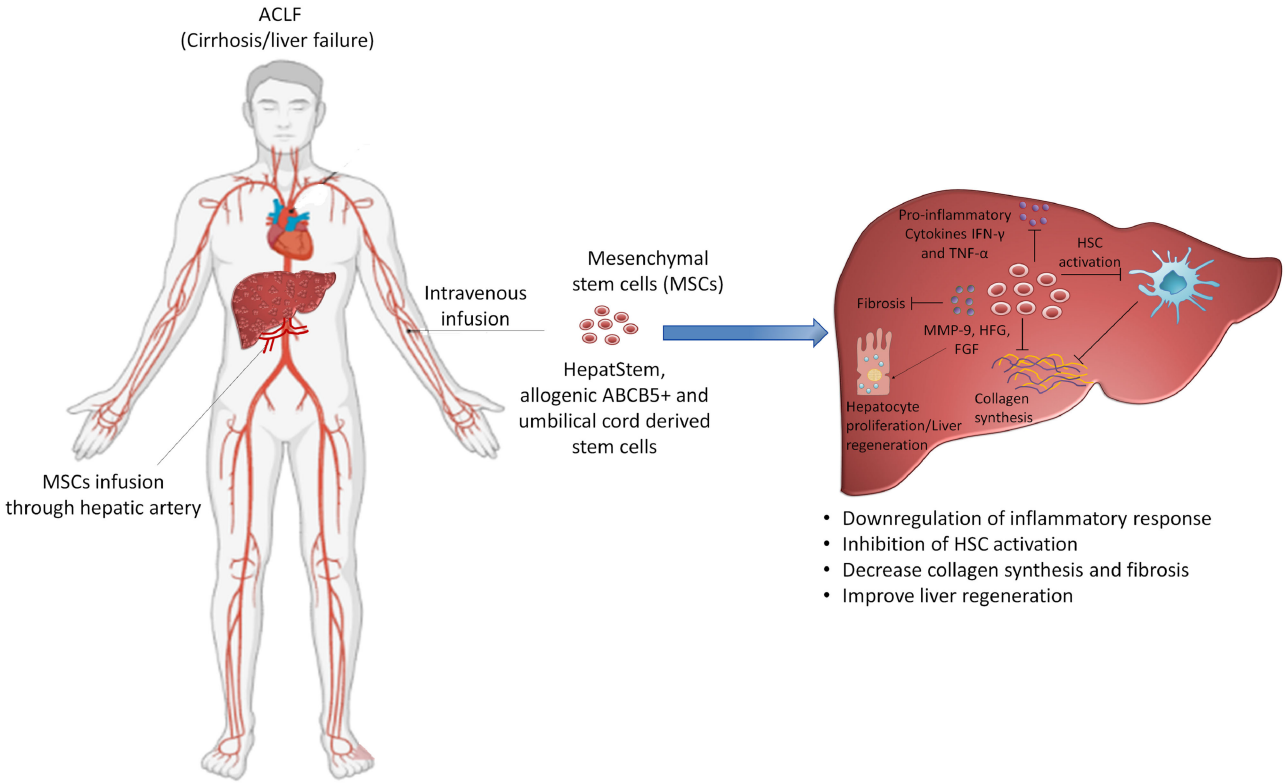


FIGURE 4 | Targeting stem cell therapy for the treatment of ACLF. Use of mesenchymal stem cells is under consideration and is being investigated in clinical trials for the treatment of ACLF. Mesenchymal stem cells possess different functions including the suppression of hyperactive inflammatory response, inhibition of hepatic stellate cell activation and collagen synthesis, reducing fibrosis and inducing liver regeneration. The diverse role of mesenchymal stem cells may benefit patients with ACLF and emerge as a potential therapeutic option for these patients. HSC: hepatic stellate cells. Some parts of this figure (Human structure) were prepared with the help of BioRender.

TABLE 1 | Clinical trials for the treatment of ACLF.

Drug/Therapy	Target	Phase	Trial number
HepaStem	Inhibits HSC activation, reduce collagen secretion and downregulation of pro-inflammatory environment.	II	NCT04229901
HepaStem	Mentioned above	II	NCT02946554
Umbilical cord derived MSCs	Improves liver fibrosis and regeneration	II	NCT04822922
Allogenic ABCB5-positive MSCs	Suppress inflammation and improve wound healing	I/II	NCT03860155
Combination of Simvastatin plus Rifaximin	Simvastatin reduce HSC activation and proliferation, increase liver sinusoidal function and decrease inflammation, Rifaximin prevent hepatic encephalopathy	IIIII	NCT03780673
Ribavirin	Hepatitis E virus infection	II	NCT01698723
PEG3350	Hepatic encephalopathy	IV	NCT03987893
Branched Chain Amino Acids	Hepatic encephalopathy	I	NCT04238416
Thymosin-α1	Treats immune suppression	N/A	NCT03082885
RL-1 Novel Human-derived bio artificial liver treatment	Improves hepatic function	N/A	NCT04195282
Albumin Plus Midodrine vs. Albumin	Reduces incidence of paracentesis induced circulatory dysfunctions	N/A	NCT04474262
Glucocorticoids	Inhibits inflammation	N/A	NCT01344174

HSC, Hepatic stellate cells; MSCs, Mesenchymal stem cells; N/A, Not applicable.

patients with sAH underwent liver transplant due to the steroid non-response (175, 176). Six-month survival of these patients was greatly improved as compared with those who did not receive a liver transplant, suggesting the requirement of early liver transplantation in patients with sAH (177, 178). **Table 1** illustrates different ongoing clinical trials for the treatment of patients with ACLF.

CONCLUSION

Acute-on-chronic liver failure is a distinct entity that differs from decompensated cirrhosis in terms of clinical presentation, pathophysiology, and prognosis. Despite the progress in the understanding of pathophysiological mechanisms in ACLF, there is no specific treatment available for these patients. Also, ACLF develops as a consequence of underlying CLD, compensated, and decompensated cirrhosis; hence, it is difficult for the clinicians to identify this syndrome at the initial stage, limiting the chances of recovery. Patients with ACLF have numerous complications that require separate diagnosis and treatment. Moreover, heterogeneity in the definition of ACLF in the Eastern and Western world restricts from exact characterization and universally accepted definition, which may also lead to differences in therapeutic approaches. Liver transplantation is unanimously accepted and the only definitive therapy for these patients in the Eastern and Western world, irrespective of different ACLF definitions and regional disparity.

Recently, stem cell technology has provided new hope to identify expandable sources that can induce liver regeneration in patients with ACLF. Ongoing clinical trials are investigating the efficacy of MSCs for the treatment of ACLF as they can reduce the inflammatory response, HSC activation, collagen secretion, and fibrosis, thereby improving liver regeneration. If the approach of regenerative medicine succeeds in the field of ACLF, it will be a milestone in providing new treatment options. Since, systemic inflammation is a major contributory factor in the worsening of ACLF condition, therapeutic approaches specifically targeting excessive inflammation are also warranted for better outcomes.

AUTHOR CONTRIBUTIONS

AK: conceptualized, drafted, edited the manuscript, and prepared the illustrations. SK: reviewed and edited the manuscript. Both authors approved the submitted version of the manuscript.

REFERENCES

1. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology.* (2013) 144:1426–37:37 e1–9. doi: 10.1053/j.gastro.2013.02.042
2. Sarin SK, Kedarisetty CK, Abbas Z, Amarapurkar D, Bihari C, Chan AC, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) (2014). *Hepatol Int.* (2014) 8:453–71. doi: 10.1007/s12072-014-9580-2
3. Mahmud N, Kaplan DE, Taddei TH, Goldberg DS. Incidence and mortality of acute-on-chronic liver failure using two definitions in patients with compensated cirrhosis. *Hepatology.* (2019) 69:2150–63. doi: 10.1002/hep.30494
4. O'Leary JG, Reddy KR, Garcia-Tsao G, Biggins SW, Wong F, Fallon MB, et al. NACSELD acute-on-chronic liver failure (NACSELD-ACLF) score predicts 30-day survival in hospitalized patients with cirrhosis. *Hepatology.* (2018) 67:2367–74. doi: 10.1002/hep.29773
5. Huang K, Hu JH, Wang HF, He WP, Chen J, Duan XZ, et al. Survival and prognostic factors in hepatitis B virus-related acute-on-chronic liver failure. *World J Gastroenterol.* (2011) 17:3448–52. doi: 10.3748/wjg.v17.i29.3448
6. Garg H, Kumar A, Garg V, Sharma P, Sharma BC, Sarin SK. Clinical profile and predictors of mortality in patients of acute-on-chronic liver failure. *Dig Liver Dis.* (2012) 44:166–71. doi: 10.1016/j.dld.2011.08.029
7. Jia L, Xue R, Zhu Y, Zhao J, Li J, He W-P, et al. The efficacy and safety of methylprednisolone in hepatitis B virus-related acute-on-chronic liver failure: a prospective multi-center clinical trial. *BMC Med.* (2020) 18:383. doi: 10.1186/s12916-020-01814-4
8. Mookerjee RP. Acute-on-chronic liver failure: the liver and portal haemodynamics. *Curr Opin Crit Care.* (2011) 17:170–6. doi: 10.1097/MCC.0b013e328344a076
9. Mehta G, Mookerjee RP, Sharma V, Jalan R. Systemic inflammation is associated with increased intrahepatic resistance and mortality in alcohol-related acute-on-chronic liver failure. *Liver Int.* (2015) 35:724–34. doi: 10.1111/liv.12559
10. López-Sánchez GN, Dóminguez-Pérez M, Uribe M, Nuño-Lámbarri N. The fibrogenic process and the unleashing of acute-on-chronic liver failure. *Clin Mol Hepatol.* (2020) 26:7–15. doi: 10.3350/cmh.2019.0011
11. Arroyo V, Moreau R, Kamath PS, Jalan R, Ginès P, Nevens F, et al. Acute-on-chronic liver failure in cirrhosis. *Nat Rev Dis Primers.* (2016) 2:16041. doi: 10.1038/nrdp.2016.41
12. Fleming KM, Aithal GP, Card TR, West J. All-cause mortality in people with cirrhosis compared with the general population: a population-based cohort study. *Liver Int.* (2012) 32:79–84. doi: 10.1111/j.1478-3231.2011.02517.x
13. Casulleras M, Zhang IW, López-Vicario C, Clària J. Leukocytes, systemic inflammation and immunopathology in acute-on-chronic liver failure. *Cells.* (2020) 9:2632. doi: 10.3390/cells9122632
14. Arroyo V, Angeli P, Moreau R, Jalan R, Claria J, Trebicka J, et al. The systemic inflammation hypothesis: towards a new paradigm of acute decompensation and multiorgan failure in cirrhosis. *J Hepatol.* (2021) 74:670–85. doi: 10.1016/j.jhep.2020.11.048
15. Khanam A, Kottilil S. Abnormal innate immunity in acute-on-chronic liver failure: immunotargets for therapeutics. *Front Immunol.* (2020) 11:2013. doi: 10.3389/fimmu.2020.02013
16. Khanam A, Trehanpati N, Riese P, Rastogi A, Guzman CA, Sarin SK. Blockade of neutrophil's chemokine receptors CXCR1/2 abrogate liver damage in acute-on-chronic liver failure. *Front Immunol.* (2017) 8:464. doi: 10.3389/fimmu.2017.00464
17. Kedarisetty CK, Anand L, Khanam A, Kumar A, Rastogi A, Maiwall R, et al. Growth factors enhance liver regeneration in acute-on-chronic liver failure. *Hepatol Int.* (2014) 8(Suppl. 2):514–25. doi: 10.1007/s12072-014-9538-4
18. Khanam A, Riese P, Pati N, Kedarisetty C, Guzman C, Sarin S. Chemokine ligand CXCL-8 mediated activation and neutrophil influx aggravates liver injury and correlates with clinical severity indices in acute-on-chronic liver failure (ACLF):283. *Hepatology.* (2013) 58.
19. Khanam A, Trehanpati N, Riese P, Rastogi A, Guzman C, Sarin S. High CXCR-1 and CXCR-2 expressing neutrophils induce early hepatocyte death and promote liver injury in acute-on-chronic liver failure: 1762. *Hepatology.* (2015) 62.
20. Wang Y, Chen C, Qi J, Wu F, Guan J, Chen Z, et al. Altered PGE2-EP2 is associated with an excessive immune response in HBV-related acute-on-chronic liver failure. *J Transl Med.* (2019) 17:93. doi: 10.1186/s12967-019-1844-0
21. Cai Q, Liu W, Zhu M, Sheng J. Microbial infections as a trigger for acute-on-chronic liver failure: a review. *Med Sci Monit.* (2019) 25:4773–83. doi: 10.12659/MSM.915637

22. Gustot T, Jalan R. Acute-on-chronic liver failure in patients with alcohol-related liver disease. *J Hepatol.* (2019) 70:319–27. doi: 10.1016/j.jhep.2018.12.008
23. Hoshi H, Chu PS, Yoshida A, Taniki N, Morikawa R, Yamataka K, et al. Vulnerability to recurrent episodes of acute decompensation/acute-on-chronic liver failure characterizes those triggered by indeterminate precipitants in patients with liver cirrhosis. *PLoS ONE.* (2021) 16:e0250062. doi: 10.1371/journal.pone.0250062
24. Hernaez R, Solà E, Moreau R, Ginès P. Acute-on-chronic liver failure: an update. *Gut.* (2017) 66:541–53. doi: 10.1136/gutjnl-2016-312670
25. Sarin SK, Choudhury A. Acute-on-chronic liver failure: terminology, mechanisms and management. *Nat Rev Gastroenterol Hepatol.* (2016) 13:131–49. doi: 10.1038/nrgastro.2015.219
26. Claria J, Arroyo V, Moreau R. The acute-on-chronic liver failure syndrome, or when the innate immune system goes astray. *J Immunol.* (2016) 197:3755–61. doi: 10.4049/jimmunol.1600818
27. Adebayo D, Morabito V, Andreola F, Pieri G, Luong TV, Dhillon A, et al. Mechanism of cell death in acute-on-chronic liver failure: a clinico-pathologic-biomarker study. *Liver Int.* (2015) 35:2564–74. doi: 10.1111/liv.12850
28. Zhang X, Dong P, Xu L, Tian Y, Sun H, Shi H, et al. The different expression of caspase-1 in HBV-related liver disease and acts as a biomarker for acute-on-chronic liver failure. *BMC Gastroenterol.* (2019) 19:148. doi: 10.1186/s12876-019-1064-3
29. Cao Z, Chen L, Li J, Liu Y, Bao R, Liu K, et al. Serum keratin-18 fragments as cell death biomarker in association with disease progression and prognosis in hepatitis B virus-related cirrhosis. *J Viral Hepat.* (2019) 26:835–45. doi: 10.1111/jvh.13100
30. Cao Z, Li F, Xiang X, Liu K, Liu Y, Tang W, et al. Circulating cell death biomarker: good candidates of prognostic indicator for patients with hepatitis B virus related acute-on-chronic liver failure. *Sci Rep.* (2015) 5:14240. doi: 10.1038/srep14240
31. Yi RT, Niu YH, Liu HL, Zhang TY, Yang YC, Zhang Y, et al. Natural killer group 2A expressed on both peripheral CD3(-)CD56(+)NK cells and CD3(+)CD8(+)T cells plays a pivotal negative regulatory role in the progression of hepatitis B virus-related acute-on-chronic liver failure. *J Interferon Cytok Res.* (2016) 36:689–97. doi: 10.1089/jir.2015.0166
32. Khanam A, Trehanpati N, Garg V, Kumar C, Garg H, Sharma BC, et al. Altered frequencies of dendritic cells and IFN-gamma-secreting T cells with granulocyte colony-stimulating factor (G-CSF) therapy in acute-on- chronic liver failure. *Liver Int.* (2014) 34:505–13. doi: 10.1111/liv.12415
33. Khanam A, Trehanpati N, Sarin SK. Increased interleukin-23 receptor (IL-23R) expression is associated with disease severity in acute-on-chronic liver failure. *Liver Int.* (2019) 39:1062–70. doi: 10.1111/liv.14015
34. Zeng Y, Li Y, Xu Z, Gan W, Lu L, Huang X, et al. Myeloid-derived suppressor cells expansion is closely associated with disease severity and progression in HBV-related acute-on-chronic liver failure. *J Med Virol.* (2019) 91:1510–8. doi: 10.1002/jmv.25466
35. Moreau R. The pathogenesis of ACLF: the inflammatory response and immune function. *Semin Liver Dis.* (2016) 36:133–40. doi: 10.1055/s-0036-1583199
36. Khanam A, Trehanpati N, Riese P, Rastogi A, Guzmán C, Sarin S. Neutrophils induce early hepatocyte death by contact dependent and independent mechanisms and positively correlate with the disease outcome in acute-on-chronic liver failure. *Journal of Hepatology.* (2016) 64. doi: 10.1016/S0168-8278(16)00878-3
37. Cao D, Xu H, Guo G, Ruan Z, Fei L, Xie Z, et al. Intrahepatic expression of programmed death-1 and its ligands in patients with HBV-related acute-on-chronic liver failure. *Inflammation.* (2013) 36:110–20. doi: 10.1007/s10753-012-9525-7
38. Rueschenbaum S, Ciesek S, Queck A, Widera M, Schwarzkopf K, Brune B, et al. Dysregulated adaptive immunity is an early event in liver cirrhosis preceding acute-on-chronic liver failure. *Front Immunol.* (2020) 11:534731. doi: 10.3389/fimmu.2020.534731
39. Schaapman JJ, Amoros A, van der Reijden JJ, Laleman W, Zeuzem S, Banares R, et al. Genetic variants of innate immunity receptors are associated with mortality in cirrhotic patients with bacterial infection. *Liver Int.* (2020) 40:646–53. doi: 10.1111/liv.14392
40. Alcaraz-Quiles J, Titos E, Casulleras M, Pavesi M, Lopez-Vicario C, Rius B, et al. Polymorphisms in the IL-1 gene cluster influence systemic inflammation in patients at risk for acute-on-chronic liver failure. *Hepatology.* (2017) 65:202–16. doi: 10.1002/hep.28896
41. Lange CM, Moreau R. Immunodysfunction in acute-on-chronic liver failure. *Visc Med.* (2018) 34:276–82. doi: 10.1159/000488690
42. Bernsmeier C, Pop OT, Singanayagam A, Triantafyllou E, Patel VC, Weston CJ, et al. Patients with acute-on-chronic liver failure have increased numbers of regulatory immune cells expressing the receptor tyrosine kinase MERTK. *Gastroenterology.* (2015) 148:603–15 e14. doi: 10.1053/j.gastro.2014.11.045
43. Khanam A, Trehanpati N, Shrivastava S, Garg V, Sakhuja P, Sarin S. Increased expression of regulatory T cells in liver ineffective in downregulating the inflammatory activation of T cells in acute on chronic liver failure (ACLF):1643. *Hepatology.* (2010) 52.
44. Martin-Mateos R, Alvarez-Mon M, Albillos A. Dysfunctional Immune response in acute-on-chronic liver failure: it takes two to Tango. *Front Immunol.* (2019) 10:973. doi: 10.3389/fimmu.2019.00973
45. Markwick LJ, Riva A, Ryan JM, Cooksley H, Palma E, Tranah TH, et al. Blockade of PD1 and TIM3 restores innate and adaptive immunity in patients with acute alcoholic hepatitis. *Gastroenterology.* (2015) 148:590–602 e10. doi: 10.1053/j.gastro.2014.11.041
46. Wasmuth HE, Kunz D, Yagmur E, Timmer-Stranghoner A, Vidacek D, Siewert E, et al. Patients with acute on chronic liver failure display "sepsis-like" immune paralysis. *J Hepatol.* (2005) 42:195–201. doi: 10.1016/j.jhep.2004.10.019
47. Triantafyllou E, Woollard KJ, McPhail MJW, Antoniades CG, Possamai LA. The role of monocytes and macrophages in acute and acute-on-chronic liver failure. *Front Immunol.* (2018) 9:2948. doi: 10.3389/fimmu.2018.02948
48. Bernsmeier C, Triantafyllou E, Brenig R, Lebosse FJ, Singanayagam A, Patel VC, et al. CD14(+) CD15(-) HLA-DR(-) myeloid-derived suppressor cells impair antimicrobial responses in patients with acute-on-chronic liver failure. *Gut.* (2018) 67:1155–67. doi: 10.1136/gutjnl-2017-314184
49. Engelmann C, Berg T. Management of infectious complications associated with acute-on-chronic liver failure. *Visceral Med.* (2018) 34:261–8. doi: 10.1159/000491107
50. Philips CA, Augustine P, Yerol PK, Rajesh S, Mahadevan P. Severe alcoholic hepatitis: current perspectives. *Hepat Med.* (2019) 11:97–108. doi: 10.2147/HMER.S197933
51. Bishehsari F, Magno E, Swanson G, Desai V, Voigt RM, Forsyth CB, et al. Alcohol and gut-derived inflammation. *Alcohol Res.* (2017) 38:163–71.
52. Bajaj JS. Alcohol, liver disease and the gut microbiota. *Nat Rev Gastroenterol Hepatol.* (2019) 16:235–46. doi: 10.1038/s41575-018-0099-1
53. Nagy LE. The role of innate immunity in alcoholic liver disease. *Alcohol Res Curr Rev.* (2015) 37:237–50.
54. Llorente C, Schnabl B. The gut microbiota and liver disease. *Cell Mol Gastroenterol Hepatol.* (2015) 1:275–84. doi: 10.1016/j.jcmgh.2015.04.003
55. Cichoz-Lach H, Michalak A. Oxidative stress as a crucial factor in liver diseases. *World J Gastroenterol.* (2014) 20:8082–91. doi: 10.3748/wjg.v20.i25.8082
56. Dubuquoy L, Louvet A, Lassailly G, Truant S, Boleslawski E, Artru F, et al. Progenitor cell expansion and impaired hepatocyte regeneration in explanted livers from alcoholic hepatitis. *Gut.* (2015) 64:1949–60. doi: 10.1136/gutjnl-2014-308410
57. Gauthier TW. Prenatal alcohol exposure and the developing immune system. *Alcohol Res Curr Rev.* (2015) 37:279–85.
58. Ren X, Xu Z, Liu Y, Li X, Bai S, Ding N, et al. Hepatitis B virus genotype and basal core promoter/precore mutations are associated with hepatitis B-related acute-on-chronic liver failure without pre-existing liver cirrhosis. *J Viral Hepat.* (2010) 17:887–95. doi: 10.1111/j.1365-2893.2009.01254.x
59. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology.* (2012) 142:1264–73.e1. doi: 10.1053/j.gastro.2011.12.061
60. Lei JH, Peng F, Chen Z, Xiao XQ. Is HBV viral load at admission associated with development of acute-on-chronic liver failure in patients with acute decompensation of chronic hepatitis B related cirrhosis? *BMC Infect Dis.* (2019) 19:363. doi: 10.1186/s12879-019-3988-1
61. Loomba R, Liang TJ. Hepatitis B reactivation associated with immune suppressive and biological modifier therapies: current concepts,

management strategies, and future directions. *Gastroenterology.* (2017) 152:1297–309. doi: 10.1053/j.gastro.2017.02.009
62. Park JW, Kwak KM, Kim SE, Jang MK, Kim DJ, Lee MS, et al. Differentiation of acute and chronic hepatitis B in IgM anti-HBc positive patients. *World J Gastroenterol.* (2015) 21:3953–9. doi: 10.3748/wjg.v21.i13.3953
63. Chauhan R, Kazim SN, Bhattacharjee J, Sakhuja P, Sarin SK. Basal core promoter, precore region mutations of HBV and their association with e antigen, genotype, and severity of liver disease in patients with chronic hepatitis B in India. *J Med Virol.* (2006) 78:1047–54. doi: 10.1002/jmv.20661
64. Li H, Xia Q, Zeng B, Li ST, Liu H, Li Q, et al. Submassive hepatic necrosis distinguishes HBV-associated acute on chronic liver failure from cirrhotic patients with acute decompensation. *J Hepatol.* (2015) 63:50–9. doi: 10.1016/j.jhep.2015.01.029
65. Hernaez R, Kramer JR, Liu Y, Tansel A, Natarajan Y, Hussain KB, et al. Prevalence and short-term mortality of acute-on-chronic liver failure: a national cohort study from the USA. *J Hepatol.* (2019) 70:639–47. doi: 10.1016/j.jhep.2018.12.018
66. Wu D, Zhang S, Xie Z, Chen E, Rao Q, Liu X, et al. Plasminogen as a prognostic biomarker for HBV-related acute-on-chronic liver failure. *J Clin Invest.* (2020) 130:2069–80. doi: 10.1172/JCI130197
67. Kumar A, Saraswat VA. Hepatitis E and acute-on-chronic liver failure. *J Clin Exp Hepatol.* (2013) 3:225–30. doi: 10.1016/j.jceh.2013.08.013
68. Zhang X, Ke W, Xie J, Zhao Z, Xie D, Gao Z. Comparison of effects of hepatitis E or A viral superinfection in patients with chronic hepatitis B. *Hepatol Int.* (2010) 4:615–20. doi: 10.1007/s12072-010-9204-4
69. Radha Krishna Y, Saraswat VA, Das K, Himanshu G, Yachha SK, Aggarwal R, et al. Clinical features and predictors of outcome in acute hepatitis A and hepatitis E virus hepatitis on cirrhosis. *Liver Int.* (2009) 29:392–8. doi: 10.1111/j.1478-3231.2008.01887.x
70. Pischke S, Hardtke S, Bode U, Birkner S, Chatzikyrkou C, Kauffmann W, et al. Ribavirin treatment of acute and chronic hepatitis E: a single-centre experience. *Liver Int.* (2013) 33:722–6. doi: 10.1111/liv.12114
71. Dalton HR, Kamar N. Treatment of hepatitis E virus. *Curr Opin Infect Dis.* (2016) 29:639–44. doi: 10.1097/QCO.0000000000000316
72. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: autoimmune hepatitis. *J Hepatol.* (2015) 63:971–1004. doi: 10.1016/j.jhep.2015.06.030
73. Yeoman AD, Westbrook RH, Zen Y, Bernal W, Al-Chalabi T, Wendon JA, et al. Prognosis of acute severe autoimmune hepatitis (AS-AIH): the role of corticosteroids in modifying outcome. *J Hepatol.* (2014) 61:876–82. doi: 10.1016/j.jhep.2014.05.021
74. Anand L, Choudhury A, Bihari C, Sharma BC, Kumar M, Maiwall R, et al. Flare of autoimmune hepatitis causing acute on chronic liver failure: diagnosis and response to corticosteroid therapy. *Hepatology.* (2019) 70:587–96. doi: 10.1002/hep.30205
75. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. *Hepatol Int.* (2019) 13:353–90. doi: 10.1007/s12072-019-09946-3
76. European Association for the Study of the Liver. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J Hepatol.* (2018) 69:406–60. doi: 10.1016/j.jhep.2018.08.009
77. Moller S, Bendtsen F. The pathophysiology of arterial vasodilatation and hyperdynamic circulation in cirrhosis. *Liver Int.* (2018) 38:570–80. doi: 10.1111/liv.13589
78. Garg H, Kumar A, Garg V, Kumar M, Kumar R, Sharma BC, et al. Hepatic and systemic hemodynamic derangements predict early mortality and recovery in patients with acute-on-chronic liver failure. *J Gastroenterol Hepatol.* (2013) 28:1361–7. doi: 10.1111/jgh.12191
79. Trebicka J, Gu W, Ibanez-Samaniego L, Hernandez-Gea V, Pitarch C, Garcia E, et al. Rebleeding and mortality risk are increased by ACLF but reduced by pre-emptive TIPS. *J Hepatol.* (2020) 73:1082–91. doi: 10.1016/j.jhep.2020.04.024
80. Mansour D, McPherson S. Management of decompensated cirrhosis. *Clin Med.* (2018) 18(Suppl. 2):s60–s5. doi: 10.7861/clinmedicine.18-2-s60
81. Alam A, Chun Suen K, Ma D. Acute-on-chronic liver failure: recent update. *J Biomed Res.* (2017) 31:1–18. doi: 10.7555/JBR.31.20160060
82. Romero-Gomez M, Montagnese S, Jalan R. Hepatic encephalopathy in patients with acute decompensation of cirrhosis and acute-on-chronic liver failure. *J Hepatol.* (2015) 62:437–47. doi: 10.1016/j.jhep.2014.09.005
83. Rose CF. Ammonia-lowering strategies for the treatment of hepatic encephalopathy. *Clin Pharmacol Therapeut.* (2012) 92:321–31. doi: 10.1038/clpt.2012.112
84. Lemberg A, Alejandra Fernández M. Hepatic encephalopathy, ammonia, glutamate, glutamine and oxidative stress. *Ann Hepatol.* (2009) 8:95–102. doi: 10.1016/S1665-2681(19)31785-5
85. Costa D, Simbrunner B, Jachs M, Hartl L, Bauer D, Paternostro R, et al. Systemic inflammation increases across distinct stages of advanced chronic liver disease and correlates with decompensation and mortality. *J Hepatol.* (2021) 74:819–28. doi: 10.1016/j.jhep.2020.10.004
86. Dong V, Karvellas CJ. Acute-on-chronic liver failure: objective admission and support criteria in the intensive care unit. *JHEP Rep.* (2019) 1:44–52. doi: 10.1016/j.jhepr.2019.02.005
87. Leise MD, Poterucha JJ, Kamath PS, Kim WR. Management of hepatic encephalopathy in the hospital. *Mayo Clin Proc.* (2014) 89:241–53. doi: 10.1016/j.mayocp.2013.11.009
88. Hudson M, Schuchmann M. Long-term management of hepatic encephalopathy with lactulose and/or rifaximin: a review of the evidence. *Eur J Gastroenterol Hepatol.* (2019) 31:434–50. doi: 10.1097/MEG.0000000000001311
89. Patidar KR, Bajaj JS. Antibiotics for the treatment of hepatic encephalopathy. *Metab Brain Dis.* (2013) 28:307–12. doi: 10.1007/s11011-013-9383-5
90. Mohammad RA, Regal RE, Alaniz C. Combination therapy for the treatment and prevention of hepatic encephalopathy. *Ann Pharmacother.* (2012) 46:1559–63. doi: 10.1345/aph.1R146
91. Poh Z, Chang PE. A current review of the diagnostic and treatment strategies of hepatic encephalopathy. *Int J Hepatol.* (2012) 2012:480309. doi: 10.1155/2012/480309
92. Yang L, Wu T, Li J. Bacterial infections in acute-on-chronic liver failure. *Semin Liver Dis.* (2018) 38:121–33. doi: 10.1055/s-0038-1657751
93. Fernandez J, Acevedo J, Wiest R, Gustot T, Amoros A, Deulofeu C, et al. Bacterial and fungal infections in acute-on-chronic liver failure: prevalence, characteristics and impact on prognosis. *Gut.* (2018) 67:1870–80. doi: 10.1136/gutjnl-2017-314240
94. Bunchorntavakul C, Chavalitdhamrong D. Bacterial infections other than spontaneous bacterial peritonitis in cirrhosis. *World J Hepatol.* (2012) 4:158–68. doi: 10.4254/wjh.v4.i5.158
95. Bunchorntavakul C, Chamroonkul N, Chavalitdhamrong D. Bacterial infections in cirrhosis: a critical review and practical guidance. *World J Hepatol.* (2016) 8:307–21. doi: 10.4254/wjh.v8.i6.307
96. Fernández J, Bert F, Nicolas-Chanoine MH. The challenges of multi-drug-resistance in hepatology. *J Hepatol.* (2016) 65:1043–54. doi: 10.1016/j.jhep.2016.08.006
97. Thulstrup AM, Sørensen HT, Schønheyder HC, Møller JK, Tage-Jensen U. Population-based study of the risk and short-term prognosis for bacteremia in patients with liver cirrhosis. *Clin Infect Dis.* (2000) 31:1357–61. doi: 10.1086/317494
98. Zhai XR, Tong JJ, Wang HM, Xu X, Mu XY, Chen J, et al. Infection deteriorating hepatitis B virus related acute-on-chronic liver failure: a retrospective cohort study. *BMC Gastroenterol.* (2020) 20:320. doi: 10.1186/s12876-020-01473-y
99. Mucke MM, Rumyantseva T, Mucke VT, Schwarzkopf K, Joshi S, Kempf VAJ, et al. Bacterial infection-triggered acute-on-chronic liver failure is associated with increased mortality. *Liver Int.* (2018) 38:645–53. doi: 10.1111/liv.13568
100. Liu XQ, Zhang XY, Ying Y, Zheng JM, Sun J, Zhang WH, et al. The role of prophylactic antibiotics in hepatitis B virus-related acute-on-chronic liver failure patients at risk of bacterial infection: a retrospective study. *Infect Dis Poverty.* (2021) 10:44. doi: 10.1186/s40249-021-00830-7
101. Zapater P, González-Navajas JM, Such J, Francés R. Immunomodulating effects of antibiotics used in the prophylaxis of bacterial infections in advanced cirrhosis. *World J Gastroenterol.* (2015) 21:11493–501. doi: 10.3748/wjg.v21.i41.11493
102. Medzhitov R, Schneider DS, Soares MP. Disease tolerance as a defense strategy. *Science.* (2012) 335:936–41. doi: 10.1126/science.1214935

103. Tazi KA, Bièche I, Paradis V, Guichard C, Laurendeau I, Dargère D, et al. *In vivo* altered unfolded protein response and apoptosis in livers from lipopolysaccharide-challenged cirrhotic rats. *J Hepatol.* (2007) 46:1075–88. doi: 10.1016/j.jhep.2007.01.034
104. Arab JP, Martin-Mateos RM, Shah VH. Gut-liver axis, cirrhosis and portal hypertension: the chicken and the egg. *Hepatol Int.* (2018) 12(Suppl. 1):24–33. doi: 10.1007/s12072-017-9798-x
105. Zhang X, Chen P, Gao H, Hao S, Yang M, Zhao H, et al. Bacterial infection and predictors of mortality in patients with autoimmune liver disease-associated acute-on-chronic liver failure. *Can J Gastroenterol Hepatol.* (2018) 2018:5108781. doi: 10.1155/2018/5108781
106. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med.* (2006) 34:1589–96. doi: 10.1097/01.CCM.0000217961.75225.E9
107. Verma N, Singh S, Taneja S, Duseja A, Singh V, Dhiman RK, et al. Invasive fungal infections amongst patients with acute-on-chronic liver failure at high risk for fungal infections. *Liver Int.* (2019) 39:503–13. doi: 10.1111/liv.13981
108. Wang C, Ma DQ, Luo S, Wang CM, Ding DP, Tian YY, et al. Incidence of infectious complications is associated with a high mortality in patients with hepatitis B virus-related acute-on-chronic liver failure. *World J Clin Cases.* (2019) 7:2204–16. doi: 10.12998/wjge.v7.i16.2204
109. Ferrarese A, Cattelan A, Cillo U, Gringeri E, Russo FP, Germani G, et al. Invasive fungal infection before and after liver transplantation. *World J Gastroenterol.* (2020) 26:7485–96. doi: 10.3748/wjg.v26.i47.7485
110. Lamoth F. Galactomannan and 1,3-beta-d-glucan testing for the diagnosis of invasive aspergillosis. *J Fungi (Basel).* (2016) 2(3). doi: 10.3390/jof2030022
111. Bartoletti M, Baldassarre M, Domenicali M, Lewis RE, Giannella M, Antognoli A, et al. Prognostic role of bacterial and fungal infections in patients with liver cirrhosis with and without acute-on-chronic liver failure: a prospective 2-center study. *Open Forum Infect Dis.* (2020) 7:ofaa453. doi: 10.1093/ofid/ofaa453
112. Habib S, Yarlagadda S, Carreon TA, Schader LM, Hsu CH. Fungal infection in acutely decompensated cirrhosis patients: value of model for end-stage liver disease score. *Gastroenterology Res.* (2020) 13:199–207. doi: 10.2139/ssrn.3393722
113. Philips CA, Sarin SK. Potent antiviral therapy improves survival in acute on chronic liver failure due to hepatitis B virus reactivation. *World J Gastroenterol.* (2014) 20:16037–52. doi: 10.3748/wjg.v20.i43.16037
114. Garg H, Sarin SK, Kumar M, Garg V, Sharma BC, Kumar A. Tenofovir improves the outcome in patients with spontaneous reactivation of hepatitis B presenting as acute-on-chronic liver failure. *Hepatology.* (2011) 53:774–80. doi: 10.1002/hep.24109
115. Yang J, Sun H, Liu Q. The comparative efficacy and safety of entecavir and lamivudine in patients with HBV-associated acute-on-chronic liver failure: a systematic review and meta-analysis. *Gastroenterol Res Pract.* (2016) 2016:5802674. doi: 10.1155/2016/5802674
116. Li J, Hu C, Chen Y, Zhang R, Fu S, Zhou M, et al. Short-term and long-term safety and efficacy of tenofovir alafenamide, tenofovir disoproxil fumarate and entecavir treatment of acute-on-chronic liver failure associated with hepatitis B. *BMC Infect Dis.* (2021) 21:567. doi: 10.1186/s12879-021-06237-x
117. Zhang X, An Y, Jiang X, Xu M, Xu L, Chen S, et al. Entecavir versus Lamivudine therapy for patients with chronic hepatitis B-associated liver failure: a meta-analysis. *Hepat Mon.* (2014) 14:e19164. doi: 10.5812/hepatmon.19164
118. Yang J, Chen G, Chen X, Zhang H, Jiang D, Yang G. Initial combination antiviral therapy with lamivudine and adefovir dipivoxil decreases short-term fatality rate of hepatitis-B-virus-related acute-on-chronic liver failure. *Virol J.* (2015) 12:97. doi: 10.1186/s12985-015-0323-3
119. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int.* (2016) 10:1–98. doi: 10.1007/s12072-015-9675-4
120. Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology.* (2018) 67:1560–99. doi: 10.1002/hep.29800
121. EASL 2017. Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol.* (2017). 67:370–98. doi: 10.1016/j.jhep.2017.03.021
122. Kumar R, Mehta G, Jalan R. Acute-on-chronic liver failure. *Clin Med.* (2020) 20:501–4. doi: 10.7861/clinmed.2020-0631
123. Kim W, Kim DJ. Severe alcoholic hepatitis-current concepts, diagnosis and treatment options. *World J Hepatol.* (2014) 6:688–95. doi: 10.4254/wjh.v6.i10.688
124. Louvet A, Naveau S, Abdelnour M, Ramond MJ, Diaz E, Fartoux L, et al. The Lille model: a new tool for therapeutic strategy in patients with severe alcoholic hepatitis treated with steroids. *Hepatology.* (2007) 45:1348–54. doi: 10.1002/hep.21607
125. Foncea CG, Sporea I, Lupusoru R, Moga TV, Bende F, Sirli R, et al. Day-4 Lille score is a good prognostic factor and early predictor in assessing therapy response in patients with liver cirrhosis and severe alcoholic hepatitis. *J Clin Med.* (2021) 10:2338. doi: 10.3390/jcm10112338
126. Zaccherini G, Weiss E, Moreau R. Acute-on-chronic liver failure: definitions, pathophysiology and principles of treatment. *JHEP Rep.* (2021) 3:100176. doi: 10.1016/j.jhepr.2020.100176
127. Vuittonet CL, Halse M, Leggio L, Fricchione SB, Brickley M, Haass-Koffler CL, et al. Pharmacotherapy for alcoholic patients with alcoholic liver disease. *Am J Health Syst Pharm.* (2014) 71:1265–76. doi: 10.2146/ajhp140028
128. Saberi B, Dadabhai AS, Jang YY, Gurakar A, Mezey E. Current management of alcoholic hepatitis and future therapies. *J Clin Transl Hepatol.* (2016) 4:113–22. doi: 10.14218/JCTH.2016.00006
129. Zein CO, Yerian LM, Gogate P, Lopez R, Kirwan JP, Feldstein AE, et al. Pentoxifylline improves nonalcoholic steatohepatitis: a randomized placebo-controlled trial. *Hepatology.* (2011) 54:1610–9. doi: 10.1002/hep.24544
130. Raetsch C, Jia JD, Boigk G, Bauer M, Hahn EG, Riecken EO, et al. Pentoxifylline downregulates profibrogenic cytokines and procollagen I expression in rat secondary biliary fibrosis. *Gut.* (2002) 50:241–7. doi: 10.1136/gut.50.2.241
131. Tyagi P, Sharma P, Sharma BC, Puri AS, Kumar A, Sarin SK. Prevention of hepatorenal syndrome in patients with cirrhosis and ascites: a pilot randomized control trial between pentoxifylline and placebo. *Eur J Gastroenterol Hepatol.* (2011) 23:210–7. doi: 10.1097/MEG.0b013e3283435d76
132. De B, Mandal S, Sau D, Mani S, Chatterjee S, Mondal S, et al. Pentoxifylline plus prednisolone versus pentoxifylline only for severe alcoholic hepatitis: a randomized controlled clinical trial. *Ann Med Health Sci Res.* (2014) 4:810–6. doi: 10.4103/2141-9248.141562
133. O'Shea RS, Dasarathy S, McCullough AJ. Alcoholic liver disease. *Hepatology.* (2010) 51:307–28. doi: 10.1002/hep.23258
134. European Association for the Study of Liver. EASL clinical practical guidelines: management of alcoholic liver disease. *J Hepatol.* (2012) 57:399–420. doi: 10.1016/j.jhep.2012.04.004
135. de Andrade KQ, Moura FA, dos Santos JM, de Araújo OR, de Farias Santos JC, Goulart MO. Oxidative stress and inflammation in hepatic diseases: therapeutic possibilities of n-acetylcysteine. *Int J Mol Sci.* (2015) 16:30269–308. doi: 10.3390/ijms161226225
136. Cai Z, Lou Q, Wang F, Li E, Sun J, Fang H, et al. N-acetylcysteine protects against liver injure induced by carbon tetrachloride via activation of the Nrf2/HO-1 pathway. *Int J Clin Exp Pathol.* (2015) 8:8655–62.
137. Nguyen-Khac E, Thevenot T, Piquet MA, Benferhat S, Goria O, Chatelain D, et al. Glucocorticoids plus N-acetylcysteine in severe alcoholic hepatitis. *N Engl J Med.* (2011) 365:1781–9. doi: 10.1056/NEJMoa 1101214
138. Amjad W, Alukal J, Doycheva I, Zhang T, Maheshwari A, Yoo H, et al. A combination of n-acetylcysteine and prednisone has no benefit over prednisone alone in severe alcoholic hepatitis: a retrospective analysis. *Dig Dis Sci.* (2020) 65:3726–33. doi: 10.1007/s10620-020-06142-4
139. Singh V, Keisham A, Bhalla A, Sharma N, Agarwal R, Sharma R, et al. Efficacy of granulocyte colony-stimulating factor and n-acetylcysteine therapies in patients with severe alcoholic hepatitis. *Clin Gastroenterol Hepatol.* (2018) 16:1650–6.e2. doi: 10.1016/j.cgh.2018.01.040
140. Moreno C, Langlet P, Hittelet A, Lasser L, Degré D, Evrard S, et al. Enteral nutrition with or without N-acetylcysteine in the treatment of severe acute alcoholic hepatitis: a randomized multicenter controlled trial. *J Hepatol.* (2010) 53:1117–22. doi: 10.1016/j.jhep.2010.05.030
141. Fernandez J, Acevedo J, Castro M, Garcia O, de Lope CR, Roca D, et al. Prevalence and risk factors of infections by multiresistant

bacteria in cirrhosis: a prospective study. *Hepatology.* (2012) 55:1551–61. doi: 10.1002/hep.25532
142. Piano S, Fasolato S, Salinas F, Romano A, Tonon M, Morando F, et al. The empirical antibiotic treatment of nosocomial spontaneous bacterial peritonitis: results of a randomized, controlled clinical trial. *Hepatology.* (2016) 63:1299–309. doi: 10.1002/hep.27941
143. Fiore M, Maraolo AE, Gentile I, Borgia G, Leone S, Sansone P, et al. Nosocomial spontaneous bacterial peritonitis antibiotic treatment in the era of multi-drug resistance pathogens: a systematic review. *World J Gastroenterol.* (2017) 23:4654–60. doi: 10.3748/wjg.v23.i25.4654
144. Zaccherini G, Tufoni M, Bernardi M. Albumin administration is efficacious in the management of patients with cirrhosis: a systematic review of the literature. *Hepat Med.* (2020) 12:153–72. doi: 10.2147/HMER.S264231
145. Cao Y, Fan Y, Wang Y, Liu X, Xie W. Acute-on-chronic liver failure in a patient with candida endophthalmitis: a case report. *J Clin Transl Hepatol.* (2021) 9:447–51. doi: 10.14218/JCTH.2020.00092
146. Prodanovic H, Cracco C, Massard J, Barrault C, Thabut D, Duguet A, et al. Invasive pulmonary aspergillosis in patients with decompensated cirrhosis: case series. *BMC Gastroenterol.* (2007) 7:2. doi: 10.1186/1471-230X-7-2
147. Chen J, Yang Q, Huang J, Li L. Risk factors for invasive pulmonary aspergillosis and hospital mortality in acute-on-chronic liver failure patients: a retrospective-cohort study. *Int J Med Sci.* (2013) 10:1625–31. doi: 10.7150/ijms.6824
148. Wang W, Zhao CY, Zhou JY, Wang YD, Shen C, Zhou DF, et al. Invasive pulmonary aspergillosis in patients with HBV-related liver failure. *Eur J Clin Microbiol Infect Dis.* (2011) 30:661–7. doi: 10.1007/s10096-010-1137-2
149. Gustot T, Maillart E, Bocci M, Surin R, Trépo E, Degré D, et al. Invasive aspergillosis in patients with severe alcoholic hepatitis. *J Hepatol.* (2014) 60:267–74. doi: 10.1016/j.jhep.2013.09.011
150. Wu Z, Ling Z, Shao F, Sheng J, Li L. Invasive pulmonary aspergillosis in patients with acute-on-chronic liver failure. *J Int Med Res.* (2012) 40:1958–65. doi: 10.1177/030006051204000537
151. Patterson TF, Thompson GR, 3rd, Denning DW, Fishman JA, Hadley S, Herbrecht R, et al. Executive summary: practice guidelines for the diagnosis and management of aspergillosis: 2016. Update by the Infectious Diseases Society of America. *Clin Infect Dis.* (2016) 63:433–42. doi: 10.1093/cid/ciw444
152. Dolton MJ, McLachlan AJ. Voriconazole pharmacokinetics and exposure-response relationships: assessing the links between exposure, efficacy and toxicity. *Int J Antimicrob Agents.* (2014) 44:183–93. doi: 10.1016/j.ijantimicag.2014.05.019
153. Gao J, Zhang Q, Wu Y, Li Y, Qi T, Zhu C, et al. Improving survival of acute-on-chronic liver failure patients complicated with invasive pulmonary aspergillosis. *Sci Rep.* (2018) 8:876. doi: 10.1038/s41598-018-19320-2
154. Vipani A, Lindenmeyer CC, Sundaram V. Treatment of severe acute on chronic liver failure: management of organ failures, investigational therapeutics, and the role of liver transplantation. *J Clin Gastroenterol.* (2021) 55:667–76. doi: 10.1097/MCG.0000000000001568
155. Xue R, Meng Q, Dong J, Li J, Yao Q, Zhu Y, et al. Clinical performance of stem cell therapy in patients with acute-on-chronic liver failure: a systematic review and meta-analysis. *J Transl Med.* (2018) 16:126. doi: 10.1186/s12967-018-1464-0
156. Nevens F, Gustot T, Laterre PF, Lasser LL, Haralampiev LE, Vargas V, et al. A phase II study of human allogeneic liver-derived progenitor cell therapy for acute-on-chronic liver failure and acute decompensation. *JHEP Rep.* (2021) 3:100291. doi: 10.1016/j.jhepr.2021.100291
157. Feng Y, Wang AT, Jia HH, Zhao M, Yu H. A brief analysis of mesenchymal stem cells as biological drugs for the treatment of acute-on-chronic liver failure (ACLF): safety and potency. *Curr Stem Cell Res Ther.* (2020) 15:202–10. doi: 10.2174/1574888X15666200101124317
158. Pittenger MF, Discher DE, Péault BM, Phinney DG, Hare JM, Caplan AI. Mesenchymal stem cell perspective: cell biology to clinical progress. *NPJ Regen Med.* (2019) 4:22. doi: 10.1038/s41536-019-0083-6
159. Luan Y, Kong X, Feng Y. Mesenchymal stem cells therapy for acute liver failure: recent advances and future perspectives. *Liver Res.* (2021) 5:53–61. doi: 10.1016/j.livres.2021.03.003
160. Eom YW, Shim KY, Baik SK. Mesenchymal stem cell therapy for liver fibrosis. *Korean J Internal Med.* (2015) 30:580–9. doi: 10.3904/kjim.2015.30.5.580
161. Cho KA, Woo SY, Seoh JY, Han HS, Ryu KH. Mesenchymal stem cells restore CCl4-induced liver injury by an antioxidative process. *Cell Biol Int.* (2012) 36:1267–74. doi: 10.1042/CBI20110634
162. Quintanilha LF, Takami T, Hirose Y, Fujisawa K, Murata Y, Yamamoto N, et al. Canine mesenchymal stem cells show antioxidant properties against thioacetamide-induced liver injury *in vitro* and *in vivo*. *Hepatol Res.* (2014) 44:E206–17. doi: 10.1111/hepr.12204
163. He Y, Guo X, Lan T, Xia J, Wang J, Li B, et al. Human umbilical cord-derived mesenchymal stem cells improve the function of liver in rats with acute-on-chronic liver failure via downregulating Notch and Stat1/Stat3 signaling. *Stem Cell Res Ther.* (2021) 12:396. doi: 10.1186/s13287-021-02468-6
164. Shi M, Zhang Z, Xu R, Lin H, Fu J, Zou Z, et al. Human mesenchymal stem cell transfusion is safe and improves liver function in acute-on-chronic liver failure patients. *Stem Cells Transl Med.* (2012) 1:725–31. doi: 10.5966/sctm.2012-0034
165. Bai YQ, Yang YX, Yang YG, Ding SZ, Jin FL, Cao MB, et al. Outcomes of autologous bone marrow mononuclear cell transplantation in decompensated liver cirrhosis. *World J Gastroenterol.* (2014) 20:8660–6. doi: 10.3748/wjg.v20.i26.8660
166. Garg V, Garg H, Khan A, Trehanpati N, Kumar A, Sharma BC, et al. Granulocyte colony-stimulating factor mobilizes CD34(+) cells and improves survival of patients with acute-on-chronic liver failure. *Gastroenterology.* (2012) 142:505–12 e1. doi: 10.1053/j.gastro.2011.11.027
167. Kedarisetty CK, Anand L, Bhardwaj A, Bhadoria AS, Kumar G, Vyas AK, et al. Combination of granulocyte colony-stimulating factor and erythropoietin improves outcomes of patients with decompensated cirrhosis. *Gastroenterology.* (2015) 148:1362–70 e7. doi: 10.1053/j.gastro.2015.02.054
168. Engelmann C, Herber A, Franke A, Bruns T, Schiefke I, Zipprich A, et al. Granulocyte-Colony Stimulating Factor (G-CSF) to treat acute-on-chronic liver failure, a multicenter randomized trial (GRAFT study). *J Hepatol.* (2021). doi: 10.1136/gutjnl-2021-BASL.10
169. Choudhary NS, Saraf N, Saigal S, Soin AS. Liver transplantation for acute on chronic liver failure. *J Clin Exp Hepatol.* (2017) 7:247–52. doi: 10.1016/j.jceh.2017.08.001
170. O'Leary JG, Lepe R, Davis GL. Indications for liver transplantation. *Gastroenterology.* (2008) 134:1764–76. doi: 10.1053/j.gastro.2008.02.028
171. Agbim U, Sharma A, Maliakkal B, Karri S, Yazawa M, Goldkamp W, et al. Outcomes of liver transplant recipients with acute-on-chronic liver failure based on EASL-CLIF consortium definition: a single-center study. *Transplant Direct.* (2020) 6:e544. doi: 10.1097/TXD.0000000000000984
172. Sundaram V, Kogachi S, Wong RJ, Karvellas CJ, Fortune BE, Mahmud N, et al. Effect of the clinical course of acute-on-chronic liver failure prior to liver transplantation on post-transplant survival. *J Hepatol.* (2020) 72:481–8. doi: 10.1016/j.jhep.2019.10.013
173. Shipley LC, Singal AK. Liver transplantation for alcoholic hepatitis. *Transl Gastroenterol Hepatol.* (2020) 5:26. doi: 10.21037/tgh.2019.11.17
174. Obed A, Stern S, Jarrad A, Lorf T. Six month abstinence rule for liver transplantation in severe alcoholic liver disease patients. *World J Gastroenterol.* (2015) 21:4423–6. doi: 10.3748/wjg.v21.i14.4423
175. Marroni CA, Fleck AM Jr., Fernandes SA, Galant LH, Mucenic M, et al. Liver transplantation and alcoholic liver disease: History, controversies, and considerations. *World J Gastroenterol.* (2018) 24:2785–805. doi: 10.3748/wjg.v24.i26.2785

176. Mitchell MC, Kerr T, Herlong HF. Current management and future treatment of alcoholic hepatitis. *Gastroenterol Hepatol.* (2020) 16:178–89.
177. Mathurin P, Moreno C, Samuel D, Dumortier J, Salleron J, Durand F, et al. Early liver transplantation for severe alcoholic hepatitis. *N Engl J Med.* (2011) 365:1790–800. doi: 10.1056/NEJMoa1105703
178. Mellinger JL, Stine JG. Early liver transplantation for severe alcoholic hepatitis. *Dig Dis Sci.* (2020) 65:1608–14. doi: 10.1007/s10620-020-06159-9

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 10 November 2021
doi: 10.3389/fmed.2021.763370

Proton Pump Inhibitor Therapy Does Not Affect Prognosis of Cirrhosis Patients With Acute Decompensation and Acute-on-Chronic Liver Failure: A Single-Center Prospective Study

Shanshan Sun[1†], Wenyi Ye[2†], Ruihong Zhao[1], Jianhua Hu[1], Xuan Zhang[1], Meifang Yang[1], Hong Zhao[1] and Jifang Sheng[1*]*

[1] State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, [2] Department of Traditional Chinese Internal Medicine, The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, China

OPEN ACCESS

Edited by:
Yu-Chen Fan,
Shandong University, China

Reviewed by:
Qiao Yang,
Sir Run Run Shaw Hospital, China
Yubao Zheng,
Third Affiliated Hospital of Sun Yat-sen University, China

***Correspondence:**
Hong Zhao
zjuzhaohong@zju.edu.cn
Jifang Sheng
jifang_sheng@zju.edu.cn

[†] These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 23 August 2021
Accepted: 07 October 2021
Published: 10 November 2021

Citation:
Sun S, Ye W, Zhao R, Hu J, Zhang X, Yang M, Zhao H and Sheng J (2021) Proton Pump Inhibitor Therapy Does Not Affect Prognosis of Cirrhosis Patients With Acute Decompensation and Acute-on-Chronic Liver Failure: A Single-Center Prospective Study. Front. Med. 8:763370. doi: 10.3389/fmed.2021.763370

Background: The aim of this study was to investigate the impact of proton pump inhibitor (PPI) therapy on complications and prognosis in cirrhosis patients with and without acute-on-chronic liver failure (ACLF).

Materials and Methods: Cirrhosis patients with acute decompensation (AD) ($n = 489$) admitted in our center were enrolled in this prospective observational cohort study. According to treatment received, patients were identified as users or nonusers of PPI. Clinical and laboratory data, complications during hospitalization, and overall survival were recorded in all the patients.

Results: Of the 489 patients, 299 (61.1%) patients received PPI therapy. The logistic regression analysis showed that age, albumin, history of previous hepatic encephalopathy (HE), and the chronic liver failure-sequential organ failure assessment (CLIF-SOFA) score were independent risk factors for HE in patients with decompensated cirrhosis [odds ratio (OR) = 1.07, 95% CI: 1.03–1.12, $p = 0.001$; OR = 1.13, 95% CI: 1.04–1.24, $p = 0.006$; OR = 242.52, 95% CI: 40.17–1464.11, $p < 0.001$; and OR = 2.89, 95% CI: 2.11–3.96, $p < 0.001$, respectively]. Previous severe liver injury and previous bacterial infections were independent risk factors for spontaneous bacterial peritonitis (SBP) in patients with decompensated cirrhosis (OR = 3.43, 95% CI: 1.16–10.17, $p = 0.026$ and OR = 6.47, 95% CI: 2.29–18.29, $p < 0.001$, respectively). The multivariate Cox proportional hazards regression model showed that the type and dose of the PPI used were not related to 28-day and 90-day mortality in cirrhosis patients with AD or ACLF.

Conclusion: PPI use does not appear to increase mortality or the risk of HE and SBP in the hospitalized cirrhosis patients with and without ACLF.

Keywords: proton pump inhibitor, acute decompensated cirrhosis, acute-on-chronic liver failure, prognosis, complications

INTRODUCTION

Proton pump inhibitors (PPIs) are commonly used in cirrhosis patients to treat gastrointestinal disorders, especially gastrointestinal bleeding (1–3). Gastrointestinal hemorrhage may be due to bleeding varices or ulcers or portal hypertensive gastropathy and is one of the most serious complications seen in acute-on-chronic liver failure (ACLF) (4–6). While PPI is useful in these cases, the drug may alter the composition of the gut microbiota and increase the risk of spontaneous bacterial peritonitis (SBP), hepatic encephalopathy (HE), *Clostridium difficile* infection, and mortality (7–14). Although some recent studies have indicated that PPI therapy does not increase the risk of SBP or mortality in cirrhosis, these studies did not include patients with ACLF (15–18). The aim of this study was to assess whether PPI therapy increases the incidence of SBP and HE and decreases survival in cirrhosis patients with and without ACLF.

MATERIALS AND METHODS

Patients

Patients diagnosed as cirrhosis with acute decompensation (AD) and hospitalized in the Department of Infectious Diseases at the First Affiliated Hospital, School of Medicine, Zhejiang University, Zhejiang, China, between February 2014 and March 2015 were eligible for inclusion in this study. Patients were excluded if they: (1) were $<$ 18 years old; (2) were pregnant; (3) had evidence of infection with human immunodeficiency virus, Epstein-Barr virus, or human cytomegalovirus; (4) had aplastic anemia, myelodysplastic syndrome, thrombocytopenia, hemophilia, or disseminated intravascular coagulation; (5) were currently using immunosuppressant medications or adrenocortical hormones; (6) had hepatocellular carcinoma or other malignant tumors; (7) had chronic renal disease or other serious comorbidity; or (8) had history of liver transplantation (19, 20). The diagnosis of liver cirrhosis was based on clinical evidence, endoscopic or radiologic examination, and liver biopsy (6, 21). Cirrhosis patients were diagnosed with AD if they had more than one of the following major complications: ascites, encephalopathy, gastrointestinal hemorrhage, severe liver injury, and infection (20). Severe liver injury was diagnosed if alanine aminotransferase (ALT) was $\geq$ five times the normal upper limit or more than twice the baseline value along with elevation of serum bilirubin to $\geq$ 85 μmol/l and international normalized ratio (INR) to $\geq$ 1.5 at any time during the preceding 1 month (21). The chronic liver failure-sequential organ failure assessment (CLIF-SOFA) score was used to diagnose organ failure (6). The diagnosis of ACLF was based on the criteria proposed by the Chinese Group on the Study of Severe Hepatitis B (COSSH) (22). The diagnosis of gastrointestinal bleeding and bacterial infections was made as previously described (1, 23, 24). PPI treatment was generally used in patients with gastrointestinal bleeding and gastric ulcer, but was extended in patients receiving endoscopic variceal ligation and those manifesting gastrointestinal disturbances such as hiccups, epigastric pain, nausea, or vomiting. For this study, "PPI use" was defined as intravenous administration of any PPI for at least 6 days with daily dose higher than that recommended by the WHO (13). Patients receiving lower doses were considered to be nonusers. PPI doses are classified according to the cumulative defined daily dose (cDDD) (25).

Informed consent for participation in this study was obtained from each participant or his or her legal surrogate. This study was approved by the Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University, Zhejiang, China and all the procedures were in accordance with the latest version of the Declaration of Helsinki.

Data Collection

Demographic data, clinical history, reason for admission, physical examination findings, laboratory results, cirrhosis complications, events of organ failure, and prognosis were recorded. Potential precipitating factors for SBP and HE were noted. Survival at 28 days and 90 days following enrollment was recorded. Survival information was obtained by telephonic interview.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corporation, Armonk, New York, USA) was used for the data analysis. Categorical variables were summarized as numbers and percentages and compared by using the chi-squared test. Continuous variables were summarized as means $\pm$ SDs or medians (and interquartile ranges) and compared by using the Student's *t*-test or the Mann–Whitney *U* test. Variables found to be associated with SBP and HE on the univariate analysis ($p < 0.10$) were entered into the multivariate logistic regression analysis to identify the independent risk factors for SBP or HE. Baseline characteristics were compared between patients receiving and not receiving PPI therapy before and after propensity score matching (PSM). We performed PSM to adjust differences in baseline characteristics of decompensated cirrhosis and ACLF including gender, age, leukocyte count, platelet count, hemoglobin, serum bilirubin, albumin, aspartate aminotransferase (AST), ALT, INR, creatinine, serum sodium, the CLIF-SOFA score, and the model for end-stage liver disease (MELD) score by matching non-PPI users with comparable patients with PPI users in a ratio of 1:1 with a caliper of 0.1 of the SD. A standardized difference $<$ 0.1 indicated a good balance between PPI and non-PPI groups. The multivariate Cox proportional hazards regression model was used to analyze the risk factors for 28-day and 90-day mortality. For the multivariate analysis, the probability for stepwise entry was at $p = 0.05$ and for removal at $p = 0.10$; only variables with $p < 0.05$ were retained in the final model. Statistical significance was at $p \leq 0.05$.

RESULTS

Basic Characteristics of PPI Users and Non-users

A total of 489 acute decompensated patients with liver cirrhosis were selected in this study (**Figure 1**). Among these 489 patients (409 patients without ACLF and 80 patients with ACLF), PPI therapy was used in 299 (61.1%) patients: 256/409 (62.6%) patients without ACLF and 43/80 (53.8%) patients with ACLF

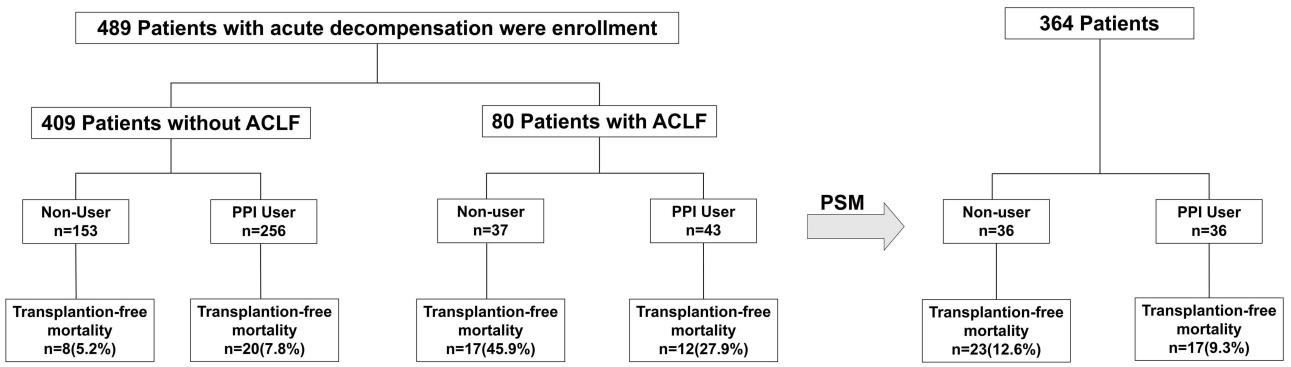


FIGURE 1 | Flowchart showing patient selection for the study. A total of 489 acute decompensated patients with liver cirrhosis were selected and then propensity score matched with 1:1. PPI, proton pump inhibitor; ACLF, acute-on-chronic liver failure.

TABLE 1 | Baseline characteristics of the enrolled patients receiving and not receiving proton pump inhibitor (PPI) therapy.

	Before PSM			After PSM		
Variables	**Non-PPI group (n = 190)**	**PPI group (n = 299)**	***P* value**	**Non-PPI group (n = 182)**	**PPI group (n = 182)**	***P* value**
Age (years)	55.47 ± 13.21	53.60 ± 11.52	0.081	55.48 ± 13.21	52.26 ± 11.17	0.020
Sex (male%)	141 (74.2%)	239 (79.9%)	0.139	135 (74.2%)	157 (86.3%)	0.004
Hypertension (%)	38 (20.0%)	47 (15.7%)	0.224	37 (20.3%)	21 (11.5%)	0.022
Etiology of cirrhosis						
HBV	108 (56.8%)	188 (62.9%)	0.184	104 (57.1%)	126 (69.2%)	0.017
Alcohol	38 (20.0%)	50 (16.7%)	0.358	36 (19.8%)	28 (15.4%)	0.271
HBV plus alcohol	1 (0.5%)	3 (1.0%)	0.568	1 (0.5%)	2 (1.1%)	0.563
Others	43 (22.6%)	58 (19.4%)	0.389	41 (22.5%)	26 (14.3%)	0.043
Previous de–compensation events[a] (%)						
Ascites[a]	89 (46.8%)	108 (36.1%)	0.019	87 (47.8%)	52 (28.6%)	0.000
Upper gastrointestinal bleeding[a]	29 (15.3%)	73 (24.4%)	0.015	25 (13.7%)	57 (31.3%)	0.000
Hepatic encephalopathy[a]	12 (6.3%)	10 (3.3%)	0.123	11 (6.0%)	6 (3.3%)	0.215
Bacterial infections[a]	18 (9.5%)	19 (6.4%)	0.204	15 (8.2%)	12 (6.6%)	0.549
Severe liver injury[a]	23 (12.1%)	24 (8.0%)	0.136	22 (12.1%)	12 (6.6%)	0.072
Complications of cirrhosis during hospital (%)						
Hepatic encephalopathy	24 (12.6%)	26 (8.7%)	0.162	20 (11.0%)	16 (8.8%)	0.483
SBP	9 (4.7%)	12 (4.0%)	0.701	9 (4.9%)	9 (4.9%)	1.000
MELD score	14.81 ± 8.34	13.10 ± 8.22	0.017	14.81 ± 8.34	11.42 ± 8.20	0.000
Laboratory data						
Leukocyte count (10^9/L)	4.4 (2.7–6.6)	4.7 (2.8–7.4)	0.693	4.4 (2.7–6.6)	4.8 (2.6–8.2)	0.652
Platelet count (10^9/L)	68 (41–103)	71 (45–108)	0.372	68 (41–103)	73 (47–116)	0.150
Hemoglobin (g/L)	105 (90–122)	94 (74–113)	0.000	105 (90–122)	80 (68–99)	0.000
Serum bilirubin (μmol/L)	48 (22–185)	35 (19–138)	0.041	48 (22–185)	28 (17–117)	0.000
Albumin (g/L)	29.1 (25.8–32.5)	29.3 (25.6–33.0)	0.650	29.1 (25.8–32.5)	30.2 (26.9–33.4)	0.114
AST (IU/L)	59 (33–101)	47 (29–91)	0.075	59 (33–101)	40 (27–73)	0.002
ALT (IU/L)	35 (19–66)	26 (17–51)	0.026	35 (19–66)	24 (15–46)	0.006
INR	1.4 (1.2–1.7)	1.3 (1.2–1.6)	0.007	1.4 (1.2–1.7)	1.3 (1.2–1.6)	0.000
Creatinine (μmol/L)	71 (59–93)	73 (59–92)	0.753	71 (59–93)	73 (58–88)	0.778
Serum sodium (mmol/L)	139 (136–141)	139 (136–142)	0.069	139 (136–141)	140 (136–142)	0.000
CLIF–SOFA score	5.08 ± 2.60	4.85 ± 2.71	0.224	5.09 ±2.55	4.60 ± 2.89	0.017
28–day mortality	25 (13.2%)	32 (10.7%)	0.902	23 (12.6%)	17 (9.3%)	0.681
90–day mortality	36 (18.9%)	59 (19.7%)	0.439	34 (18.7%)	32 (17.6%)	0.836

Data are expressed as mean ± SD, median (Q1–Q3), or number (percentage). Comparisons between the groups were performed by the Student's t-test, the Mann–Whitney U test, or the chi-squared test. [a]Within the prior 3 months before the hospital admission related to the study. AST, aspartate aminotransferase; ALT, alanine aminotransferase; HBV, hepatitis B virus; INR, international normalized ratio; MELD score, model for end-stage liver disease score; SBP, spontaneous bacterial peritonitis.

(**Figure 1**; **Table 1**). The PPIs included omeprazole ($n = 98$; daily dose 40–80 mg), pantoprazole ($n = 132$; daily dose 40–80 mg), and lansoprazole ($n = 69$; daily dose 30–60 mg).

Proton pump inhibitor non-users ($n = 190$) and users ($n = 299$) did not differ significantly with respect to the baseline features (hepatitis B, 56.8 vs. 62.9%); (alcohol abuse, 20.0 vs. 16.7%); age (55.47 ± 13.21 vs. 53.60 ± 11.52 years); gender composition (male, 74.2 vs. 79.9%); prevalence of hypertension (20.0 vs. 15.7%); and history of HE (6.3 vs. 3.3%), bacterial infections (9.5 vs. 6.4%), or severe liver injury (12.1 vs. 8.0%) in the preceding 3 months. Gastrointestinal bleeding in the 3 months prior to admission was significantly more common in PPI users than in nonusers (24.4 vs. 15.3%, $p = 0.015$), while ascites in the 3 months prior to admission was significantly less common in PPI users (36.1 vs. 46.8%, $p = 0.019$). The incidence of HE (8.7 vs. 12.6%) and SBP (4.0 vs. 4.7%) during hospitalization was not differ significantly between PPI users and nonusers (**Table 1**). PPI users had significantly lower hemoglobin, serum bilirubin, ALT, INR, and the MELD score ($p = 0$, $p = 0.041$, $p = 0.026$, $p = 0.007$, and $p = 0.017$, respectively) (**Table 1**).

Then, we matched 489 patients with acute decompensated cirrhosis with 1:1. After PSM, there are 182 patients in PPI user group and non-user group, respectively (**Table 1**). Compared with the decompensated cirrhosis patients before PSM, some baseline characteristics have changed. PPI users were younger

TABLE 2 | Baseline characteristics of ACLF patients receiving and not receiving PPI therapy.

	Before PSM			After PSM		
Variables	**Non-PPI group ($n = 37$)**	**PPI group ($n = 43$)**	**P value**	**Non-PPI group ($n = 36$)**	**PPI group ($n = 36$)**	**P value**
Age (years)	51.19 ± 11.87	51.07 ± 12.67	0.896	51.19 ± 11.87	52.58 ± 12.59	0.673
Sex (male%)	31 (83.8%)	36 (83.7%)	0.994	30 (83.3%)	31 (86.1%)	0.745
Hypertension (%)	9 (24.3%)	5 (11.6%)	0.139	9 (25.0%)	2 (5.6%)	0.023
Etiology of cirrhosis						
HBV	27 (73.0%)	30 (69.8%)	0.754	26 (72.2%)	25 (69.4%)	0.797
Alcohol	3 (8.1%)	6 (14.0%)	0.412	3 (8.3%)	6 (16.7%)	0.288
HBV plus alcohol	0	0	1.000	0	0	1.000
Others	7 (18.9%)	7 (16.3%)	0.758	7 (19.4%)	5 (13.9%)	0.530
Previous de–compensation events[a] (%)						
Ascites[a]	17 (45.9%)	23 (53.5%)	0.504	16 (44.4%)	20 (55.6%)	0.349
Upper gastrointestinal bleeding[a]	3 (8.1%)	1 (2.3%)	0.240	2 (5.6%)	1 (2.8%)	0.558
Hepatic encephalopathy[a]	3 (8.1%)	1 (2.3%)	0.240	3 (8.3%)	1 (2.8%)	0.307
Bacterial infections[a]	2 (5.4%)	3 (7.0%)	0.774	1 (2.8%)	3 (8.3%)	0.307
Severe liver injury[a]	14 (37.8%)	12 (27.9%)	0.347	14 (38.9%)	10 (27.8%)	0.321
Complications of cirrhosis during hospital (%)						
Hepatic encephalopathy	6 (16.2%)	6 (14.0%)	0.779	5 (13.9%)	6 (16.7%)	0.745
SBP	1 (2.7%)	2 (4.7%)	0.649	1 (2.8%)	2 (5.6%)	0.558
MELD score	25.32 ± 5.79	25.86 ± 6.58	0.945	25.32 ± 5.79	25.48 ± 6.28	0.964
Laboratory data						
Leukocyte count (10^9/L)	6.5 (4.7–9.4)	6.9 (3.8–10.8)	0.753	6.5 (4.7–9.4)	6.5 (3.9–9.7)	0.685
Platelet count (10^9/L)	82 (52–124)	62 (43–100)	0.208	82 (52–124)	62 (42–103)	0.235
Hemoglobin (g/L)	117 (97–142)	109 (98–128)	0.194	117 (97–141)	108 (91–127)	0.112
Serum bilirubin (μmol/L)	391 (275–485)	396 (294–495)	0.579	391 (279–485)	396 (291–497)	0.744
Albumin (g/L)	31.9 (28.5–33.3)	30.0 (26.4–33.9)	0.320	31.9 (28.5–33.3)	29.4 (25.6–33.3)	0.087
AST (IU/L)	139 (91–275)	118 (64–219)	0.316	139 (91–275)	178 (66–217)	0.292
ALT (IU/L)	105 (66–306)	71 (27–204)	0.075	105 (66–306)	78 (26–184)	0.066
INR	2.0 (1.7–2.4)	2.2 (1.6–2.6)	0.954	2 (1.7–2.4)	2.0 (1.6–2.5)	0.665
Creatinine (μmol/L)	65 (57–95)	70 (57–103)	0.810	65 (57–95)	73 (56–115)	0.510
Serum sodium (mmol/L)	137 (134–140)	136 (132–139)	0.539	137 (134–140)	137 (132–139)	0.923
CLIF–SOFA score	8.33 ± 1.39	8.90 ± 1.97	0.400	8.33 ± 1.39	9.08 ± 2.05	0.121
28–day mortality	17 (45.9%)	12 (27.9%)	0.482	17 (45.9%)	10 (27.8%)	0.091
90–day mortality	21 (56.8%)	19 (44.2%)	0.887	21 (56.8%)	17 (47.2%)	0.348

Data are expressed as mean ± SD, median (Q1–Q3), or number (percentage). Comparisons between the groups were performed by using the Student's t-test, the Mann–Whitney U test, or the chi-squared test. [a]Within the prior 3 months before the hospital admission related to the study. ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLIF-SOAF score, chronic liver failure-sequential organ failure assessment score; HBV, hepatitis B virus; INR, international normalized ratio; MELD score, model for end-stage liver disease score.

than nonusers (52.26 ± 11.17 vs. 55.48 ± 13.21 years, $p = 0.020$) included more male individuals (86.3 vs. 74.2%, $p = 0.004$) and fewer hypertension patients (11.5 vs. 20.3%, $p = 0.022$). Among PPI users, decompensated cirrhosis was more commonly caused by hepatitis B virus alone compared to PPI non-users (69.2 vs. 57.1%, $p = 0.005$) other than by other causes (14.3 vs. 22.5%, $p = 0.043$). In addition, PPI users had remarkably higher serum sodium level ($p = 0.000$), lower AST level, and the CLIF-SOFA score ($p = 0.002$ and $p = 0.017$). Other baseline characteristics did not change significantly.

We also analyzed 80 patients with ACLF in detail and matched them by the same method as above (**Table 2**). Among patients with ACLF, there were no significant differences between PPI users and non-users in mean age, gender composition, prevalence of hypertension, etiology of cirrhosis, incidence of previous decompensation, complications of cirrhosis during hospital stay, laboratory data, and scores of the MELD and the CLIF-SOFA. Similarly, PPI users and non-users had comparable 28-day mortality (27.8 vs. 45.9%, 0.482) and 90-day mortality rates (47.2 vs. 56.8%, 0.887). After PSM, other features did not change significantly, except for the lower prevalence of hypertension in PPI users (25.0 vs. 5.6%, $p = 0.023$).

TABLE 3 | Risk factors associated with HE and SBP in decompensated cirrhosis patients.

Variables	OR	95%CI	*P* value
Risk of HE			
Age	1.07	1.03–1.12	0.001
Albumin	1.13	1.04–1.24	0.006
Previous HE[a]	242.52	40.17–1464.11	<0.001
CLIF–SOFA	2.89	2.11–3.96	<0.001
Risk of SBP			
Previous severe liver injury[a]	3.43	1.16–10.17	0.026
Previous bacterial infections[a]	6.47	2.29–18.29	<0.001

Statistical analysis was performed by using the logistic regression analysis. For HE and SBP, the variables entered into the multivariate analysis were gender, age, previous ascites, previous upper gastrointestinal bleeding, previous hepatic encephalopathy (HE), previous bacterial infections, previous severe liver injury, the MELD score, leukocyte count, platelet count, hemoglobin, serum bilirubin, albumin, AST, ALT, INR, creatinine, serum sodium, the CLIF-SOFA score, and PPI use. [a]Within the prior 3 months before the hospital admission related to the study. OR, odds ratio; SBP, spontaneous bacterial peritonitis.

Proton Pump Inhibitor Therapy and the Complications of Cirrhosis During Hospitalization

The proportion of patients developing HE during hospitalization was comparable between PPI users and nonusers before and after PSM (8.7 vs. 12.6%, $p = 0.162$; 8.8 vs. 11.0%, $p = 0.483$) (**Table 1**).

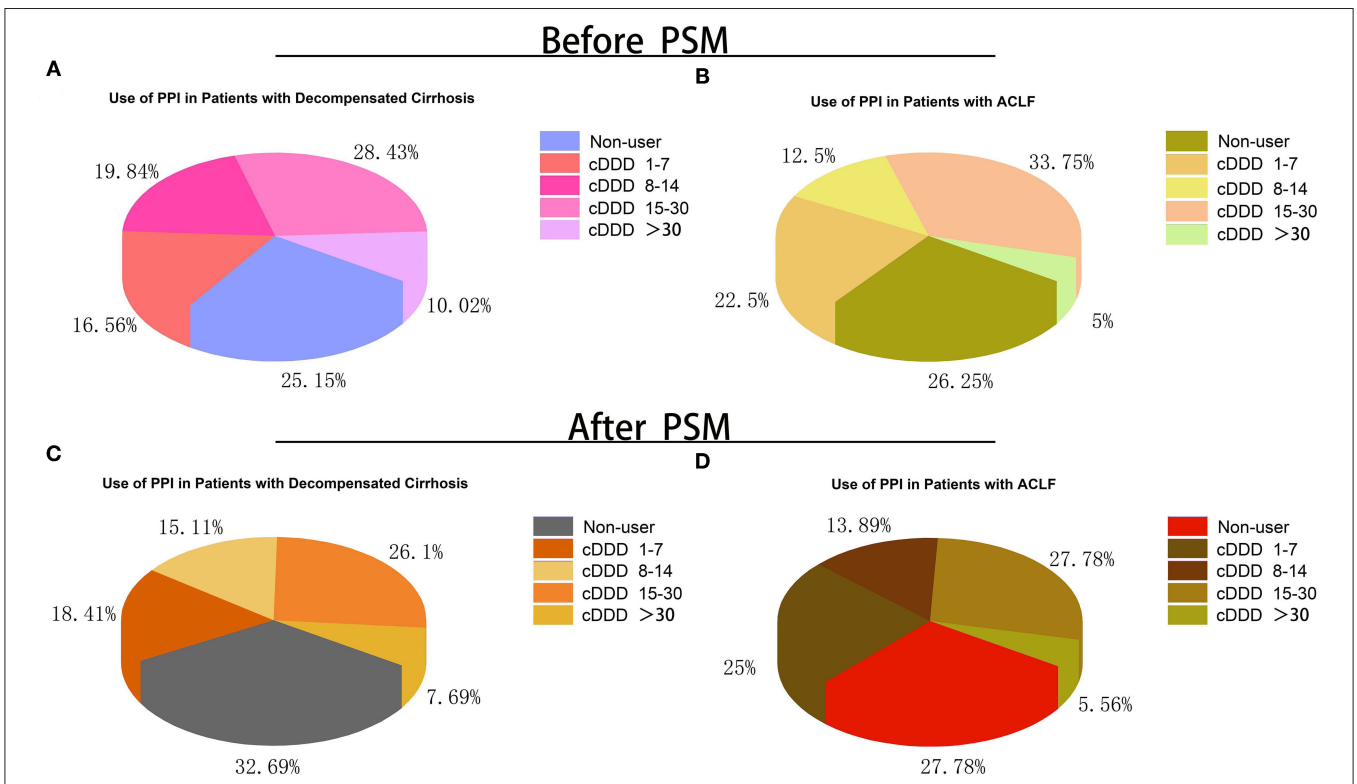


FIGURE 2 | Proportion of patients with different doses and without PPI treatment during hospitalization. **(A)** Proportion of patients with different doses and without PPI treatment in acute decompensated cirrhosis before PSM; **(B)** Proportion of patients with different doses and without PPI treatment in ACLF before PSM; **(C)** Proportion of patients with different doses and without PPI treatment in acute decompensated cirrhosis after PSM; **(D)** Proportion of patients with different doses and without PPI treatment in ACLF after PSM. cDDD, cumulative defined daily dose; PPI, proton pump inhibitor; ACLF, acute-on-chronic liver failure; PSM, propensity score matching.

This was true even when patients with ACLF (14.0 vs. 16.2%, $p = 0.779$; 16.7 vs. 13.9%, $p = 0.745$) (**Table 2**). On the multivariate analysis, age [odds ratio (OR) = 1.07, 95% CI: 1.03–1.12, $p = 0.001$], albumin (OR = 1.13, 95% CI: 1.04–1.24, $p = 0.006$), history of previous HE (OR = 242.52, 95% CI: 40.17–1464.11, $p < 0.001$) and the CLIF-SOFA score (OR =2.89, 95% CI: 2.11–3.96, $p < 0.001$) were significantly associated with the risk of developing HE in decompensated cirrhosis patients during hospitalization (**Table 3**).

The proportion of patients developing SBP during hospitalization was comparable between PPI users and nonusers (4.0 vs. 4.7%, $p = 0.701$; 4.9 vs. 4.9%, $p = 1$) (**Table 1**). This was true even when patients with ACLF were considered separately (4.0 vs. 2.7%, $p = 0.649$; 5.6 vs. 2.8%, $p = 0.558$) (**Table 2**). On the multivariate analysis, history of previous severe liver injury (OR = 3.43, 95% CI: 1.16–10.17, $p = 0.026$) and previous bacterial infections (OR = 6.47, 95% CI: 2.29–18.29, $p < 0.001$) were independent risk factors for SBP in patients with decompensated cirrhosis (**Table 3**).

The cDDD of PPI in patients with decompensated cirrhosis and ACLF is summarized in **Figure 2**. The cDDDs of PPI in patients with decompensated cirrhosis and ACLF were divided into four groups: 1–7, 8–14, 15–30, and >30 patients. Among all the patients with decompensated cirrhosis, 25.15% were not treated with PPI. Especially, among all the patients with ACLF, 26.25% were not treated with PPI. Before and after PSM, the group of cDDD 15–30 patients accounted for the highest proportion of PPI users in both the decompensated cirrhosis group and ACLF group. But, the proportion of patients with cDDD > 30 patients was the smallest.

Then, we further discussed the relationship between the patients with HE and SBP during hospitalization and the cDDD of PPI (**Figure 3**). Before PSM, cDDD of 32% patients with HE and cDDD of 29% patients with SBP in decompensated cirrhosis were 15–30 patients, which had the highest proportion. The PPI non-user and cDDD 15–30 patients complicated with HE accounted for a high proportion of 33% in ACLF. After PSM of patients with decompensated cirrhosis, the PPI non-users and cDDD 15–30 patients complicated with HE accounted for a high proportion of 31%, while cDDD 1–7 patients and cDDD 15–30 patients with SBP accounted for a high proportion of 28%. ACLF patients with SBP had the same proportion in the three cDDD groups (1–7, 8–14, and 15–30 patients). Meanwhile, the type and dose of the PPI used were not related to the risk of developing HE or SBP (**Table 3**).

Risk Factors for Survival

After median follow-up of 23 months (IQR 6–26 months), the 28-day and 90-day mortality rates were 11.7% (57/489) and 19.4% (95/489), respectively (**Table 1**; **Figure 1**).

The multivariate Cox proportional hazards regression model did not show significant association between the type and dose of the PPI and 28-day and 90-day mortality (**Table 4**; **Figures 4**, **5**).

TABLE 4 | Risk factors associated with mortality in decompensated cirrhosis patients.

Variables	HR	95%CI	*P* value
28-day			
Age	1.04	1.01–1.07	0.007
Leucocyte count	1.07	1.00–1.15	0.042
Previous severe liver injury[a]	2.07	1.17–3.64	0.012
MELD	1.11	1.07–1.16	<0.001
90-day			
Age	1.04	1.01–1.06	0.002
Leucocyte count	1.13	1.05–1.21	<0.001
MELD	1.11	1.07–1.15	<0.001

Statistical analysis was performed by using the Cox proportional hazards regression model. For mortality, variables entered into the multivariate Cox proportional hazards regression model were gender, age, previous ascites, previous upper gastrointestinal bleeding, previous HE, previous bacterial infections, previous severe liver injury, the MELD score, leukocyte count, platelet count, hemoglobin, serum bilirubin, albumin, AST, ALT, INR, creatinine, serum sodium, the CLIF-SOFA score, and PPI use. [a]Within the prior 3 months before the hospital admission related to the study. ACLF, acute-on-chronic liver failure; HR, hazard ratio; MELD score, model for end-stage liver disease score; INR, international normalized ratio.

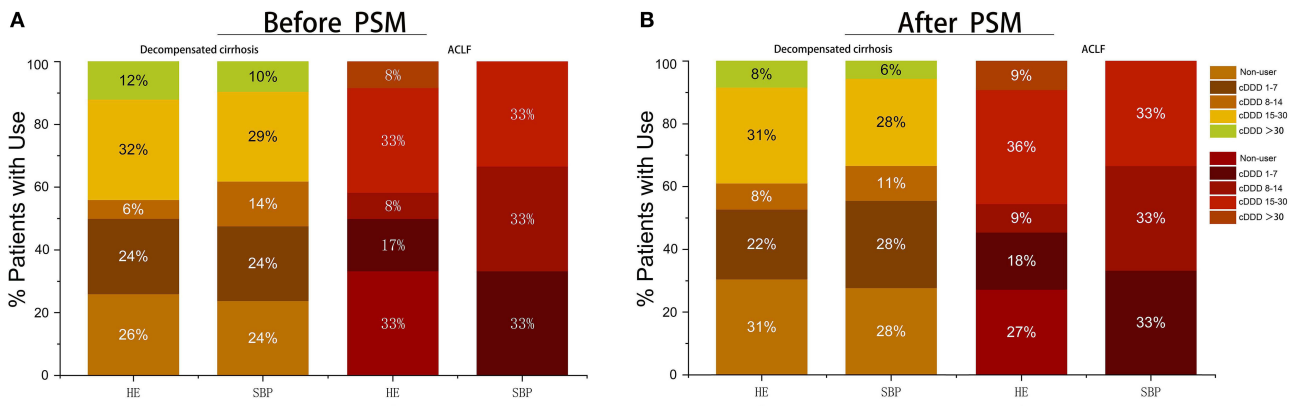


FIGURE 3 | Proportion of patients with different doses and without PPI treatment who suffer from complications during hospitalization. **(A)** Proportion of patients with different doses and without PPI treatment before PSM; **(B)** Proportion of patients with different doses and without PPI treatment after PSM. cDDD, cumulative defined daily dose; PPI, proton pump inhibitor; ACLF, acute-on-chronic liver failure.

The *p*-value of the cumulative incidence of mortality death between patients with decompensated liver cirrhosis and the dose of the PPI was 0.018 before PSM, but after PSM, there was no statistical significance. However, age, leukocyte count, and the high MELD score were independent predictors of 28-day and 90-day mortality in cirrhosis patients with AD (**Table 4**). In addition, previous severe liver injury was an independent predictor of 28-day mortality (**Table 4**).

DISCUSSION

This study aimed to investigate the impact of PPI therapy on complications and prognosis in cirrhosis patients with and without ACLF. PPI therapy is used in as much as 46–78% of cirrhosis patients (7). In this study, PPI was used in 61.1% of cirrhosis patients including 62.6% of patients without ACLF and 53.8% of patients with ACLF. Thus, consistent with previous studies, our results also demonstrate that PPI use is common in cirrhosis patients with and without ACLF (2). The proportions of patients with history of previous gastrointestinal bleeding and low hemoglobin were higher among PPI users than among non-users. This is not surprising because PPIs are commonly used in cirrhosis patients with gastrointestinal bleeding (2, 26).

The type of PPI and the dose were not associated with the risk of HE and SBP. Previous reports have indicated that treatment with acid-suppressing drugs increase the incidence of SBP and HE (7, 11, 14). The explanation offered was that PPIs might promote small intestinal bacterial overgrowth with subsequent bacterial translocation (7, 27). Another explanation was that PPIs may inhibit the functions of neutrophils and natural killer cells (28). However, the association between PPI and SBP has not been consistently demonstrated (15, 21, 29). In one prospective multicenter study of many cirrhosis patients with AD, PPI use did not increase the risk of SBP (17). Our result is in accordance with the conclusion of an earlier meta-analysis that did not support the association between PPI use and SBP or HE (18).

In this study, age, albumin, history of previous HE, and the CLIF-SOFA score were significantly associated with HE during hospitalization. In addition, previous severe liver injury and previous bacterial infections were shown to be independent risk

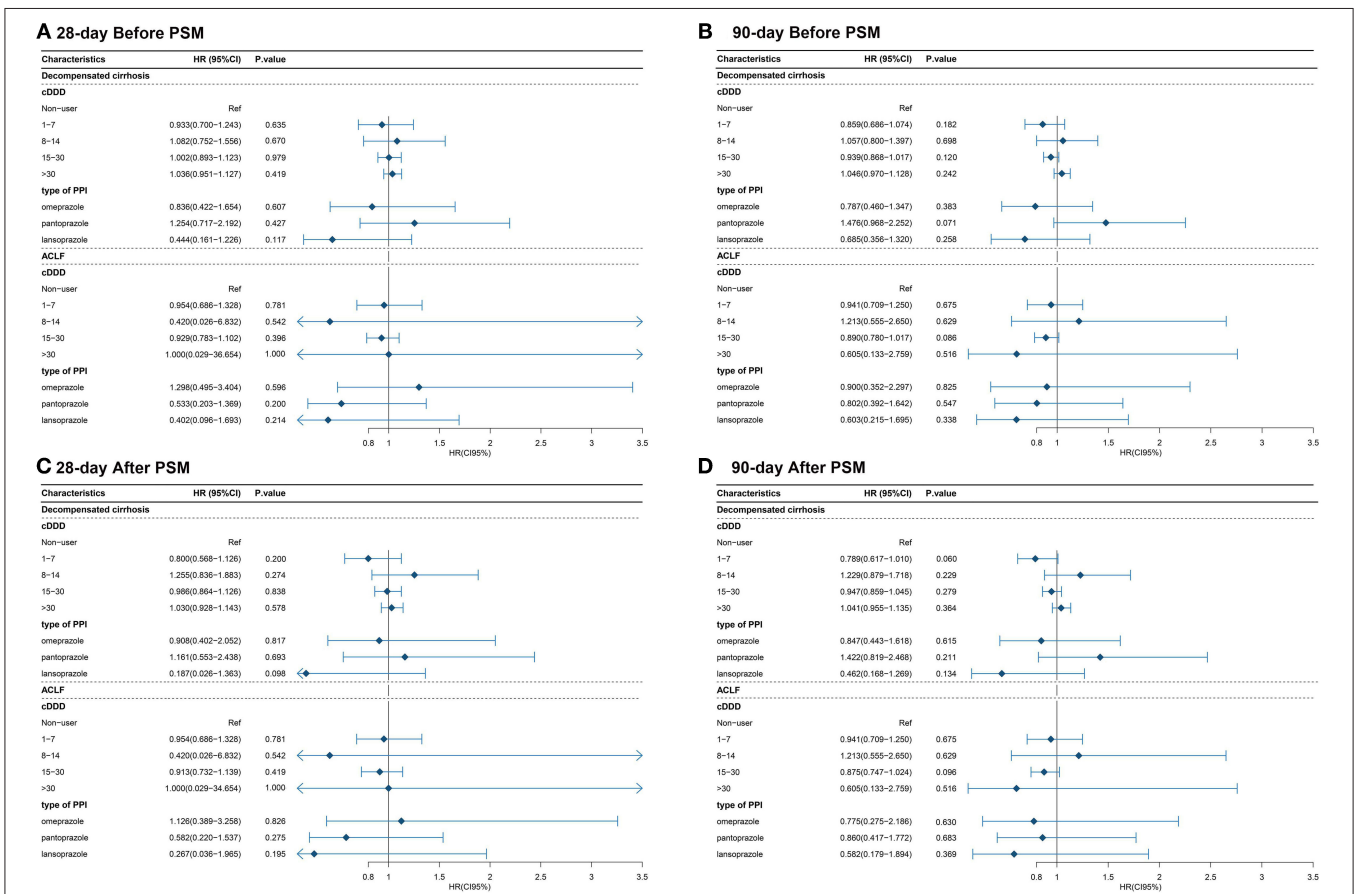


FIGURE 4 | The relationship between the type and dose of the PPI therapy and 28-day and 90-day mortality **(A)** The relationship between the type and dose of the PPI therapy and 28-day mortality before PSM; **(B)** The relationship between the type and dose of the PPI therapy and 90-day mortality before PSM; **(C)** The relationship between the type and dose of the PPI therapy and 28-day mortality after PSM; **(D)** The relationship between the type and dose of the PPI therapy and 90-day mortality after PSM. cDDD, cumulative defined daily dose; PPI, proton pump inhibitor; ACLF, acute-on-chronic liver failure.

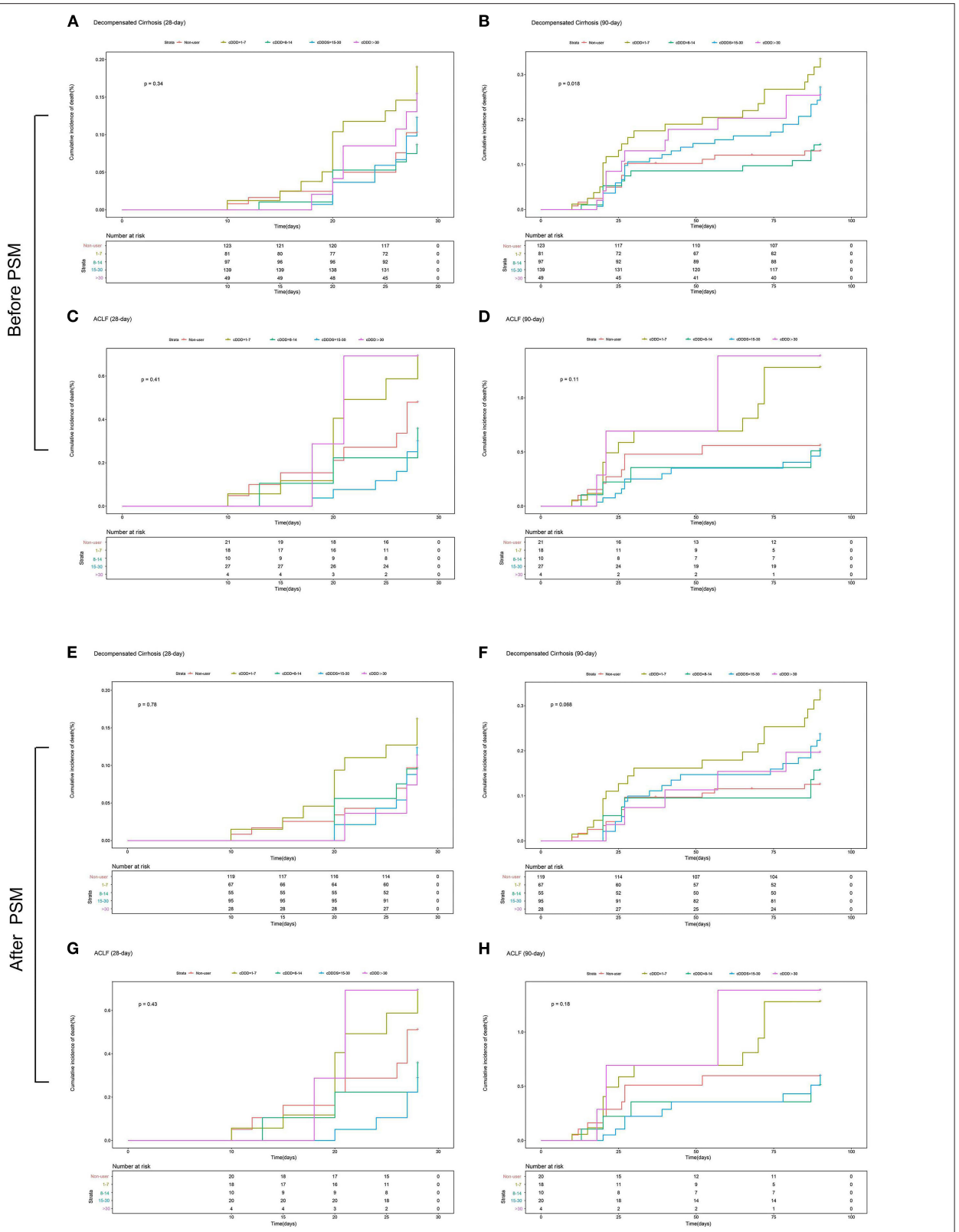


FIGURE 5 | The 28-day or 90-day cumulative incidence of death of patients with and without ACLF with different doses or without PPI treatment during hospitalization. **(A)** The 28-day cumulative incidence of death of patients with decompensated cirrhosis with different doses or without PPI treatment before PSM;

(Continued)

FIGURE 5 | (B) The 90-day cumulative incidence of death of patients with decompensated cirrhosis with different doses or without PPI treatment before PSM; **(C)** The 28-day cumulative incidence of death of patients with ACLF with different doses or without PPI treatment before PSM; **(D)** The 90-day cumulative incidence of death of patients with ACLF with different doses or without PPI treatment before PSM; **(E)** The 28-day cumulative incidence of death of patients with decompensated cirrhosis with different doses or without PPI treatment after PSM; **(F)** The 90-day cumulative incidence of death of patients with decompensated cirrhosis with different doses or without PPI treatment after PSM; **(G)** The 28-day cumulative incidence of death of patients with ACLF with different doses or without PPI treatment after PSM; **(H)** The 90-day cumulative incidence of death of patients with ACLF with different doses or without PPI treatment after PSM. cDDD, cumulative defined daily dose; PPI, proton pump inhibitor; ACLF, acute-on-chronic liver failure.

factors for SBP in cirrhosis patients. A previous study has also shown that history of HE is associated with increased incidence of HE during hospitalization (30).

This study found that older age, high leukocyte count, and the high MELD score independently predicted the 28-day and 90-day mortality in cirrhosis patients with AD. Previous studies have also found that age, leukocyte count, and the MELD score have to be independently associated with the risk of death in cirrhosis (31, 32). In this study, PPI therapy was not associated with the 28-day and 90-day mortality in cirrhosis patients with AD or ACLF. Others have also reported that PPI use does not increase the mortality rate in cirrhosis patients with or without ACLF (16, 18).

In conclusion, PPI therapy does not appear to increase the risk of HE or SBP or to shorten survival in cirrhosis patients with or without ACLF. Further prospective multicenter studies with large samples are necessary to confirm our findings and to clarify how PPI therapy is related to complications and disease progression in cirrhosis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital, School of Medicine of Zhejiang University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JS and HZ conceived and designed the study and draw the manuscript. SS, WY, and RZ collected and analyzed the data. JH, XZ, and MY participated in the data interpretation. SS, HZ, and JS revised the manuscript. All authors contributed to the manuscript and approved the submitted version of the manuscript.

FUNDING

This study was supported by the National Natural Science Foundation of China (Grant No. 81971982) and the Special Anti-epidemic Project of Zhejiang Provincial Department of Education (Y202043368).

ACKNOWLEDGMENTS

We thank all the colleagues who provide enthusiastic help for the study in the First Affiliated Hospital, Zhejiang University, Zhejiang, China.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.763370/full#supplementary-material

REFERENCES

1. Gralnek IM, Dumonceau JM, Kuipers EJ, Lanas A, Sanders DS, Kurien M, et al. Diagnosis and management of nonvariceal upper gastrointestinal hemorrhage: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. *Endoscopy.* (2015) 47:a1–46. doi: 10.1055/s-0034-1393172
2. Lu Y, Adam V, Teich V, Barkun A. timing or dosing of intravenous proton pump inhibitors in acute upper gastrointestinal bleeding has low impact on costs. *Am J Gastroenterol.* (2016) 111:1389–98. doi: 10.1038/ajg.2016.157
3. Scarpignato C, Gatta L, Zullo A, Blandizzi C. Effective and safe proton pump inhibitor therapy in acid-related diseases - A position paper addressing benefits and potential harms of acid suppression. *BMC Med.* (2016) 14:179. doi: 10.1186/s12916-016-0718-z
4. Arroyo V, Moreau R, Jalan R, Gines P. Acute-on-chronic liver failure: a new syndrome that will re-classify cirrhosis. *J Hepatol.* (2015) 62:S131–43. doi: 10.1016/j.jhep.2014.11.045
5. Jalan R, Saliba F, Pavesi M, Amoros A, Moreau R, Ginès P, et al. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. *J Hepatol.* (2014) 61:1038–47. doi: 10.1016/j.jhep.2014.06.012
6. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology.* (2013) 144:1426–37. doi: 10.1053/j.gastro.2013.02.042
7. Dam G, Vilstrup H, Watson H, Jepsen P. Proton pump inhibitors as a risk factor for hepatic encephalopathy and spontaneous bacterial peritonitis

in patients with cirrhosis with ascites. *Hepatology.* (2016) 64:1265–72. doi: 10.1002/hep.28737
8. Imhann F, Bonder MJ, Vich Vila A, Fu J, Mujagic Z, Vork L, et al. Proton pump inhibitors affect the gut microbiome. *Gut.* (2016) 65:740–8. doi: 10.1136/gutjnl-2015-310376
9. Jackson MA, Goodrich JK, Maxan ME, Freedberg DE, Abrams JA, Poole AC, et al. Proton pump inhibitors alter the composition of the gut microbiota. *Gut.* (2016) 65:749–56. doi: 10.1136/gutjnl-2015-310861
10. Lin ZN, Zuo YQ, Hu P. Association of proton pump inhibitor therapy with hepatic encephalopathy in hepatitis b virus-related acute-on-chronic liver failure. *Hepat Mon.* (2014) 14:e16258. doi: 10.5812/hepatmon.16258
11. Min YW, Lim KS, Min BH, Gwak GY, Paik YH, Choi MS, et al. Proton pump inhibitor use significantly increases the risk of spontaneous bacterial peritonitis in 1965 patients with cirrhosis and ascites: a propensity score matched cohort study. *Aliment Pharmacol Ther.* (2014) 40:695–704. doi: 10.1111/apt.12875
12. Ratelle M, Perreault S, Villeneuve JP, Tremblay L. Association between proton pump inhibitor use and spontaneous bacterial peritonitis in cirrhotic patients with ascites. *Can J Gastroenterol Hepatol.* (2014) 28:330–4. doi: 10.1155/2014/751921
13. Tripathi D, Stanley AJ, Hayes PC, Patch D, Millson C, Mehrzad H, et al. UK guidelines on the management of variceal haemorrhage in cirrhotic patients. *Gut.* (2015) 64:1680–704. doi: 10.1136/gutjnl-2015-309262
14. Tsai CF, Chen MH, Wang YP, Chu CJ, Huang YH, Lin HC, et al. Proton pump inhibitors increase risk for hepatic encephalopathy in patients with cirrhosis in a population study. *Gastroenterology.* (2017) 152:134–41. doi: 10.1053/j.gastro.2016.09.007
15. Kim JH, Lim KS, Min YW, Lee H, Min BH, Rhee PL, et al. Proton pump inhibitors do not increase the risk for recurrent spontaneous bacterial peritonitis in patients with cirrhosis. *J Gastroenterol Hepatol.* (2017) 32:1064–70. doi: 10.1111/jgh.13637
16. Mandorfer M, Bota S, Schwabl P, Bucsics T, Pfisterer N, Summereder C, et al. Proton pump inhibitor intake neither predisposes to spontaneous bacterial peritonitis or other infections nor increases mortality in patients with cirrhosis and ascites. *PLoS ONE.* (2014) 9:e110503. doi: 10.1371/journal.pone.0110503
17. Terg R, Casciato P, Garbe C, Cartier M, Stieben T, Mendizabal M, et al. Proton pump inhibitor therapy does not increase the incidence of spontaneous bacterial peritonitis in cirrhosis: a multicenter prospective study. *J Hepatol.* (2015) 62:1056–60. doi: 10.1016/j.jhep.2014.11.036
18. Yu T, Tang Y, Jiang L, Zheng Y, Xiong W, Lin L. Proton pump inhibitor therapy and its association with spontaneous bacterial peritonitis incidence and mortality: a meta-analysis. *Dig Liver Dis.* (2016) 48:353–9. doi: 10.1016/j.dld.2015.12.009
19. Shi Y, Yan H, Zhou Z, Fang H, Li J, Ye H, et al. The clinical course of cirrhosis patients hospitalized for acute hepatic deterioration: a prospective bicentric study. *Medicine.* (2015) 94:e2031. doi: 10.1097/MD.0000000000002031
20. Shi Y, Yang Y, Hu Y, Wu W, Yang Q, Zheng M, et al. Acute-on-chronic liver failure precipitated by hepatic injury is distinct from that precipitated by extrahepatic insults. *Hepatology.* (2015) 62:232–42. doi: 10.1002/hep.27795
21. Bajaj JS, O'Leary JG, Reddy KR, Wong F, Olson JC, Subramanian RM, et al. Second infections independently increase mortality in hospitalized patients with cirrhosis: the North American consortium for the study of end-stage liver disease (NACSELD) experience. *Hepatology.* (2012) 56:2328–35. doi: 10.1002/hep.25947
22. Wu T, Li J, Shao L, Xin J, Jiang L, Zhou Q, et al. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure. *Gut.* (2018) 67:2181–91. doi: 10.1136/gutjnl-2017-314641
23. Fernandez J, Acevedo J, Wiest R, Gustot T, Amoros A, Deulofeu C, et al. Bacterial and fungal infections in acute-on-chronic liver failure: prevalence, characteristics and impact on prognosis. *Gut.* (2018) 67:1870–80. doi: 10.1136/gutjnl-2017-314240
24. Wiest R, Krag A, Gerbes A. Spontaneous bacterial peritonitis: recent guidelines and beyond. *Gut.* (2012) 61:297–310. doi: 10.1136/gutjnl-2011-300779
25. Roza MADe, Kai L, Kam JW, Chan YH, Kwek A, Ang TL, et al. Proton pump inhibitor use increases mortality and hepatic decompensation in liver cirrhosis. *World J Gastroenterol.* (2019) 25:4933–44. doi: 10.3748/wjg.v25.i33.4933
26. Ray WA, Chung CP, Murray KT, Smalley WE, Daugherty JR, Dupont WD, et al. Association of proton pump inhibitors with reduced risk of warfarin-related serious upper gastrointestinal bleeding. *Gastroenterology.* (2016) 151:1105–12e10. doi: 10.1053/j.gastro.2016.08.054
27. Bauer TM, Steinbruckner B, Brinkmann FE, Ditzen AK, Schwacha H, Aponte JJ, et al. Small intestinal bacterial overgrowth in patients with cirrhosis: prevalence and relation with spontaneous bacterial peritonitis. *Am J Gastroenterol.* (2001) 96:2962–7. doi: 10.1111/j.1572-0241.2001.04668.x
28. Zedtwitz-Liebenstein K, Wenisch C, Patruta S, Parschalk B, Daxbock F, Graninger W. Omeprazole treatment diminishes intra- and extracellular neutrophil reactive oxygen production and bactericidal activity. *Crit Care Med.* (2002) 30:1118–22. doi: 10.1097/00003246-200205000-00026
29. D. Law and M. Pimentel. Proton pump inhibitor therapy does not affect hydrogen production on lactulose breath test in subjects with IBS. *Dig Dis Sci.* (2010) 55:2302–8. doi: 10.1007/s10620-009-1010-2
30. Patidar KR, Thacker LR, Wade JB, Sterling RK, Sanyal AJ, Siddiqui MS, et al. Covert hepatic encephalopathy is independently associated with poor survival and increased risk of hospitalization. *Am J Gastroenterol.* (2014) 109:1757–63. doi: 10.1038/ajg.2014.264
31. Ampuero J, Simon M, Montoliu C, Jover R, Serra MA, Cordoba J, et al. Minimal hepatic encephalopathy and critical flicker frequency are associated with survival of patients with cirrhosis. *Gastroenterology.* (2015) 149:1483–9. doi: 10.1053/j.gastro.2015.07.067
32. Maiwall R, Kumar S, Chaudhary AK, Maras J, Wani Z, Kumar C, et al. Serum ferritin predicts early mortality in patients with decompensated cirrhosis. *J Hepatol.* (2014) 61:43–50. doi: 10.1016/j.jhep.2014.03.027

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.

The reviewer QY declared a shared affiliation with the authors to the handling editor at time of review.

frontiers in Medicine

SYSTEMATIC REVIEW
published: 16 November 2021
doi: 10.3389/fmed.2021.784240

Therapeutic Effect and Safety of Granulocyte Colony-Stimulating Factor Therapy for Acute-On-Chronic Liver Failure: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

Xiaoxue Hou[1†], *Yuwen Li*[2†], *Hui Yuan*[1], *Jinyuan Cai*[1], *Rui Liu*[3], *Jun Li*[1] and *Chuanlong Zhu*[3,4*]

[1] Department of Infectious Disease, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China, [2] Department of Pediatrics, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China, [3] Department of Tropical Diseases, The Second Affiliated Hospital of Hainan Medical University, Haikou, China, [4] Laboratory of Infectious Disease, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China

Background and Aims: Granulocyte colony-stimulating factor (G-CSF) has been proposed as a therapeutic option for patients with acute-on-chronic liver failure (ACLF). However, its clinical efficacy remains debatable. This study aimed to synthesize available evidence on the efficacy of G-CSF in ALCF.

Methods: The Cochrane Library, CNKI, MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL), and ClinicalTrials.gov were searched from inception until September 2021. After qualitative evaluation of the included literature, the included studies were analyzed.

Results: Seven studies were included in this meta-analysis. Overall, G-CSF therapy was not associated with a reduced risk of death (30-day survival, OR = 1.55, 95% CI: 1.00, 2.38, $P = 0.05$; 60-day survival, OR = 1.50, 95% CI: 0.95, 2.36, $P = 0.08$; 90-day survival, OR = 1.61, 95% CI: 0.99, 2.62, $P = 0.05$) or complication including occurrence of infections infection (OR = 0.66, 95% CI: 0.41, 1.05, $P = 0.08$), bleeding (OR = 1.50, 95% CI: 0.58, 3.89, $P = 0.41$), and hepatorenal syndrome (OR = 0.56, 95% CI: 0.25, 1.24, $P = 0.15$). Moreover, it had no obvious beneficial effects on the model of end-stage liver disease score (30-day SMD = −3.31, 95%CI: −7.42, 0.81, $P = 0.12$; 60-day SMD = −1.23, 95% CI: −5.21, 2.75, $P = 0.54$; 90-day SMD = −2.29, 95%CI: −4.94, 0.37, $P = 0.09$). Sensitivity analyses showed that patients in Asia had improved survival (30-day OR = 2.76, 95%CI: 1.43, 5.35, $P = 0.003$; 60-day OR = 2.83, 95% CI: 1.39, 5.73, $P = 0.004$; 90-day OR = 2.92, 95% CI: 1.34, 6.36, $P = 0.007$).

OPEN ACCESS

Edited by:
Yu-Chen Fan,
Shandong University, China

Reviewed by:
Yu Shi,
Zhejiang University, China
Mengfei Liu,
Mayo Clinic, United States
Enqiang Chen,
Sichuan University, China

***Correspondence:**
Chuanlong Zhu
chuanlong@yahoo.com

[†]*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *27 September 2021*
Accepted: *26 October 2021*
Published: *16 November 2021*

Citation:
Hou X, Li Y, Yuan H, Cai J, Liu R, Li J and Zhu C (2021) Therapeutic Effect and Safety of Granulocyte Colony-Stimulating Factor Therapy for Acute-On-Chronic Liver Failure: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Front. Med. 8:784240. doi: 10.3389/fmed.2021.784240

Conclusions: Our findings suggest that, currently, G-CSF cannot be recommended for the treatment of ACLF.

Keywords: granulocyte colony-stimulating factor, acute-on-chronic liver failure, end stage liver disease, hepatic insufficiency, randomized controlled trial

INTRODUCTION

Acute-on-chronic liver failure (ACLF) is a kind of short-term liver damage due to acute causes of chronic liver disease; it is accompanied by the failure of one or more extrahepatic organs, and the short-term risk of death is high (1). Currently, there is no specific treatment for ACLF, and liver transplantation is the definitive treatment for ACLF. However, many patients cannot benefit from liver transplantation because of limited organ availability, high cost, transplant-related complications, and lifetime immunity-related side effects (2, 3). Therefore, alternative treatment strategies for liver transplantation are being sought.

Granulocyte colony-stimulating factor (G-CSF) is a glycoprotein that can mobilize immune cells, stimulate bone marrow and stem cell production, and it has immune regulation and regeneration capabilities. Previous studies have been reported encouraging results on the use of G-CSF in animal models. G-CSF is found to mobilize hematopoietic stem cells, induce liver regeneration, and improve survival. However, Engelmann et al. (4) found that G-CSF increased Toll-like receptor-mediated inflammation, which led to an increase in mortality. In human studies, a few small randomized clinical trials have demonstrated not only improvement in liver function with G-CSF but also significant survival benefit compared with standard medical therapy for ACLF (5–7). On the contrary, other clinical trials reported that the use of G-CSF in ACLF patients did not result in survival benefits (4, 8), which has caused widespread concern. Therefore, in this study, we conducted a meta-analysis of randomized controlled trials (RCTs) to compare the risk of death and infection between ACLF patients treated with G-CSF and ACLF patients who did not receive G-CSF.

METHODS

Literature Search

We searched for RCTs involving ACLF patients treated with G-CSF from electronic medical databases, including the Cochrane Library, CNKI, MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL), and ClinicalTrials.gov, from inception to September 14, 2021. Key search terms were "granulocyte colony-stimulating factor," "acute-on-chronic liver failure," "hepatic insufficiency," "end stage liver disease," and "randomized controlled trial." MeSH terms and free-text terms, as well as variations of root words, were combined within each database. No language restrictions were applied during the search. The reference lists of eligible articles and relevant review articles were also checked to identify additional studies. The detailed search strategies are outlined in **Figure 1**.

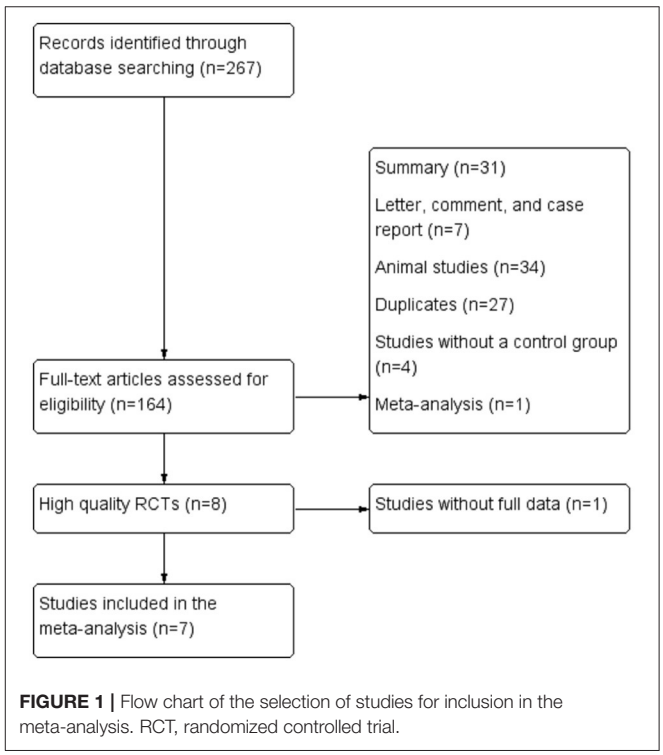


FIGURE 1 | Flow chart of the selection of studies for inclusion in the meta-analysis. RCT, randomized controlled trial.

Study Inclusion and Exclusion Criteria

The inclusion criteria were as follows: (1) RCTs, (2) patients diagnosed with ACLF, (3) patients in the experimental group received G-CSF therapy, and patients in the control group received conventional treatment, and (4) availability of clinical outcomes. The primary outcomes were short-term survival rate. The secondary outcomes included the scores of Model for End-Stage Liver Disease (MELD), risk of bleeding, occurrence of infections and hepatorenal syndrome. The exclusion criteria were (1) animal-based review articles or case reports, and (2) research in which valid data could not be extracted from the full text.

Data Extraction and Quality Score

Data extraction was performed independently by two authors using standardized data collection forms, and disagreements were resolved through discussion with another author. The following details were extracted from the included studies using a predefined data form: study first author, year of

Abbreviations: ACLF, acute-on-chronic liver failure; CI, confidence interval; G-CSF, granulocyte colony-stimulating factor; OR, odds ratio; RCT, randomized controlled trial.

TABLE 1 | Basic characteristics of included literature.

Authors	Country	Etiology	Sample size (Exp/Con)	Group of patients/ study design	G-CSF doses	Age (years, range)	Sex ratio (No. of males, %)	MELD score at baseline	CTP score at baseline	Number of people surviving 30 days	Number of people surviving 60 days	Number of people surviving 90 days	Number of infections	Number of HRS	Number of bleeding
Garg et al. (5)	India, Asia	AH and HBV	23	G-CSF	5 μg/kg/day (5 days) and every 3 days (1 month); subcutaneously	40 (30–65)*	20 (87%)	29 (21–40)*	12 (11–14)*	–	16	–	3	–	2
			24	Placebo+ SMT		40 (19–55)*	21 (87%)	31.5 (20–40)*	12 (10–14)*	–	7	–	10	–	2
Duan et al. (6)	China, Asia	HBV	27	G-CSF+SMT	5μg/kg/days; 6 days; subcutaneously	43.5(29–63)*	22 (81.5%)	25.11 ± 3.30**	12.17 ± 1.47**	–	–	13	3	2	5
			28	SMT		45.9(22–65)*	22 (78.6%)	26.30 ± 4.12**	12.25 ± 1.29**	–	–	6	7	6	3
Xiang et al. (11)	China, Asia	HBV	49	G-CSF+SMT	300 μg/days subcutaneously	41.72 ± 10.11**	40 (83.3%)	23.78 ± 3.68**	–	45	–	–	12	4	–
			50	SMT		45.62 ± 10.36**	42 (84%)	24.62 ± 4.45**	–	32	–	–	16	4	–
Saha et al. (9)	India, Asia	HBV	16	G-CSF+SMT	5 μg/kg/day (5 days); subcutaneously	48 (22–62)*	16(100%)	24.5 (21–32)*	12 (10–14)*	–	14	14	–	1	0
			16	SMT		39 (18–55)*	12(75%)	25.5 (21–35)*	12 (10–13)*	–	13	8	–	3	2
Sharma et al. (10)	India, Asia	HEV and HAV	15	G-CSF+SMT	5 mcg/kg/day (5 days); subcutaneously	7.53 ± 3.7**	7(46.6%)	–	12 ± 1.4**	10	8	–	–	–	–
			16	SMT		6.31 ± 4.9**	12(75%)	–	12.75 ± 0.85**	6	6	–	–	–	–
Haque et al. (12)	Japan, Asia	HBV	22	G-CSF+EPO	5 μg/kg/day(6 days); subcutaneously	42.64 ± 10.39**	19(86.4%)	27.64 ± 4.6**	–	14	11	8	1	5	4
			17	SMT		42.18 ± 13.06**	12(70.6%)	29.47 ± 5.5**	–	10	5	5	3	5	0
Engelmann et al. (8)	Multicentric, Europe	AH	88	G-CSF+SMT	5 μg/kg/day (5 days) and every 3 days (1 month); subcutaneously	54.4 ± 10.2**	50 (56.8%)	24.4 ± 6.3**	–	42	29	27	71	–	–
			88	SMT		57.1 ± 9.6**	61 (69.3%)	24.5 ± 6.1**	–	43	31	26	69	–	–

Con, control group; Exp, experimental group; G-CSF, granulocyte colony-stimulating factor; SMT, standard treatment; EPO, erythropoietin; AH, Alcoholic hepatitis; HBV, Hepatitis B virus; HEV, hepatitis E virus; AVH, acute viral hepatitis.

**Expressed as median.*

***Expressed as mean ± SD.*

FIGURE 2 | Assessment of risk of bias.

publication, country, and the number of participants in the experimental and control groups, the specific etiology of liver failure, the dose, administration, and duration of G-CSF treatment. Each trial was assessed using the Cochrane risk of bias tool. The standard criteria included the following domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other bias.

Statistical Analysis

Review Manager (version 5.4) software was used for the data merging and processing. For categorical variables in the study, the standardized mean difference (SMD) and odds ratio (OR) was used as the effect index to calculate the combined value and 95% confidence interval (CI). The Mantel-Haenszel test was used to test the heterogeneity of the included studies. If $I^2 \leq 50\%$ ($P \geq 0.05$), indicating that the differences in the studies were not statistically significant, the fixed-effects model

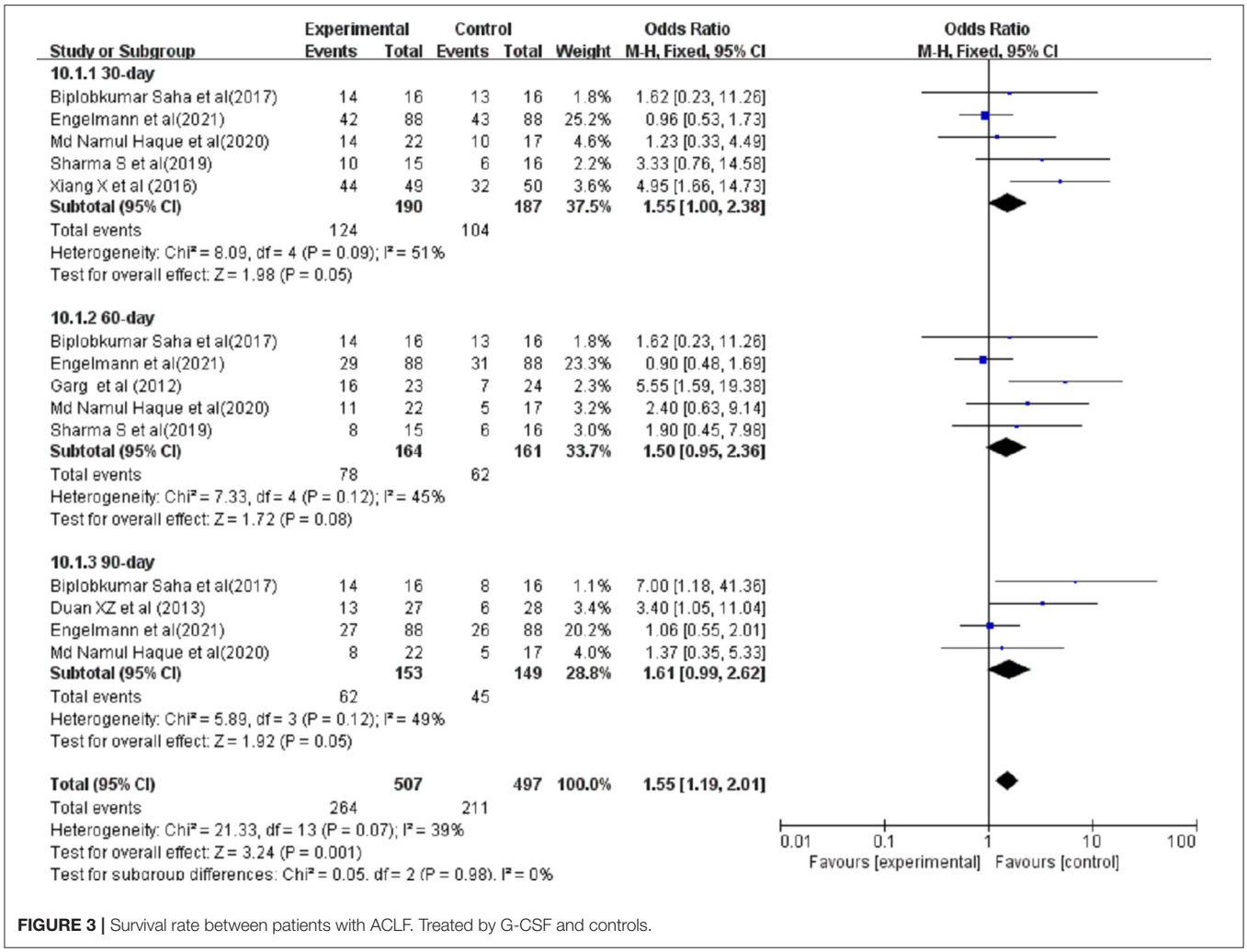


FIGURE 3 | Survival rate between patients with ACLF. Treated by G-CSF and controls.

was used for analysis. If $I^2 > 50\%$ ($P < 0.05$), indicating that there was significant heterogeneity, the random-effects model was used. Obvious clinical heterogeneity was evaluated by removing a single study and repeating the meta-analysis. Statistical significance was set as $P < 0.05$. Publication bias was evaluated with a funnel plot.

RESULTS

Selection of Eligible Studies

Following the search strategy described in **Figure 1**, 267 studies were initially identified based on the assessment of the titles and abstracts. We excluded 260 studies considering the predefined criteria. Seven eligible studies were finally included in the meta-analysis (5, 6, 8–12).

Characteristics of the Included Studies

Seven studies involving 479 ACLF patients included G-CSF treatment ($n = 240$) and control ($n = 239$) groups. The baseline characteristics, including the study design, treatment methods for each group, sample sizes of each group (**Table 1**).

Quality Assessment of the Included Studies

The details of the risk of bias tool are shown in **Figure 2**.

Survival Rate

Seven studies were included in the analysis of survival rate, compared with conventional treatment, G-CSF therapy was not associated with a reduced risk of death (30-day survival, OR = 1.55, 95% CI: 1.00, 2.38, $P = 0.05$; 60-day survival, OR = 1.50, 95% CI: 0.95, 2.36, $P = 0.08$; 90-day survival, OR = 1.61, 95% CI: 0.99, 2.62, $P = 0.05$) (**Figure 3**).

Clinical Severity Indices

The scores of Model for End-Stage Liver Disease (MELD) in ACLF patients for meta-analysis were available from three studies (**Figure 4**). Before and after treatment, no significant difference was observed between the experimental and control groups (Before treatment: SMD = −1.36, 95% CI: −3.05, 0.32, $P = 0.11$; After treatment: 30-day SMD = −3.31, 95%CI: −7.42, 0.81, $P = 0.12$; 60-day SMD = −1.23, 95% CI: −5.21,2.75, $P = 0.54$;90-day SMD = −2.29, 95%CI: −4.94,0.37, $P = 0.09$).

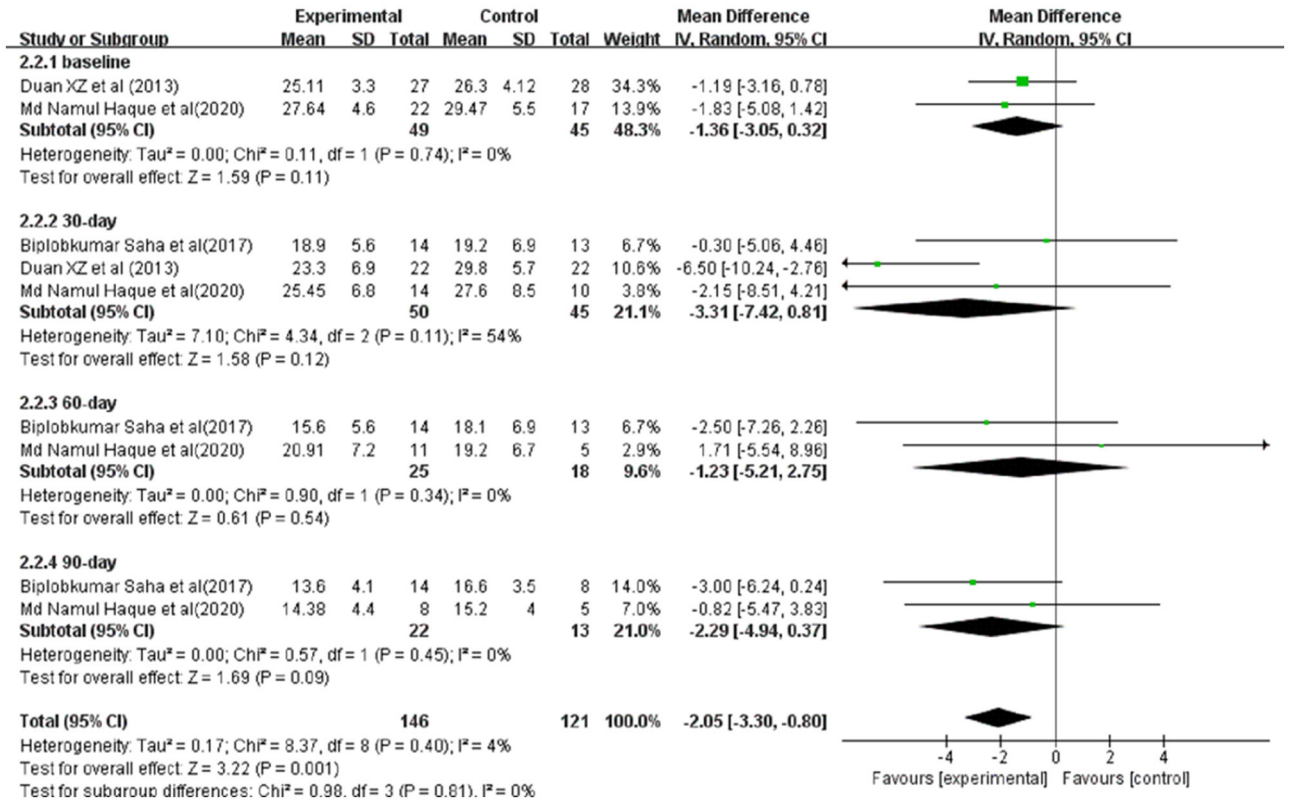


FIGURE 4 | Improvement in clinical severity indices between patients with ACLF. Treated by G-CSF and controls.

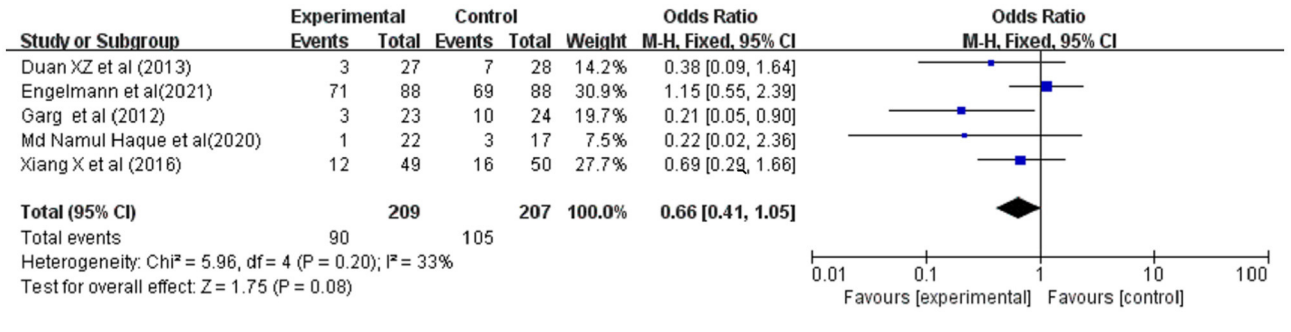


FIGURE 5 | Pooled estimate rate for infection between patients with ACLF. Treated by G-CSF and controls.

Occurrence of Infections

Secondary infections were reported in 5 studies involving 416 ACLF patients, 209 were in the G-CSF treatment group and 207 were in the control group. Owing to the low heterogeneity (I^2 = 33%), a fixed-effects model was adopted. The meta-analysis showed that patients receiving G-CSF did not have a significantly reduced risk of infections compared with traditional treatment (OR = 0.66, 95% CI: 0.41, 1.05, P = 0.08) (**Figure 5).**

Occurrence of Bleeding

Bleeding was reported in 4 studies involving 162 ACLF patients, 83 were in the G-CSF treatment group and 79 were in the control group. Owing to the low heterogeneity (I^2 = 9%), a fixed-effects model was adopted. The meta-analysis showed that compared with traditional treatment, patients receiving G-CSF did not have a significantly reduced risk of bleeding (OR = 1.50, 95% CI: 0.58, 3.89, P = 0.41) (**Figure 6**).

Occurrence of Hepatorenal Syndrome

Hepatorenal syndrome (HRS) were reported in 4 studies involving 214 ACLF patients, 109 were in the G-CSF treatment group and 105 were in the control group. Owing to the low heterogeneity (I^2 = 0%), a fixed-effects model was adopted. The meta-analysis showed that compared with traditional treatment,

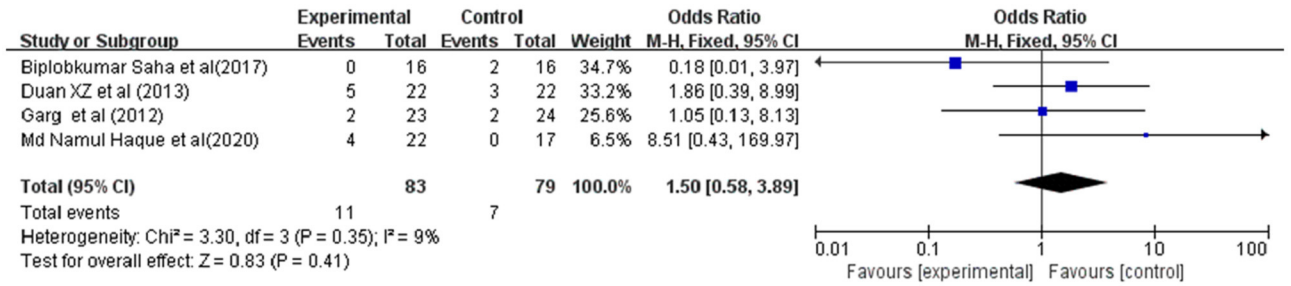


FIGURE 6 | Pooled estimate rate for bleeding between patients with ACLF. Treated by G-CSF and controls.

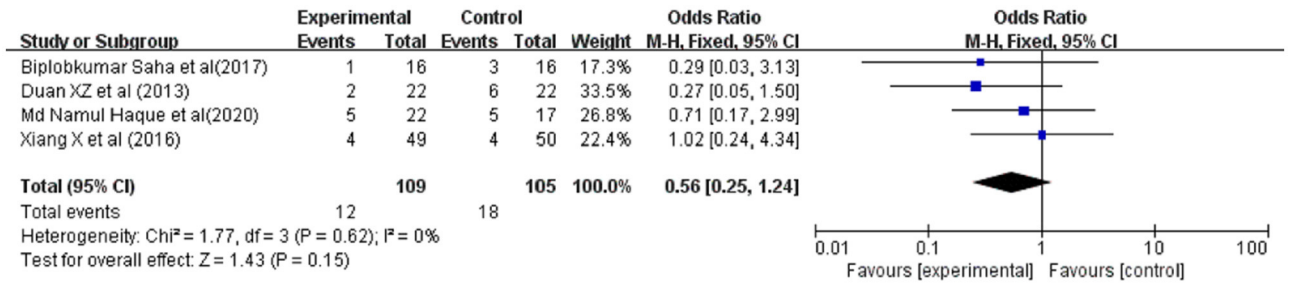


FIGURE 7 | Pooled estimate rate for Hepatorenal syndrome between patients with ACLF. Treated by G-CSF and controls.

patients receiving G-CSF did not have a significantly reduced risk of HRS (OR = 0.56, 95% CI: 0.25, 1.24, $P = 0.15$) (**Figure 7**).

Sensitivity Analyses

The sensitivity analysis showed that after excluding European studies, 4 Asian studies remained with low heterogeneity (30-day and 60-day $I^2 = 0\%$; 90-day $I^2 = 9\%$); considering these Asian studies, the patients' survival rate improved after the injection of G-CSF (30-day OR = 2.76, 95%CI: 1.43, 5.35, $P = 0.003$;60-day OR = 2.83, 95% CI: 1.39, 5.73, $P = 0.004$) (**Figures 8A–C**).

Risk of Publication Bias

Funnel plots of survival rate meta-analyses demonstrated asymmetry and suggested the presence of publication bias (**Figures 9A–C**).

DISCUSSION

ACLF is characterized by organ failure and high short-term mortality. Currently, there are no specific therapies for ACLF. Liver transplantation is the ultimate treatment for those who are acceptable candidates, but is limited by organ shortage and high frequency of contraindications in this group of patients (2, 3). Previous studies have shown that G-CSF can reduce the short-term mortality of ACFL patients (13). Yet the newly published clinical trial does not seem to support this conclusion (8). In this study, we conducted a comprehensive meta-analysis of 7 RCTs to evaluate the efficacy and safety of G-CSF in the treatment of ACLF patients. We failed to find a significant beneficial effect of G-CSF for patients with ACLF, unlike noted in a previous meta-analysis (14). Through sensitivity analysis of sources of heterogeneity, we found that etiologies of ACLF differ by region, with reactivation of hepatitis B more common in Asia, whereas alcoholic hepatitis are reportedly more common in European. We speculate the ultimate treatment outcome of patient may depend on the etiology of ACLF. In addition, different ACLF diagnostic criteria have led to considerable regional differences in ACLF recognition, onset and treatment timing, and final prognosis. In this meta-analysis, the admission criteria for ACLF patients in the Asian study were in accordance with APASL, and the admission criteria for patients in the European study were in accordance with the EASL-CLIF criteria. An intriguing and important finding of this study was significantly different disease progression among patients with ACLF at enrollment defined by APASL or EASL-CLIF Consortium. This may also affect the therapeutic effect of G-CSF on different ACLF patients.

Currently, it has been shown that G-CSF stimulates liver regeneration. G-CSF can stimulate bone marrow to release stem cells (CD34+), which could migrate to liver and differentiate into mature hepatocytes. It can also reduce the production of interferon-gamma, improve the local microenvironment of liver, promote liver repair, and improve liver injury. This translates into improved liver function, decreased risk of complications of liver disease, reduced risk of infections, and improved survival (15). The effect of G-CSF on liver regeneration may explain the survival benefit which was observed in Asian studies. The role of G-CSF in the latter stages of ACLF is limited due to the exhausted and destructed state of bone marrow ecology.

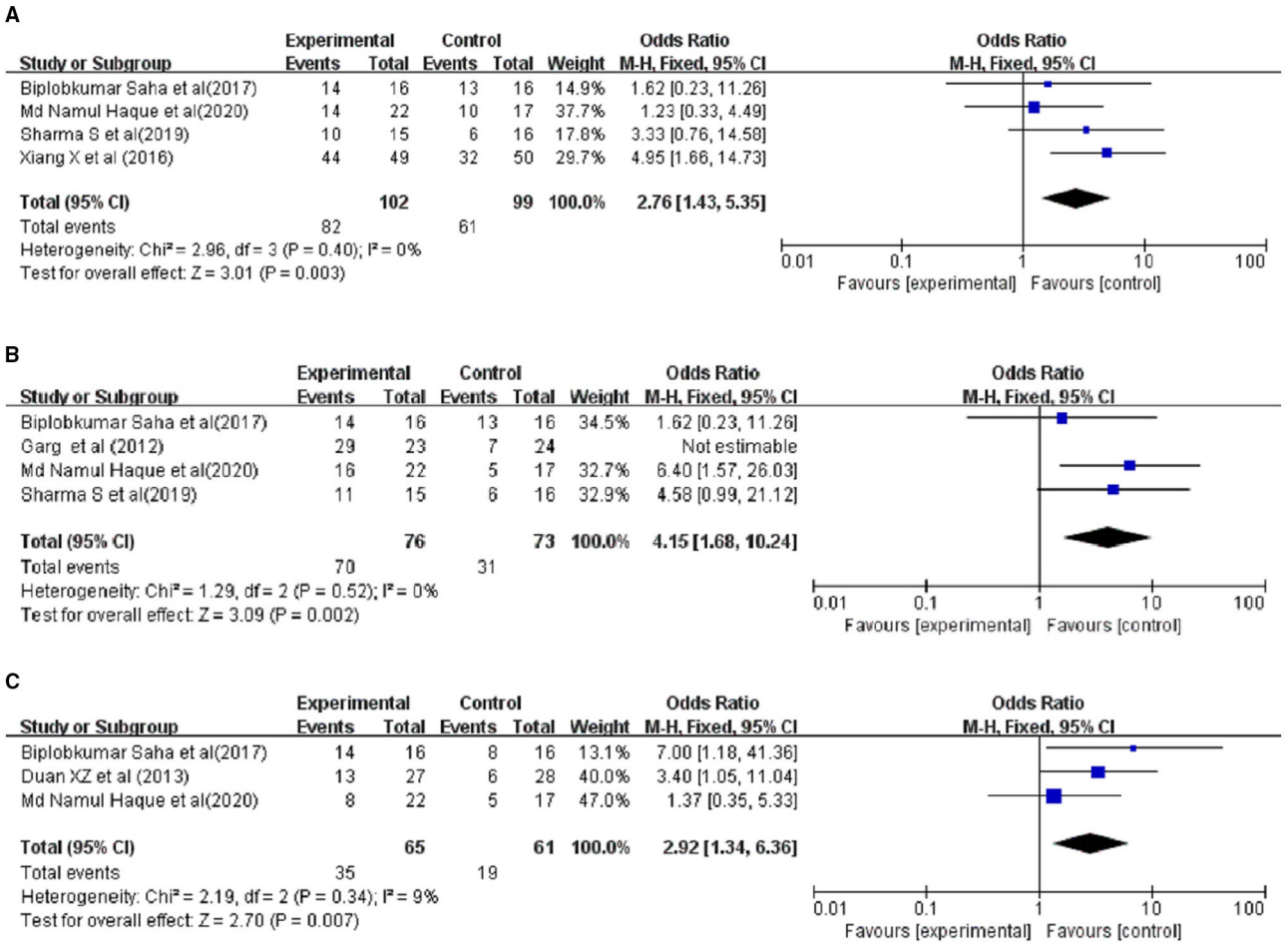


FIGURE 8 | Sensitivity analysis. Treated by G-CSF and controls. **(A)** 30-day; **(B)** 60-day; **(C)** 90-day.

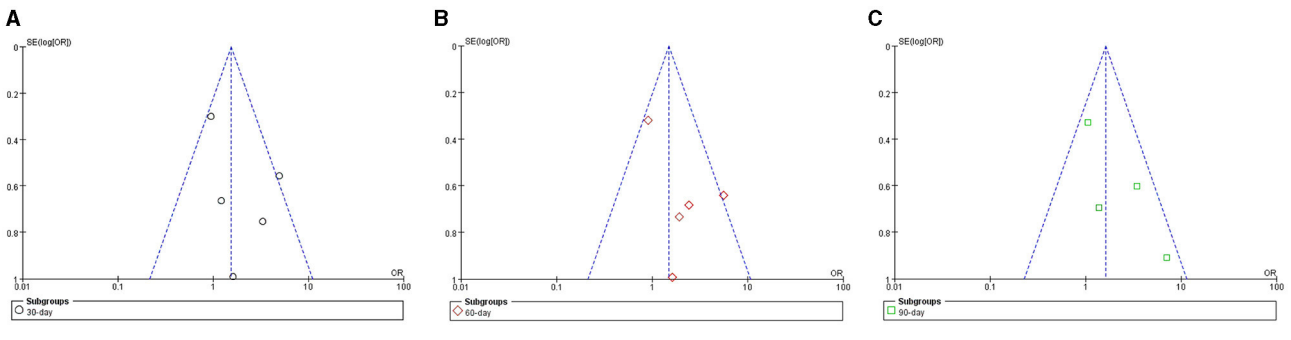


FIGURE 9 | Funnel plot of meta-analyses for survival rate in ACLF patients. **(A)** 30-day; **(B)** 60-day; **(C)** 90-day.

In the Asian regional study included in this meta-analysis, G-CSF was generally used in the early stages of ACLF. In the included European RCTs trial, we found that ∼70% of patients had cardiopulmonary failure and severe sepsis at the time of enrollment (8), which means they were in the end-stage of ACLF. Whether this condition affects our final results needs further exploration. In addition, studies have found that G-CSF requires a non-inflammatory environment to exert its protective effects on the liver. ACLF is characterized by increased white blood cell counts and plasma C-reactive protein levels. Patients often have a strong systemic inflammatory response (8, 16), and we speculate that G-CSF does not play a beneficial role in ACLF patients. Moreover, there were fewer studies in the European region in this study. Thus, more European clinical trials are

needed to determine whether current results from the included European region trials were non-comprehensive. There were no clear conclusions concerning the usefulness of G-CSF in those with ACLF, although survival benefits were observed in Asian patients compared to European patients. The conflicting results between Asian and European studies led to a high degree of overall heterogeneity in the analysis, and it is unclear whether this difference can be explained by ethnic differences or patient selection. Based on our results, we do not recommend G-CSF as a definitive treatment for patients with ACLF.

The Model for End-Stage Liver Disease (MELD) has been established as a reliable indicator of short-term survival in patients with end-stage liver disease. In this meta-analysis, we comprehensively analyzed the clinical severity indices of ACLF. The results suggested that G-CSF therapy may not improve MELD scores, unlike what is noted in a previous meta-analysis (17). Also, patients with G-CSF therapy did not achieve significantly lower bleeding risk and the occurrence of HRS compared with standard medical treatment. ACLF has marked pathophysiological features, namely, susceptibility to infection. Moreover, bacterial infection is a major challenge for its treatment (18, 19). G-CSF is an immunomodulatory glycoprotein that exerts anti-inflammatory and immunomodulatory effects in the body, thereby reducing the occurrence of bacteremia and infection. This benefit of G-CSF may be particularly important in patients with ACLF. Nonetheless, in this meta-analysis, there was no significant difference in the risk of infection among patients receiving treatment. We found that in the study of Engelmann et al. almost 40% of patients had bacterial infection at enrollment (8). The role of G-CSF for ACLF patients with severe bacterial infection is debatable. G-CSF is helpful to prevent development of bacterial infection, but is not beneficial to treat it. It is important to clarify whether this affected the final results.

Several limitations of this meta-analysis should be mentioned. First of all, the high heterogeneity in some aggregate estimation results may have hindered the establishment of reliable conclusions and recommendations. Sensitivity analyses indicated that regions and different types of liver disease may be the main cause of heterogeneity. However, it is unclear whether other factors would lead to the results of the study, such as the degree of ACLF progress, differences between studies, and the dose and duration of G-CSF injections. Moreover, some data cannot be obtained from each study, resulting in limited strength of evidence for the results obtained. Secondly, the total sample size is small, which may affect the reliability of the analysis results to a certain extent, and has limited significance for clinical guidance. Lastly, different trials use different outcome parameters at different measurement time points to evaluate the treatment effect, making it difficult to use a limited statistical sample size at a specific time point to summarize reliable results.

No clear conclusion could be drawn regarding the usefulness of G-CSF in ACLF, although survival benefits were observed in Asian patients. The conflicting results between regions and different etiology of liver disease lead to a high degree of overall heterogeneity in the analysis. It is unclear whether these differences can be explained by ethnic differences or different liver failure causes. Moreover, different diagnostic criteria for ACLF caused different patient prognosis (20). We need more RCTs and high-quality literature are required to clarify the usefulness of G-CSF for ACLF treatment. In conclusion, based on our results, we do not recommend G-CSF as a definitive treatment for patients with ACLF.

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.

AUTHOR CONTRIBUTIONS

XH, YL, and CZ conceived and designed the study. XH, HY, and JC selected the studies, collected the data, and drafted and revised the paper. XH, RL, and JL analyzed data. All authors interpreted the results, read, and approved the final version of the manuscript.

FUNDING

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81770591 and 81800778), Science and Technology Plan of Hainan Province (Clinical Research Center) (LCYX202103), and Hainan Province Clinical Medical Center.

REFERENCES

1. Allen AM, Kim WR, Moriarty JP, Shah ND, Larson JJ, Kamath PS. Time trends in the health care burden and mortality of acute on chronic liver failure in the United States. *Hepatology*. (2016) 64:2165–72. doi: 10.1002/hep.28812
2. Benítez C, Wolff R. Current status and future challenges of liver transplantation programs in Chile. *Liver Transpl*. (2018) 24:1757–61. doi: 10.1002/lt.25332
3. Belli LS, Duvoux C, Artzner T, Bernal W, Conti S, Cortesi PA, et al. Liver transplantation for patients with acute-on-chronic liver failure (ACLF) in Europe: results of the ELITA/EF-CLIF collaborative study (ECLIS). *J Hepatol*. (2021) 75:610–22. doi: 10.1016/j.jhep.2021.03.030
4. Engelmann C, Martino VD, Kerbert AJC, Weil-Verhoeven D, Aehling NF, Herber A, et al. The current status of granulocyte-colony stimulating factor to treat acute-on-chronic liver failure. *Semin Liver Dis*. (2021) 41:298–307. doi: 10.1055/s-0041-1723034
5. Garg V, Garg H, Khan A, Trehanpati N, Kumar A, Sharma BC, Sakhuja P, Sarin SK. Granulocyte colony-stimulating factor mobilizes CD34(+) cells and improves survival of patients with acute-on-chronic liver failure. *Gastroenterology*. (2012) 142:505–512.e1. doi: 10.1053/j.gastro.2011.11.027
6. Duan XZ, Liu FF, Tong JJ, Yang HZ, Chen J, Liu XY, et al. Granulocyte-colony stimulating factor therapy improves survival in patients with hepatitis B virus-associated acute-on-chronic liver failure. *World J Gastroenterol*. (2013) 19:1104–10. doi: 10.3748/wjg.v19.i7.1104

7. Verma N, Kaur A, Sharma R, Bhalla A, Sharma N, De A, et al. Outcomes after multiple courses of granulocyte colony-stimulating factor and growth hormone in decompensated cirrhosis: a randomized trial. *Hepatology.* (2018) 68:1559–73. doi: 10.1002/hep.29763
8. Engelmann C, Herber A, Franke A, Bruns T, Schiefke I, Zipprich A, et al. Granulocyte-Colony Stimulating Factor (G-CSF) to treat acute-on-chronic liver failure, a multicenter randomized trial (GRAFT study). *J Hepatol.* (2021). doi: 10.1016/j.jhep.2021.07.033
9. Saha BK, Mahtab MA, Akbar SMF, Noor-E-Alam SM, Mamun AA, Hossain SMS, et al. Therapeutic implications of granulocyte colony stimulating factor in patients with acute-on-chronic liver failure: increased survival and containment of liver damage. *Hepatol Int.* (2017) 11:540–6. doi: 10.1007/s12072-017-9814-1
10. Sharma S, Lal SB, Sachdeva M, Bhatia A, Varma N. Role of granulocyte colony stimulating factor on the short-term outcome of children with acute on chronic liver failure. *J Clin Exp Hepatol.* (2020) 10:201–10. doi: 10.1016/j.jceh.2019.10.001
11. Xu X, Liu XY, Chen J, Xiao L, Tong JJ, Guan CD, et al. Randomized controlled clinical study of granulocyte colony stimulating factor in the treatment of chronic hepatitis B-related acute liver failure. *Infect Dis Inf.* (2016) 29:279–83.
12. Haque MN, Al-Mahtab M, Das DC, Mohammad NS, Mamun AA, Khan MSI, et al. Effect of granulocyte colony-stimulating factor and erythropoietin on patients with acute-on-chronic liver failure. *Euroasian J Hepatogastroenterol.* (2020) 10:64–7. doi: 10.5005/jp-journals-10018-1330
13. Simonetto DA, Shah VH, Kamath PS. Improving survival in ACLF: growing evidence for use of G-CSF. *Hepatol Int.* (2017) 11:473–5. doi: 10.1007/s12072-017-9834-x
14. Chavez-Tapia NC, Mendiola-Pastrana I, Ornelas-Arroyo VJ, Noreña-Herrera C, Vidaña-Perez D, Delgado-Sanchez G, et al. Granulocyte-colony stimulating factor for acute-on-chronic liver failure: systematic review and meta-analysis. *Ann Hepatol.* (2015) 14:631–41. doi: 10.1016/S1665-2681(19)30757-4
15. Rathi S, Hussaini T, Yoshida EM. Granulocyte colony stimulating factor: a potential therapeutic rescue in severe alcoholic hepatitis and decompensated cirrhosis. *Ann Hepatol.* (2021) 20:100211. doi: 10.1016/j.aohep.2020.04.011
16. Gustot T. Beneficial role of G-CSF in acute-on-chronic liver failure: effects on liver regeneration, inflammation/immunoparalysis or both? *Liver Int.* (2014) 34:484–6. doi: 10.1111/liv.12356
17. Chen P, Wang YY, Chen C, Guan J, Zhu HH, Chen Z. The immunological roles in acute-on-chronic liver failure: an update. *Hepatobiliary Pancreat Dis Int.* (2019)18:403–11. doi: 10.1016/j.hbpd.2019.07.003
18. Bernal W, Jalan R, Quaglia A, Simpson K, Wendon J, Burroughs A. Acute-on-chronic liver failure. *Lancet.* (2015) 386:1576–87. doi: 10.1016/S0140-6736(15)00309-8
19. Zhang Q, Li Y, Han T, Nie C, Cai J, Liu H, Liu Y. Comparison of current diagnostic criteria for acute-on-chronic liver failure. *PLoS ONE.* 10:e0122158. doi: 10.1371/journal.pone.0122158
20. Yang Q, Yang Y, Shi Y, Lv F, He J, Chen Z. Effects of granulocyte colony-stimulating factor on patients with liver failure: a meta-analysis. *J Clin Transl Hepatol.* (2016) 4:90–6. doi: 10.14218/JCTH.2016.00012

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 16 November 2021
doi: 10.3389/fmed.2021.779744

Plasma Exchange-Based Non-bioartificial Liver Support System Improves the Short-Term Outcomes of Patients With Hepatitis B Virus-Associated Acute-on-Chronic Liver Failure: A Multicenter Prospective Cohort Study

Yuan-yuan Chen[1†], Hai Li[2†], Bao-yan Xu[3†], Xin Zheng[4†], Bei-ling Li[5†], Xian-bo Wang[6†], Yan Huang[7], Yan-hang Gao[8], Zhi-ping Qian[9], Feng Liu[10], Xiao-bo Lu[11], Jia Shang[12], Hai Li[13], Shao-yang Wang[14], Yin-hua Zhang[1], Zhong-ji Meng[1] and Chinese Chronic Liver Failure (CLIF) Consortium*

[1] Department of Infectious Diseases, Hubei Clinical Research Center for Precise Diagnosis and Therapy of Liver Cancer, Taihe Hospital, Hubei University of Medicine, Shiyan, China, [2] Key Laboratory of Gastroenterology and Hepatology, Department of Gastroenterology, Renji Hospital, School of Medicine, Shanghai Institute of Digestive Disease, Shanghai Jiao Tong University, Chinese Ministry of Health (Shanghai Jiao Tong University), Shanghai, China, [3] Department of Infectious Diseases, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China, [4] Department of Infectious Diseases, Institute of Infection and Immunology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [5] Hepatology Unit, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China, [6] Center of Integrative Medicine, Beijing Ditan Hospital, Capital Medical University, Beijing, China, [7] Hunan Key Laboratory of Viral Hepatitis, Department of Infectious Disease, Xiangya Hospital, Central South University, Changsha, China, [8] Department of Hepatology, The First Hospital of Jilin University, Changchun, China, [9] Department of Liver Intensive Care Unit, Shanghai Public Health Clinical Centre, Fudan University, Shanghai, China, [10] Department of Infectious Diseases and Hepatology, The Second Hospital of Shandong University, Jinan, China, [11] Liver Disease Center, First Affiliated Hospital of Xinjiang Medical University, Urumqi, China, [12] Department of Infectious Diseases, Henan Provincial People's Hospital, Zhengzhou, China, [13] Infectious Disease Center, Affiliated Hospital of Logistics University of People's Armed Police Force, Tianjin, China, [14] Department of Infectious Diseases, Fuzhou General Hospital of Nanjing Military Command, Fuzhou, China

Background and aims: Hepatitis B virus-associated acute-on-chronic liver failure (HBV-ACLF) is a complicated syndrome with extremely high short-term mortality. Whether plasma exchange (PE) improves HBV-ACLF outcomes remains controversial. Here, PE-based non-bioartificial liver support system (NB-ALSS) effects on short-term HBV-ACLF patient outcomes were investigated.

Materials and methods: HBV-ACLF patients from Chinese Acute-on-chronic Liver Failure (CATCH-LIFE) cohort receiving standard medical therapy (SMT) alone or PE-based NB-ALSS in addition to SMT were allocated to SMT and SMT+PE groups, respectively; propensity score matching (PSM) was used to eliminate confounding bias. Short-term (28/90-day and 1-year) survival rates were calculated (Kaplan-Meier).

OPEN ACCESS

Edited by:
Yu Shi,
Zhejiang University, China

Reviewed by:
Su Lin,
First Affiliated Hospital of Fujian Medical University, China
Lang Bai,
Sichuan University, China

****Correspondence:***
Zhong-ji Meng
zhongji.meng@163.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *19 September 2021*
Accepted: *13 October 2021*
Published: *16 November 2021*

Citation:
Chen Y-y, Li H, Xu B-y, Zheng X, Li B-l, Wang X-b, Huang Y, Gao Y-h, Qian Z-p, Liu F, Lu X-b, Shang J, Li H, Wang S-y, Zhang Y-h, Meng Z-j and Chinese Chronic Liver Failure (CLIF) Consortium (2021) Plasma Exchange-Based Non-bioartificial Liver Support System Improves the Short-Term Outcomes of Patients With Hepatitis B Virus-Associated Acute-on-Chronic Liver Failure: A Multicenter Prospective Cohort Study. Front. Med. 8:779744. doi: 10.3389/fmed.2021.779744

Results: In total, 524 patients with HBV-ACLF were enrolled in this study; 358 received SMT alone (SMT group), and the remaining 166 received PE-based NB-ALSS in addition to SMT (SMT+PE group). PSM generated 166 pairs of cases. In the SMT+PE group, 28-day, 90-day, and 1-year survival rates were 11.90, 8.00, and 10.90%, respectively, higher than those in the SMT group. Subgroup analysis revealed that PE-based NB-ALSS had the best efficacy in patients with ACLF grade 2 or MELD scores of 30–40 (MELD grade 3). In MELD grade 3 patients who received SMT+PE, 28-day, 90-day, and 1-year survival rates were improved by 18.60, 14.20, and 20.10%, respectively. According to multivariate Cox regression analysis, PE-based NB-ALSS was the only independent protective factor for HBV-ACLF patient prognosis at 28 days, 90 days, and 1 year (28 days, HR = 0.516, $p = 0.001$; 90 days, HR = 0.663, $p = 0.010$; 1 year, HR = 0.610, $p = 0.051$). For those who received SMT+PE therapy, PE-based NB-ALSS therapy frequency was the only independent protective factor for short-term prognosis (28-day, HR = 0.597, $p = 0.001$; 90-day, HR = 0.772, $p = 0.018$).

Conclusions: This multicenter prospective study showed that the addition of PE-based NB-ALSS to SMT improves short-term (28/90 days and 1-year) outcomes in patients with HBV-ACLF, especially in MELD grade 3 patients. Optimization of PE-based NB-ALSS may improve prognosis or even save lives among HBV-ACLF patients.

Keywords: acute-on-chronic liver failure, hepatitis B virus, plasma exchange, survival rate, propensity score matching

INTRODUCTION

Acute-on-chronic liver failure (ACLF) is a clinical syndrome that occurs on the basis of chronic liver disease and is characterized by rapidly progressing organ failure and high short-term mortality (1). China is a high endemic area of hepatitis B virus (HBV). Indeed, there are 78 million HBV carriers and 28 million chronic hepatitis B (CHB) patients in China (2), and HBV-associated acute-on-chronic liver failure (HBV-ACLF) is the most common type of liver failure. A study by Xie et al. showed that 96.5% of ACLF patients in Southwest China were HBV-ACLF (3); in contrast, alcoholism and hepatitis C virus (HCV) infection are the most common causes of ACLF in Western developed countries (4, 5). The 28-day mortality is >15% in all ACLF patients and 40% in HBV-ACLF patients (6, 7).

Shi et al.'s study showed that HBV reactivation is the most common inducement of HBV-ACLF (8). The occurrence of drug resistance to nucleos(t)ide analogs (NAs) and NAs withdrawal without authorization are the main causes of HBV reactivation (9). Li et al. found that the immune metabolic disorder caused by HBV was the core axis of the occurrence and progression of HBV-ACLF (10). Bacterial infection is another common inducement of HBV-ACLF. Innate immune disorders and subsequent systemic inflammatory response syndrome (SIRS) not only drive the occurrence and progression of HBV-ACLF (11–13), but also lead to multiple organ failure in HBV-ACLF patients (14), and eventually damage the defense system of host immune cells and impair immune function, rendering HBV-ACLF patients more prone to secondary infection, which will further aggravate organ dysfunction and cause a sudden rise in the mortality of these patients (15–17).

Liver transplantation (LT) is currently the most effective therapy for ACLF. However, the shortage of donors and the high cost limit its clinical application (18, 19). Although NAs effectively inhibit HBV replication in CHB patients and reduce the 90-day mortality of HBV-ACLF patients, they are effective only in patients with a MELD score of 20–30 (20, 21). Various non-bioartificial liver support systems (NB-ALSSs) have since the late 1950's been utilized to treat liver failure, and plasma exchange (PE) is the most commonly applied mode of NB-ALSS in China. PE removes HBV-ACLF patient plasma rich in toxic metabolites and pro-inflammatory cytokines and compensates with the same volume of fresh frozen plasma supplemented with coagulation factors, albumin, immunoglobulin and other essential components to improve the liver microenvironment and to facilitate liver regeneration and functional recovery (22–24). A number of studies have shown that NB-ALSS, especially PE, is able to prolong the survival of patients with acute liver failure (ALF) or ACLF and improve their short-term outcomes (22, 25, 26). Case-control studies have shown that PE prolongs the survival of patients with ACLF and thus plays a role in bridging LT but that it does not improve short-term outcomes in patients with ACLF (27–29). There were also studies showing that PE improves the laboratory parameters and clinical symptoms of ACLF patients, without reporting whether PE improve outcomes (30, 31). Klementina et al. conducted a network meta-analysis and found that only PE therapy significantly improved 90-day outcomes in ACLF patients (32). Therefore, it remains

controversial whether PE-based NB-ALSS therapy improves the outcomes of patients with HBV-ACLF.

Based on the two large prospective multicenter cohorts of the Chinese acute-on-chronic liver failure (CATCH-LIFE) study (33, 34), the present study focused on whether PE-based NB-ALSS therapy improves short-term (28/90 days and 1-year) outcomes of patients with HBV-ACLF and examined factors influencing the efficacy of PE-based NB-ALSS therapy for these patients.

MATERIALS AND METHODS

Patients

The HBV-ACLF patients enrolled in this study were all from the CATCH-LIFE study. The CATCH-LIFE study currently includes two prospective multicenter cohorts (Development Cohort NCT02457637 and Validation Cohort NCT03641872) of patients with acute exacerbation of chronic liver disease (33, 34). Patients in both cohorts were followed up for more than 1 year. The ethics committee of Renji Hospital and Shiyan Taihe Hospital approved this study.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows:

(1) HBV infection (HBV surface antigen positive $\geq$ 6 months), a history of abnormal liver function, with or without cirrhosis;
(2) Meeting the EASL-ACLF diagnostic criteria (7):

No ACLF: (1) patients with no organ failure; (2) patients with single "non-kidney" organ failure (i.e., single failure of the liver, coagulation, circulation or respiration) and serum creatinine (Cr) $<$ 1.5 mg/dl and no hepatic encephalopathy (HE); and (3) patients with single cerebral failure and serum Cr $<$ 1.5 mg/dl.

ACLF grade 1: (1) patients with single kidney failure; (2) patients with single failure of the liver, coagulation, circulation or respiration and serum Cr ranging from 1.5 to 1.9 mg/dl and/or HE grade 1 or grade 2; and (3) patients with single cerebral failure and serum Cr ranging from 1.5 to 1.9 mg/dl.

ACLF grade 2: patients with two organ failures of any combination.

ACLF grade 3: patients with three or more organ failures of any combination.

The exclusion criteria were as follows:

(1) Hepatocellular carcinoma or other liver malignancies before or during admission.
(2) Extrahepatic malignancies or severe chronic extrahepatic disease.
(3) Age younger than 18 or older than 80 years.
(4) Pregnancy.
(5) HIV infection.

Data Retrieval

All clinical data for the enrolled patients were extracted from the CATCH-LIFE study database, including demographic data (age, gender) and the patient's cirrhosis status. Laboratory data of the patients at the time of diagnosis of ACLF and on days 4, 7, 14, 21, and 28 included the following: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (Tbil), international normalized ratio (INR), and serum Cr levels; HBV-DNA load; HE grade; ascites; coinfection; and 28-day, 90-day, and 1-year outcomes (death was the outcome event). The frequency, combination pattern, and plasma volume per use of PE-based NB-ALSS therapy were recorded. Clinical data at the time of ACLF diagnosis were used as baseline data. The MELD score of each patient was calculated according to the formula: MELD score = [9.57 $\times$ ln(Cr mg/dl) + 3.78 $\times$ ln(Tbil mg/dl) + 11.20 $\times$ ln(INR) + 6.43 (except for cholestatic and alcoholic liver disease, 6.43 should be added)] (35). The HBV-ACLF patients were divided into four grades according to the correlation between the MELD score and the risk of death (4): MELD grade 1, MELD scores $<$ 20; MELD grade 2, MELD scores of 20 to $<$ 30; MELD grade 3, MELD scores of 30 to $<$ 40; and MELD grade 4, MELD scores $\geq$ 40. The HBV-ACLF patients were also classified into three types based on cirrhotic status: Type A, HBV-ACLF patients without cirrhosis; Type B, HBV-ACLF patients based on compensatory cirrhosis; and Type C, HBV-ACLF patients based on decompensated cirrhosis (36). In addition, SIRS scores were calculated according to the consensus of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee (37).

Research Grouping

The Standard Medical Therapy (SMT) Group

Patients who received SMT alone during hospitalization were enrolled in the SMT group. SMT includes the following: a high-calorie diet; enteral nutrition is recommended; correction of hypoproteinemia; correction of water-electrolyte and acid-base balance; NAs for HBV-DNA-positive patients; anti-infective therapy for infection; a restricted protein diet; lactulose, L-ornithine aspartate for HE; diuretics and tolvaptan for ascites; vasoactive drugs; maintenance of arterial blood pressure and water restriction for hepatorenal syndrome; and oxygen therapy for hepatopulmonary syndrome.

The SMT+PE Group

Patients who received at least one PE-based NB-ALSS therapy in addition to SMT during hospitalization were enrolled in the SMT+PE group. PE-based NB-ALSS includes PE alone or in combination with hemofiltration (HF), a double plasma molecular adsorption system (DPMAS), plasma diafiltration (PDF), plasma bilirubin adsorption (PBA), continuous renal replacement therapy (CRRT), or plasma perfusion (PP).

Statistical Analysis

Normally and non-normally distributed continuous variables are presented as means $\pm$ standard deviations (SDs) and medians (interquartile ranges, IQRs), respectively. Categorical variables are shown as *n* (%). To compare differences between two groups, *t*-tests, chi-square tests and Mann-Whitney *U*-tests were used. Propensity score matching analysis was employed to eliminate confounding bias between two groups. The propensity score was calculated according to age, sex, ACLF type, ACLF grade, HE grade, Tbil, serum sodium (Na^+), Cr, INR, infection, ascites, respiratory failure, and circulatory failure, and the nearest neighbor 1:1 matching scheme was adopted. The 28-day, 90-day,

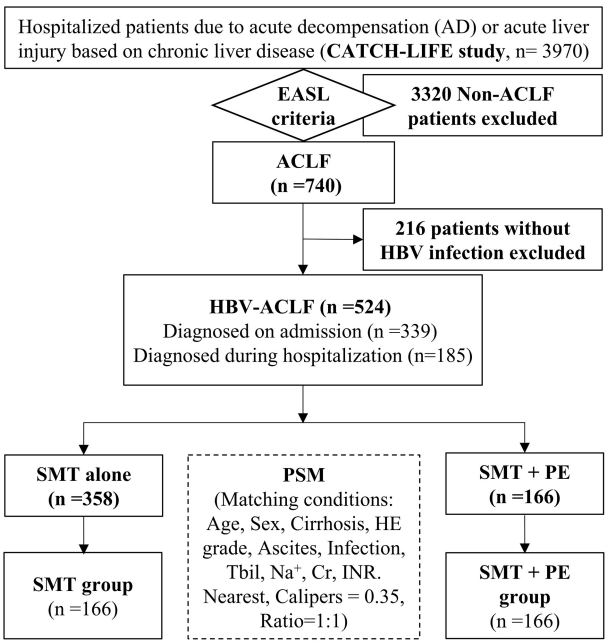


FIGURE 1 | Flow chart of the study. HBV, hepatitis B virus; ACLF, acute-on-chronic liver failure; SMT, standard medical therapy; PE, plasma exchange; PSM, propensity score match; HE, hepatic encephalopathy; Tbil, total bilirubin; Na^+, serum sodium; Cr, serum creatinine; INR, international normalized ratio; EASL, European Association for The Study of Liver Diseases.

and 1-year survival rates of HBV-ACLF patients were calculated using Kaplan-Meier survival curves. The log-rank test was used to compare survival rates between groups and univariate and multivariate Cox regression analyses were used to determine independent prognostic factors for HBV-ACLF patients and the factors affecting PE-based NB-ALSS efficacy. SPSS software, version 24.0 (SPSS, Inc., Chicago, IL, USA) was used for the data analysis. Figures were produced using GraphPad Prism software, version 6.0 (GraphPad, LLC, San Diego, USA). A two-tailed p-value < 0.05 was considered statistically significant.

RESULTS

Patient Characteristics

A total of 3,970 patients with chronic liver disease admitted for acute decompensation or acute liver injury were screened for this study. Of them, 740 patients met the EASL-ACLF diagnostic criteria, 216 patients without HBV infection were excluded, and 524 HBV-ACLF patients were ultimately enrolled. Of these, 339 patients were diagnosed with ACLF on admission, and the remaining 185 developed ACLF during hospitalization (**Figure 1**). Among the 524 HBV-ACLF patients, 440 (83.97%) were male. A total of 115 (21.95%) patients exhibited no evidence of liver cirrhosis (Type A); in contrast, 299 (57.06%) had compensated cirrhosis (Type B), and 110 (20.99%) had decompensated cirrhosis (Type C). Of these, 62 (11.83%), 403 (76.91%), and 59 (11.26%) cases were classified as ACLF grade 1, 2, and 3, respectively. The numbers of patients with MELD grades 1, 2, 3, and 4 were 14 (2.67%), 166 (31.68%), 306 (58.40%), and 38 (7.25%), respectively. A total of 358 patients with HBV-ACLF received SMT alone (SMT group), whereas 166 received PE-based NB-ALSS in addition to SMT (SMT+PE group). The 166 HBV-ACLF patients in the SMT+PE group received a total of 422 PE-based NB-ALSS therapies during hospitalization at least once and up to nine times, with a median of two times. The modes of NB-ALSS therapies included PE alone 294 (69.67%) times, PE + HF 65 (15.40%) times, PE + DPMAS 45 (10.67%) times, and PE + PBA 18 (4.27%) times. A median volume of 2,000 mL fresh frozen plasma (FFP) was used for single PE-based NB-ALSS therapy, and the median time of initiation of PE-based NB-ALSS treatment was 5 days after admission (**Table 1**).

Overall, there were significant differences in baseline characteristics between the patients treated with SMT+PE and SMT alone, such as ACLF type, ACLF grade, alpha fetal protein (AFP), ALT, and AST. For PSM-matched 166 pairs of HBV-ACLF patients, there was no significant difference in baseline data between the SMT+PE group and SMT group, except that longer PT and higher HBV-DNA load were observed in patients in the former compared with the latter (PT, 28.60 vs. 27.20, $p = 0.008$; HBV-DNA, 4.85 log. vs. 4.31 log., $p = 0.030$) (**Table 1**).

PE-Based NB-ALSS Significantly Improves the Short-Term Survival Rates of ACLF Patients, Especially in MELD Grade 3 Patients

According to Kaplan-Meier survival analysis, among the enrolled HBV-ACLF patients, the 28-day and 1-year survival rates of patients in the SMT+PE group were significantly higher than those in the SMT group (28-day, 69.50% vs. 60.90%, $p = 0.025$; 1-year, 42.20% vs. 34.10%, $p = 0.047$) (**Figures 2A,C**). The 90-day survival rate was also higher in patients in the SMT+PE group than in the SMT group, though without a significant difference (48.70% vs. 42.80%, $p = 0.076$) (**Figure 2B**). Subgroup analysis revealed no significant difference in the survival rate of HBV-ACLF patients between the two groups at ACLF grade 1 and ACLF grade 3 (**Supplementary Figures 1G–I**). However, for ACLF grade 2 patients, 28-day, 90-day, and 1-year survival rates in the SMT+PE group were higher than those in the SMT group (**Figures 2D–F**), but the difference was not significant (28-day, 70.10 vs. 62.90%, $p = 0.073$; 90-day, 47.80 vs. 41.60%, $p = 0.111$; 1-year, 42.50 vs. 34.00%, $p = 0.069$). Subgroup analysis according to MELD grade revealed no significant difference in the survival rates of HBV-ACLF patients between the two groups at MELD grade 1, MELD grade 2, and MELD grade 4 (**Supplementary Figures 1A–F**); for MELD grade 3 patients, 28-day, 90-day, and 1-year survival rates were significantly higher in patients in the SMT+PE group than in the SMT group (28-day, 68.90 vs. 54.10%, $p = 0.005$; 90-day, 50.00 vs. 33.80%, $p = 0.002$; 1-year, 42.20 vs. 23.50%, $p = 0.001$) (**Figures 2G–I**).

Importantly, in 166 pairs of PSM-matched HBV-ACLF patients, 28-day, 90-day, and 1-year survival rates were all significantly higher in the SMT+PE group than in the SMT

TABLE 1 | Baseline characteristics of HBV-ACLF patients who received SMT alone or SMT+PE.

Parameters	Entire patients			Propensity score-matched patients		
	SMT+PE (n = 166)	SMT (n = 358)	p-value	SMT+PE (n = 166)	SMT (n = 166)	p-value
PE times	2.00 (1.00, 3.00)			2.00 (1.00, 3.00)		
Mean plasma dose	2,000 (1,600, 2,800)			2,000 (1,600, 2,800)		
Therapy timing	5.00 (3.00, 8.00)			5.00 (3.00, 8.00)		
Modes of ALSS						
PE	294 (69.67%)			294 (69.67%)		
PE + HF	65 (15.40%)			65 (15.40%)		
PE + DPMAS	45 (10.67%)			45 (10.67%)		
PE + PBA	18 (4.27%)			18 (4.27%)		
Age (year)	47.47 ± 11.40	48.31 ± 11.17	0.428	47.47 ± 11.40	47.30 ± 11.35	0.889
Sex (male)	145 (87.35%)	295 (82.40%)	0.151	145 (87.35%)	22 (8.00%)	0.87
HE (≥3)	8 (4.82%)	37 (10.34%)	0.036	8 (4.82%)	17 (10.24%)	0.061
Respiratory failure	10 (6.02%)	28 (7.82%)	0.461	10 (6.02%)	11 (6.63%)	0.813
Circulatory failure	2 (1.20%)	16 (4.47%)	0.056	2 (1.20%)	1 (0.60%)	0.562
Infection (yes)	56 (33.73%)	147 (41.06%)	0.109	56 (33.73%)	56 (33.73%)	1.00
Ascites (yes)	68 (40.96%)	143 (39.94%)	0.825	68 (40.96%)	64 (38.55%)	0.654
ACLF type (A/B/C)	49/98/19	66/201/91	<0.001	49/98/19	45/106/15	0.620
ACLF grade (1/2/3)	10/146/10	52/257/49	<0.001	10/146/10	16/132/18	0.112
MELD grade (1/2/3/4)	1/58/97/10	13/108/209/28	0.157	1/58/97/10	2/45/108/11	0.455
Meld score	31.73 ± 5.37	31.12 ± 5.60	0.241	31.73 ± 5.37	31.66 ± 4.65	0.896
Meld-Na score	32.23 ± 4.97	31.96 ± 5.41	0.589	32.23 ± 4.97	32.23 ± 4.56	0.991
SIRS score	0.81 ± 0.09	0.99 ± 0.07	0.122	0.81 ± 0.09	0.95 ± 0.10	0.318
NH3 (μmol/L)	62.00 (34.60, 83.50)	48.20 (27.90, 80.50)	0.173	62.00 (34.60, 83.50)	48.00 (28.90, 80.50)	0.332
CRP (μg/L)	12.55 (7.78, 17.70)	11.95 (6.49, 22.06)	0.644	12.55 (7.78, 17.70)	12.50 (6.49, 19.90)	0.638
PCT (μg/L)	0.40 (0.00, 0.93)	0.34 (0.00, 0.79)	0.965	0.40 (0.00, 0.93)	0.67 (0.44, 1.17)	0.333
AFP (μg/L)	56.69 (20.09, 185.30)	30.80 (6.89, 108.90)	0.001	56.69 (20.09, 185.30)	36.10 (10.96, 135.30)	0.087
CA199 (μg/L)	54.64 (15.65, 223.70)	56.70 (21.62, 150.59)	0.793	54.64 (15.65, 223.70)	57.44 (26.10, 170.73)	0.93
Log (HBV-DNA)	4.85± 2.09	4.39 ± 2.15	0.034	4.85± 2.09	4.31 ± 2.05	0.030
ALT (U/L)	188.00 (70.65, 607.50)	122.50 (46.98, 385.75)	0.002	188.00 (70.65, 607.50)	186.60 (76.15, 521.00)	0.742
AST (U/L)	192.70 (99.50, 469.00)	151.65 (73.75, 309.58)	0.003	192.70 (99.50, 469.00)	179.00 (104.23, 418.50)	0.519
GGT (U/L)	69.00 (46.25, 101.00)	58.00 (36.00, 88.00)	0.001	69.00 (46.25, 101.00)	61.00 (41.00, 94.00)	0.123
AKP (U/L)	144.65 (111.25, 199.50)	128.50 (98.00, 170.25)	0.002	144.65 (111.25, 199.50)	135.50 (108.00, 170.00)	0.056
Tbil (μmol/L)	437.48 ± 162.20	380.17 ± 188.88	0.001	437.48 ± 162.20	431.35 ± 162.76	0.731
Alb (g/L)	31.79 ± 5.70	30.95 ± 6.18	0.138	31.79 ± 5.70	31.58 ± 5.23	0.723
Na^+ (mmol/L)	135.76 ± 5.48	134.14 ± 5.87	0.003	135.76 ± 5.48	135.37 ± 4.89	0.498
K^+ (mmol/L)	3.91 ± 0.64	3.96 ± 0.74	0.481	3.91 ± 0.64	3.95 ± 0.72	0.666
Cr (μmol/L)	83.90 (60.95, 106.18)	80.95 (60.00, 149.33)	0.321	83.90 (60.95, 106.18)	71.10 (56.75, 104.43)	0.079
BUN (mmol/L)	4.44(2.99, 8.02)	6.00 (3.63, 11.96)	0.001	4.44 (2.99, 8.02)	4.88 (3.30, 7.90)	0.475
PT (s)	28.60 (21.00, 33.50)	24.70 (16.30, 31.60)	<0.001	28.60 (21.00, 33.50)	27.20 (16.35, 31.60)	0.008
INR	2.76 (2.53, 3.26)	2.75 (2.39, 3.28)	0.378	2.76 (2.53, 3.26)	2.78 (2.55, 3.41)	0.485
WBC ($\times 10^9$/L)	8.00 ± 4.17	8.26 ± 4.88	0.562	8.00 ± 4.17	8.24 ± 3.65	0.576
HGB (g/L)	117.22 ± 21.69	109.55 ± 26.10	0.001	117.22 ± 21.69	116.40 ± 24.75	0.750
PLT ($\times 10^9$/L)	82.50 (54.00, 117.00)	78.50 (50.00, 120.00)	0.507	82.50 (54.00, 117.00)	87.00 (61.70, 131.00)	0.064

HBV-ACLF, hepatitis B virus-associated acute-chronic liver failure; ACLF type: A, non-cirrhosis; B, compensatory cirrhosis; C, decompensated cirrhosis; NH3, blood ammonia; CRP, c-reactive protein; PCT, procalcitonin; AFP, alpha fetal protein; CA199, cancerantigen199; DNA, deoxyribonucleic acid; SMT, standard medical therapy; PE, plasma exchange; HF, hemofiltration; DPMAS, a double plasma molecular adsorption system; PBA, plasma bilirubin adsorption; HE, hepatic encephalopathy; Meld score, Model for end-stage liver disease score; Meld-Na score, Model for end-stage liver disease with the incorporation of serum sodium score; SIRS, systemic inflammatory response syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; AKP, alkaline phosphatase; Tbil, total bilirubin; Alb, albumin; Na^+, serum sodium; K^+, serum potassium; Cr, serum creatinine; BUN, blood urea nitrogen; PT, prothrombin time; INR, international normalized ratio; WBC, white blood cell; HGB, hemoglobin; PLT, platelet.

group (28-day, 69.50 vs. 57.60%, $p = 0.006$; 90-day, 48.70 vs. 40.70%, $p = 0.031$; 1-year, 42.20 vs. 31.30%, $p = 0.014$) (**Figures 3A–C**). Moreover, subgroup analysis based on ACLF and MELD grades showed significantly higher 28-day, 90-day, and 1-year survival rates for the SMT+PE group among patients with ACLF grade 2 (**Figures 3D–F**) and MELD grade

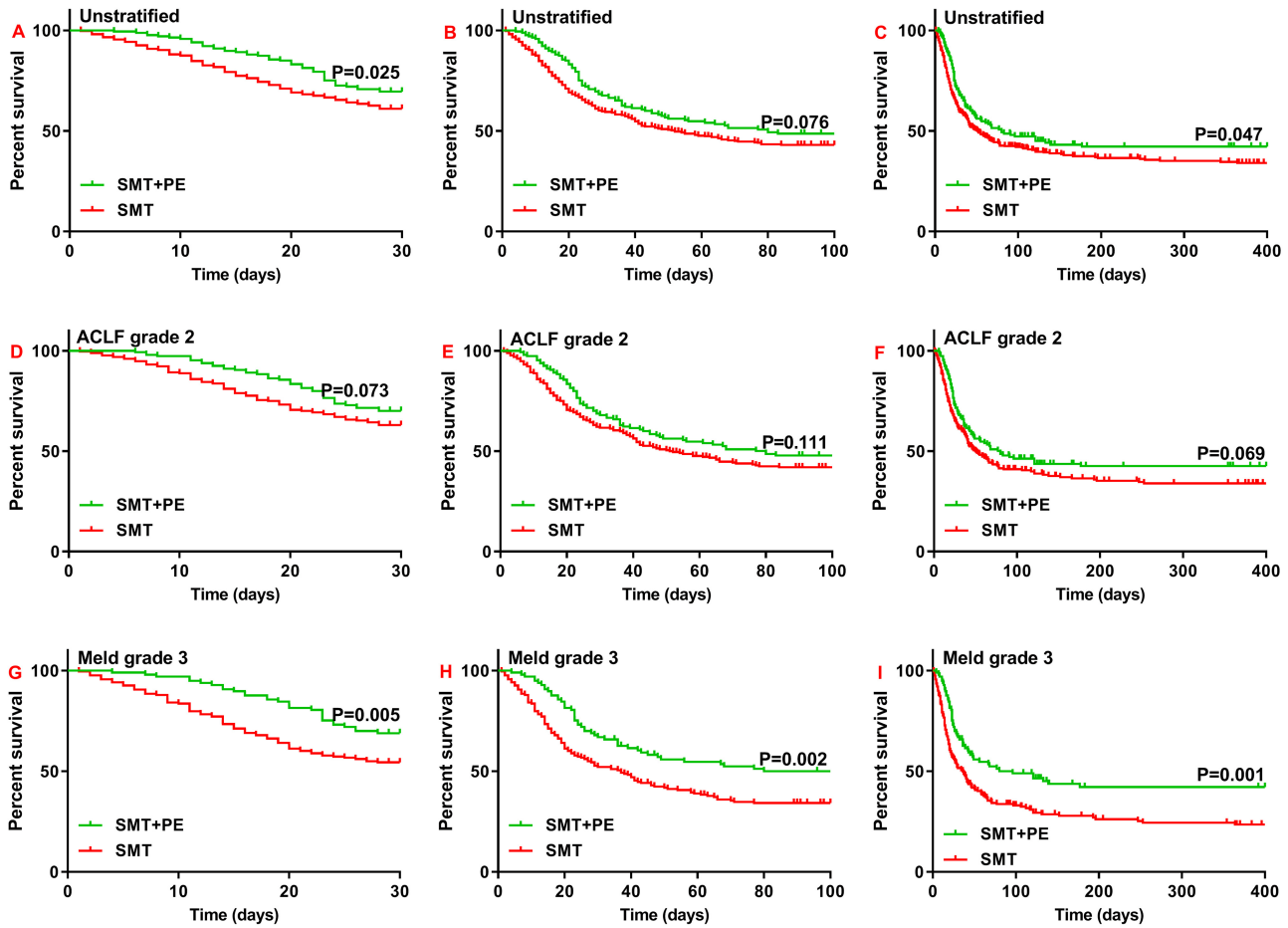


FIGURE 2 | Survival curves for patients with HBV-ACLF who received SMT alone or SMT+PE. **(A–C)** 28-day **(A)**, 90-day **(B)**, and 1-year **(C)** survival curves for patients with HBV-ACLF. **(D–F)** 28-day **(D)**, 90-day **(E)**, and 1-year **(F)** survival curves for HBV-ACLF patients with ACLF grade 2. **(G–I)** 28-day **(G)**, 90-day **(H)**, and 1-year **(I)** survival curves for HBV-ACLF patients with MELD scores of 30–40. SMT, standard medical therapy; PE, plasma exchange; HBV-ACLF, hepatitis B virus-associated acute-on-chronic liver failure.

3 (**Figures 3G–I**), especially for the latter. In addition, 28-day, 90-day, and 1-year survival rates in patients in the SMT+PE group were 18.60, 14.20, and 20.10% higher than those in the SMT group, respectively (**Table 2**). However, no significant difference was found in the short-term survival rate between the SMT group and SMT+PE group of HBV-ACLF patients with ACLF grade 1, ACLF grade 3, MELD grade 2, and MELD grade 4 (**Supplementary Figure 2**).

PE-Based NB-ALSS Does Not Lead to Continuous Improvement in the Laboratory Parameters of HBV-ACLF Patients Within 28 Days

Among 166 pairs of PSM-matched HBV-ACLF patients, significant reductions in Tbil, GGT, and PT were observed only on day 7 in those treated with SMT+PE compared with those treated with SMT (**Table 3**). Although ALT, AST, and GGT levels in the SMT+PE group were lower in patients treated with SMT+PE on days 4, 7, 14, 21, and 28, and thereafter, the difference was not significant (**Figure 4**). The alkaline phosphatase (AKP) level of HBV-ACLF patients in the SMT+PE group showed a continuous decline but fluctuated in patients in the SMT group. However, Alb displayed a gradually increasing trend in both groups, and Alb was significantly higher in the SMT+PE group than in the SMT group on day 14. There were no significant differences in Cr, blood urea nitrogen (BUN), Na^+, serum potassium (K^+), or hemoglobin (HGB) levels at each time point. Although the WBC count was significantly lower in the SMT+PE group than in the SMT group on day 4 and 7, the PLT count was significantly lower in the former on days 7, 14 and 21 (**Figure 4**). Further analysis in SIRS scores showed that SIRS scores were significantly lower in the SMT+PE group than those in the SMT group on day 7 (0.62 vs. 1.03, $p = 0.002$) and day 14 (0.64 vs. 1.01, $p = 0.035$) (**Supplementary Figure 3**).

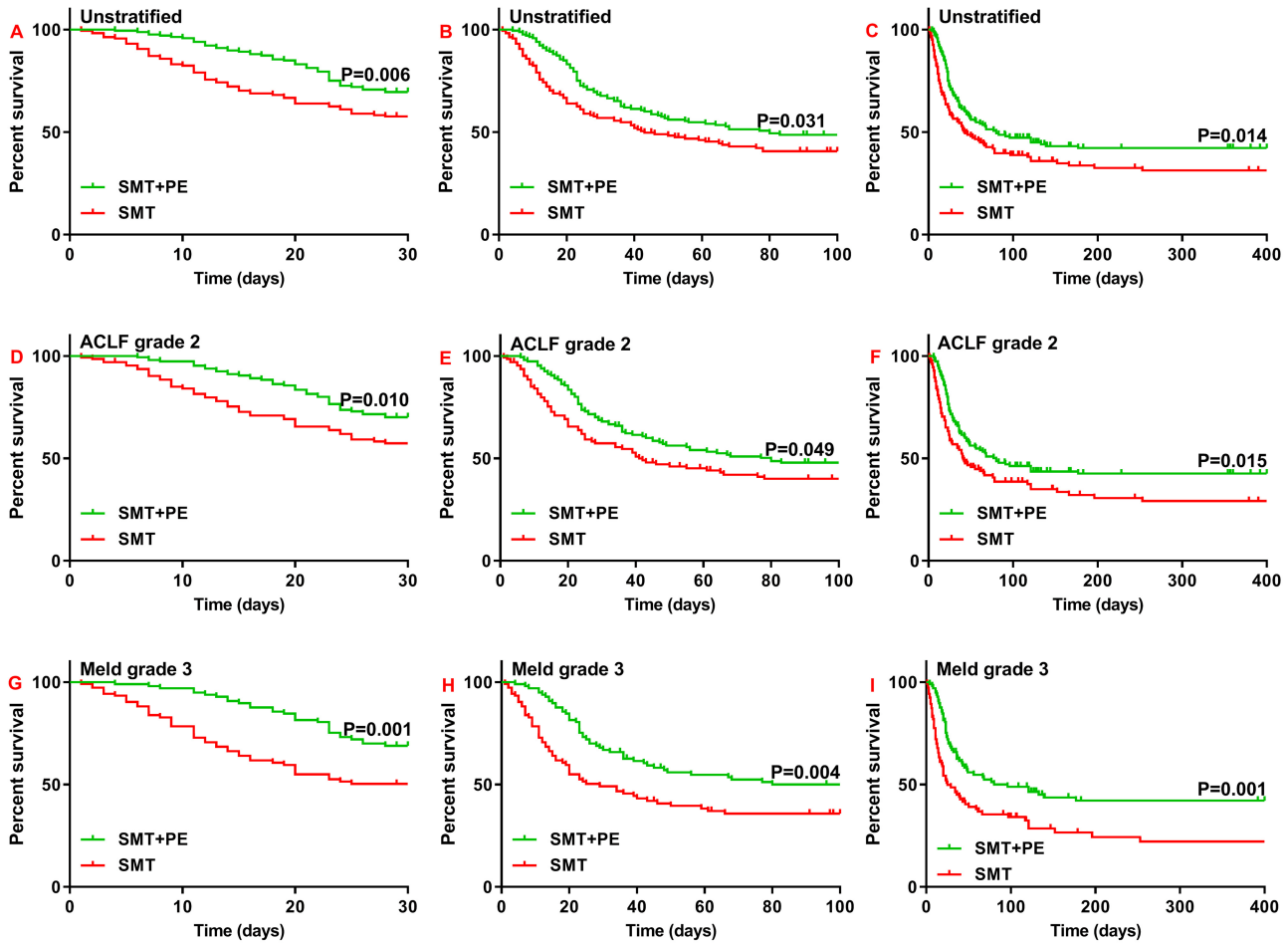


FIGURE 3 | Survival curves for 166 pairs of matched patients with HBV-ACLF who received SMT alone or SMT+PE. **(A–C)** 28-day **(A)**, 90-day **(B)**, and 1-year **(C)** survival curves for patients with HBV-ACLF. **(D–F)** 28-day **(D)**, 90-day **(E)**, and 1-year **(F)** survival curves for HBV-ACLF patients with ACLF grade 2. **(G–I)** 28-day **(G)**, 90-day **(H)**, and 1-year **(I)** survival curves for HBV-ACLF patients with MELD scores of 30 to 40. PE, plasma exchange; SMT, standard medical therapy; HBV-ACLF, hepatitis B virus-associated acute-on-chronic liver failure.

PE-Based NB-ALSS Is the Only Independent Protective Factor for Short-Term Prognosis in Patients With HBV-ACLF

Based on multivariate Cox regression analysis, PE-based NB-ALSS was the only independent protective factor for the survival of HBV-ACLF patients at 28 days, 90 days, and 1 year (28 days, HR = 0.516, $p = 0.001$; 90 days, HR = 0.663, $p = 0.010$; 1 year, HR = 0.610, $p = 0.051$). Advanced age, combined with cerebral failure (HE $\geq$ 3), respiratory failure and high MELD score were independent risk factors for survival at 28 days (**Table 4**). The independent risk factors for 90-day and 1-year survival of HBV-ACLF patients are shown in **Table 4**. The results of univariate Cox regression analysis are shown in **Supplementary Table 1**.

Frequency Is an Important Factor Affecting the Efficacy of PE-Based NB-ALSS

Multivariate Cox regression analysis showed that for HBV-ACLF patients treated with PE-based NB-ALSS, the frequency of the therapy was the only independent protective factor for 28-day and 90-day survival (28-day, HR = 0.597, $p = 0.001$; 90-day, HR = 0.772, $p = 0.018$). Respiratory failure and high INR were independent risk factors for 28-day survival, and advanced age, respiratory failure, high BUN and high INR were independent risk factors for 90-day prognosis (**Table 5**). The results of univariate Cox regression analysis are shown in **Supplementary Table 2**.

DISCUSSION

In this study, PE-based NB-ALSS significantly improved 28-day and 90-day outcomes, as well as 1-year outcomes, in HBV-ACLF patients. Nevertheless, subgroup analysis showed that not all patients with HBV-ACLF benefited from PE-based NB-ALSS. Indeed, PE-based NB-ALSS had significant efficacy only in those with ACLF grade 2 or MELD grade 3, but did not lead to continuous improvement in the laboratory parameters, such as Tbil, PT, GGT, AST, and ALT, of HBV-ACLF patients within 28 days. However, multivariate Cox regression analysis

TABLE 2 | Cumulative survival rates of HBV-ACLF patients who received SMT alone or combination therapy with SMT and PE.

		Time	Survival rate		χ^2 value	*p*-value	Survival rate difference
			SMT+PE	SMT			
Unstratified	All patients	28 days	69.50%	60.90%	5.005	0.025	8.60%
		90 days	48.70%	42.80%	3.149	0.076	5.90%
		1 year	42.20%	34.10%	3.944	0.047	8.10%
	Matched patients	28 days	69.50%	57.60%	7.508	0.006	11.90%
		90 days	48.70%	40.70%	4.633	0.031	8.00%
		1 year	42.20%	31.30%	5.991	0.014	10.90%
ACLF grade 2	All patients	28 days	70.10%	62.90%	3.219	0.073	7.20%
		90 days	47.80%	41.60%	2.537	0.111	6.20%
		1 year	42.50%	34.00%	3.312	0.069	8.50%
	Matched patients	28 days	70.10%	57.30%	6.637	0.010	12.80%
		90 days	47.80%	40.00%	3.873	0.049	7.80%
		1 year	42.50%	29.20%	5.940	0.015	13.30%
Meld grade 3	All patients	28 days	68.90%	54.10%	7.914	0.005	14.80%
		90 days	50.00%	33.80%	9.253	0.002	16.20%
		1 year	42.20%	23.50%	11.349	0.001	18.70%
	Matched patients	28 days	68.90%	50.30%	10.562	0.001	18.60%
		90 days	50.00%	35.80%	6.637	0.004	14.20%
		1 year	42.20%	22.10%	11.229	0.001	20.10%

HBV-ACLF, hepatitis B virus-associated acute-on-chronic liver failure; ACLF, acute-on-chronic liver failure; PE, plasma exchange; SMT, standard medical therapy; Meld score, Model for end-stage liver disease score.

TABLE 3 | Changes in laboratory parameters in patients with HBV-ACLF who received SMT alone or combination therapy with SMT and PE-based NB-ALSS.

Parameter	Group	Mean	SE	*t*-value	*p*-value
ΔTbil	SMT	21.38	17.12	2.039	0.043
	SMT+PE	−22.19	13.20		
ΔGGT	SMT	20.22	22.84	2.266	0.025
	SMT+PE	−26.12	7.07		
ΔPT	SMT	8.09	1.98	3.795	<0.001
	SMT+PE	0.48	0.97		

Δ, the difference in the value between day 7 and the baseline.
GGT, γ-glutamyltransferase; Tbil, total bilirubin; PT, prothrombin time.

indicated that PE-based NB-ALSS was the only independent protective factor for 28-day and 90-day prognosis in HBV-ACLF patients and that advanced age, cerebral failure, respiratory failure, and high MELD score were independent risk factors for 28-day prognosis. Additionally, advanced age, cerebral failure, respiratory failure, hyponatremia, and elevated INR were independent risk factors for 90-day prognosis, which is consistent with the results of previous studies (22, 25, 26, 38). PE-based NB-ALSS therapy was also found to be an independent protective factor for the 1-year prognosis of HBV-ACLF patients in this study.

Studies in Europe have shown that the molecular adsorbent recirculating system (MARS) can improve the short-term survival rate of patients with ACLF grades 2-3 (39). Ning Qin et al. reported that plasma perfusion (PP) combined with PE therapy significantly improves the short-term outcomes of HBV-ACLF patients, with the best efficacy for those with ACLF grades 2-3 (38). In this study, however, PE-based NB-ALSS therapy only exhibited significant efficacy for ACLF grade 2 patients, which may not only be due to the different therapeutic mechanisms of MARS and PE but also to the different disease characteristics of ACLF patients in the East and West. In fact, alcoholism and HCV infection are the most common causes of ACLF in Western countries, and renal function damage is very common in these ACLF patients, whereas HBV infection is the most common cause of ACLF in China (1, 3, 5, 40). Overall, HBV-ACLF patients are more prone to liver failure and coagulation failure but less prone to renal failure and cerebral failure (5, 6, 38). Of the 332 HBV-ACLF patients enrolled in the matched analysis in this study, 278 (83.73%) had ACLF grade 2; among them, 243 (87.41%) patients had liver failure combined with coagulation failure, 26 (7.83%) had ACLF grade 1, and 28 (8.43%) had ACLF grade 3. Patients with ACLF grade 1 mostly experience renal failure or cerebral failure with renal dysfunction. FFP is rich in coagulation factors, albumin, immunoglobulin and other essential substances and can partially replace the synthesis and detoxification functions of the liver, thereby improving liver and coagulation function in HBV-ACLF patients. Hence, PE-based NB-ALSS has a significant therapeutic effect on patients with EASL-ACLF grade 2. Although PE therapy is effective in clearing albumin-bound toxins, it cannot effectively remove water-soluble toxins of small and medium sizes. Therefore, PE therapy is not ideal for improving renal function in HBV-ACLF patients. In this study, PE-based NB-ALSS therapy showed no significant effect in ACLF grade 1 patients, which may be related to the small

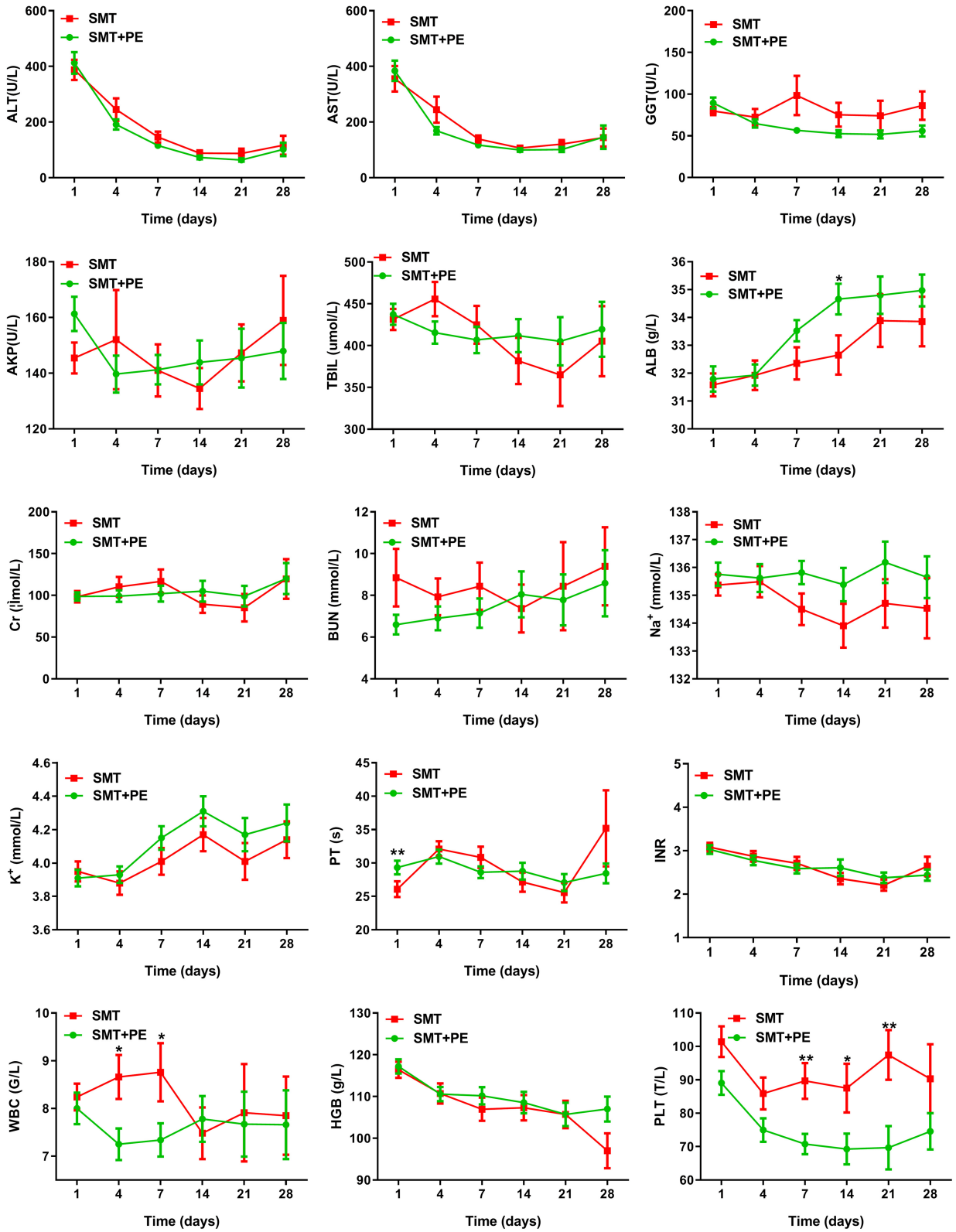


FIGURE 4 | Changes in laboratory parameters in HBV-ACLF patients in the SMT group and SMT+PE group. SMT, standard medical therapy; PE, plasma exchange; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; AKP, alkaline phosphatase; Tbil, total bilirubin; Alb, albumin; Na^+, serum sodium; K^+, serum potassium; Cr, serum creatinine; BUN, blood urea nitrogen; PT, prothrombin time; INR, international normalized ratio; WBC, white blood cell; HGB, hemoglobin; PLT, platelet; *P value < 0.05; **P value < 0.01.

TABLE 4 | Prognostic factors at 28 days, 90 days, and 1 year in HBV-ACLF patients treated with SMT alone or in combination with SMT and PE-based NB-ALSS.

Multivariate	*p* value	HR	95% CI HR		Time point
Parameter			Lower bound	Upper bound	
PE	0.001	0.516	0.353	0.755	28 days
Age	<0.001	1.05	1.032	1.068	
HE(≥3)	<0.001	3.774	2.208	6.452	
Respiratory failure	<0.001	3.645	1.955	6.798	
MELD score	<0.001	1.09	1.058	1.122	
PE	0.006	0.647	0.473	0.884	90 days
Age	<0.001	1.049	1.035	1.064	
HE(≥3)	<0.001	3.945	2.349	6.625	
Respiratory failure	0.001	2.633	1.508	4.596	
Na^+	0.002	0.949	0.919	0.98	
INR	<0.001	1.325	1.214	1.446	
PE	0.051	0.610	0.372	1.002	1 year
Age	<0.001	1.038	1.016	1.059	
HE(≥3)	0.006	3.221	1.392	7.450	
INR	<0.001	1.289	1.134	1.466	
CRP	0.004	1.017	1.005	1.028	

HBV-ACLF, hepatitis B virus-associated acute-on-chronic liver failure; PE, plasma exchange; HE, hepatic encephalopathy; Meld score, Model for end-stage liver disease score; Na^+, serum sodium; INR, international normalized ratio; CRP, c-reactive protein; HR, hazard ratio.

TABLE 5 | Prognostic factors in HBV-ACLF patients treated with PE-based NB-ALSS.

Multivariate	*p*-value	HR	95% CI HR		Time point
Parameters			Lower bound	Upper bound	
PE times	0.001	0.597	0.436	0.816	28 days
Respiratory failure	0.001	4.497	1.843	10.973	
INR	0.007	1.216	1.056	1.400	
PE times	0.018	0.772	0.623	0.957	90 days
Age	0.003	1.033	1.011	1.055	
Respiratory failure	0.022	2.829	1.159	6.905	
BUN	0.035	1.037	1.003	1.072	
INR	0.002	1.225	1.075	1.396	
Respiratory failure	0.057	3.510	0.965	12.773	1 year
CRP	0.001	1.024	1.010	1.039	
PT	<0.001	1.039	1.018	1.061	

PE, plasma exchange; HE, hepatic encephalopathy; INR, international normalized ratio; BUN, blood urea nitrogen; CRP, c-reactive protein; PT, prothrombin time; HR, hazard ratio.

number of ACLF grade 1 patients and the weak effect of PE on renal failure. For patients with ACLF grade 1, CRRT or DPMAS in addition to SMT is recommended, as is the combination of PE with CRRT or DPMAS. Patients with ACLF grade 3 underwent at least 3 organ failures, and their 28-day mortality can be as high as 78.6% without LT (7). Although studies have shown that PE can control the occurrence and development of multiple organ failure in ACLF patients, PE therapy may be ineffective for those who have already experienced multiple organ failure. Instead, it will increase the risk of infection, hemodynamic instability and HE (22, 38). In the present study, PE-based NB-ALSS therapy did not improve the outcomes of ACLF grade 3 patients, and it is recommended that such patients receive LT as soon as possible.

Among HBV-ACLF patients with MELD scores between 30 and 40, 28-day, 90-day, and 1-year survival rates increased by 14–20% in patients who received SMT+PE therapy compared with those who received SMT alone. However, in HBV-ACLF patients with MELD scores >40, SMT+PE therapy failed to improve short-term survival rates, similar to the result of Yu et al. (26). Because the MELD scores of the ACLF patients enrolled in Yu et al.'s study were all >30, their study did not investigate the effect of PE therapy in those with MELD scores <30. Among the HBV-ACLF patients included in the matching analysis in the present study, there were only 3 with MELD scores <20, which is too small for a statistical analysis. Our study cohort comprised 45 patients in the SMT group and 58 in the SMT+PE group with MELD scores between 20 and 30, and PE-based NB-ALSS therapy did not improve the short-term survival rate of these patients. However, Xia et al.'s study showed that PE-based NB-ALSS can significantly improve the short-term outcomes of HBV-ACLF patients with a MELD score $\leq$ 30. In particular, the 12-week survival rate of patients with MELD score $\leq$ 20 increased by 21%; for patients with MELD score >30, the 4–48 weeks survival rate was significantly lower in those receiving PE therapy than in those receiving SMT (41). Although the sample size (787 cases) was sufficient, Xia et al.'s study was a single-center retrospective study without PSM analysis, and the study by Yu et al. was a prospective randomized controlled study with a higher level of evidence. Therefore, PE-based NB-ALSS therapy can improve short-term outcomes in patients with HBV-ACLF but is likely to have significant efficacy in only a subset of patients. A large, prospective, multicenter randomized controlled study is needed to further define the subgroup of patients who will potentially benefit from PE-based NB-ALSS therapy.

In the study of Chen et al., PE therapy was found to significantly reduce Tbil and INR in ACLF patients (42). Yao et al. also reported that the ALT, AST, Tbil, and INR of HBV-ACLF patients were significantly reduced after PE therapy (43). In our study, there was an instantaneous and significant decrease in Tbil after PE-based NB-ALSS therapy (data not shown), but Tbil and other laboratory parameters rebounded to high levels in the absence of subsequent PE-based NB-ALSS therapy. Indeed, significantly reductions in Tbil, GGT, and PT were only detected on day 7 in PSM-matched HBV-ACLF patients who received PE-based NB-ALSS therapy. According to the consensus of the Asian Pacific Association for the Study of the Liver (APASL), patients with ACLF who develop SIRS within 7 days after disease onset will subsequently develop multiple organ failure (MOF) and are deemed to have a very poor prognosis. Therefore, the development of SIRS within 1 week is an important determinant of prognosis in patients with ACLF (44). PE therapy can significantly reduce levels of pro-inflammatory cytokines in ACLF patients, thereby preventing overactivation of the immune system (22–24, 38, 45). In this study, the WBC count showed a

slight decline in the SMT+PE group and an increase in the SMT group. The WBC count in the SMT+PE group was significantly lower than that in the SMT group on day 4 and 7, and the SIRS scores were also significantly lower in the SMT+PE group than in the SMT group on day 7 and 14. Thus, PE-based NB-ALSS therapy reduces the inflammatory response degree in HBV-ACLF patients, which may reduce the occurrence of SIRS and MOF. This finding may explain why in this study, PE-based NB-ALSS therapy improved the prognosis of HBV-ACLF patients without continuous improvement in laboratory parameters. In addition, the disease course of ACLF patients who survive for 28 days usually exceeds 60 days, and most patients begin to recover after 28 days. Nonetheless, the laboratory parameters of HBV-ACLF patients were only recorded for 28 days in this study. Overall, extending the observation period may reveal the effect of PE-based NB-ALSS therapy in improving the laboratory parameters of HBV-ACLF patients.

The platelet count of HBV-ACLF patients receiving PE-based NB-ALSS therapy continued to decrease and was significantly lower than that of patients in the SMT group. This phenomenon may be related to thrombocytopenia caused by the use of heparin (46, 47). Therefore, platelet changes and the coagulation function of ACLF patients receiving PE-based NB-ALSS therapy should be closely observed in the clinic. It is suggested that HBV-ACLF patients with platelet counts $<50 \times 10^9$/L should be given low molecular weight heparin or citrate as an anticoagulant during the PE-based NB-ALSS process to avoid heparin-induced thrombocytopenia.

In this study, the median PE-based NB-ALSS therapy frequency was 2, and multivariate Cox regression analysis showed that the frequency of the therapy was the only independent protective factor for 28-day and 90-day prognosis in HBV-ACLF patients treated with this modality. This result suggests that the more PE-based NB-ALSS therapy is administered, the better is the prognosis of HBV-ACLF patients. Surprisingly, the timing of PE-based NB-ALSS therapy in HBV-ACLF patients and the volume of FFP used per session were not significantly associated with prognosis. As most of the patients in this study received PE-based NB-ALSS therapy within 1 week after admission and the FFP volume used for each session was ∼2 L for the vast majority of patients, we were unable to evaluate the impact of the timing and FFP volume on the efficacy of the therapy. In a multicenter prospective study by Larsen FS et al., high-volume (8–12 L) PE therapy once daily for 3 consecutive days significantly improved outcomes in patients with ALF (22), and Stahl et al.'s showed that low-volume (2–3 L) PE per day was as effective as high-volume PE for ALF patients (48). These results suggest that frequent PE therapy can improve the outcomes of those with liver failure when the FFP volume is no <2 L. In this study, the frequency of PE-based NB-ALSS therapy, rather than the FFP volume, exhibited a significant impact on the therapeutic effect of the therapy, which again confirmed the results of Stahl et al.'s study. Therefore, frequent low-volume PE-based NB-ALSS therapy is recommended for HBV-ACLF patients, potentially reducing both the amount of FFP and the risk of complications such as internal environment disorder and hemodynamic instability.

The CATCH-LIFE study consisted of two prospective multicenter cohorts of acute exacerbations of chronic liver disease established by 15 tertiary hospitals. The locations of these 15 tertiary hospitals cover 95% of the population distribution in China. The HBV-ACLF patients enrolled in this study were all from CATCH-LIFE, effectively reducing selection bias and rendering the research results reliable. Nevertheless, this study has some limitations. First, this was an observational study; the timing, frequency and FFP volume of PE therapy were not carried out uniformly, and the sample size was not large enough. The optimal timing, frequency and FFP volume of PE therapy still need to be determined through a multicenter randomized controlled study. Second, only HBV-ACLF patients were enrolled, and whether the results are applicable to ACLF patients with other etiologies needs further investigation. Third, as this study found that PE-based NB-ALSS therapy could not continuously improve the laboratory parameters of HBV-ACLF patients during the 28-day observation period, the clinical observation period should be extended to further clarify the improvement effect. Finally, this study did not detect levels of inflammatory cytokines before and after PE-based NB-ALSS treatment, and thus the mechanism of PE-based NB-ALSS was not examined. Fortunately, proteomics and metabolomics studies are being conducted using blood samples from this cohort, and the results may clarify the mechanism of PE-based NB-ALSS as ACLF therapy.

In conclusion, this study showed that PE-based NB-ALSS therapy significantly improves the short-term (28/90-day and 1-year) outcomes of patients with HBV-ACLF, and that patients with EASL-ACLF grade 2 or MELD scores of 30–40 are more likely to benefit, thereby reducing their dependence on LT. Moreover, increasing the frequency of PE therapy may provide greater benefits to patients with HBV-ACLF.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the Chinese Chronic Liver Failure (CLIF) Consortium, available at: aclf_group@163.com.

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

Y-yC and Z-jM were responsible for the study concept, design, and drafted the manuscript. HL (2nd author), X-bW, YH, Z-jM, Y-hG, Z-pQ, FL, X-bL, JS, HL (13th author), S-yW, and Y-hZ performed the data acquisition. All authors had access to the data and a role in writing the manuscript, offered critical revision, and approved the final draft of the manuscript.

CHINESE (ACUTE ON) CHRONIC LIVER FAILURE CONSORTIUM (CH-CLIF. C)

Department of Gastroenterology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University—Shan Yin, Wenyi Gu, Yan Zhang, Tongyu Wang, Dandan Wu, Fuchen Dong, Bo Zeng, Liuying Chen, Shijin Wang;
Centre of Integrative Medicine, Beijing Ditan Hospital, Capital Medical University—Qun Zhang, Yixin Hou, Yuxin Li, Yunyi Huang;
Department of Infectious Diseases, Southwest Hospital, Third Military Medical University (Army Medical University)—Shuning Sun, Wenting Tan, Xiaomei Xiang, Yunjie Dan, Guohong Deng;
Department of Infectious Disease, Hunan Key Laboratory of Viral Hepatitis, Xiangya Hospital, Central South University—Jun Chen, Chengjin Liao, Xiaoxiao Liu;
Department of Infectious Diseases, Institute of Infection and Immunology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology—Jing Liu, Ling Xu, Shue Xiong, Yan Xiong, Congcong Zou;
Hepatology Unit, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University—Jinjun Chen, Congyan Zhu;
Department of Hepatology, First Hospital of Jilin University—Chang Jiang, Xiaoyu Wen, Na Gao, Chunyan Liu;
Department of Infectious Disease, Taihe Hospital, Hubei University of Medicine—Qing Lei, Sen Luo;
Department of Infectious Disease, The First Hospital of Zhejiang University—Haotang Ren; Department of Liver Intensive Care Unit, Shanghai Public Health Clinical Centre, Fudan University—Xue Mei, Jiefei Wang, Liujuan Ji;
Department of Infectious Diseases and Hepatology, Second Hospital of Shandong University—Tao Li, Xuanqiong Fang, Jing Li, Ziyu Wang;
Liver Disease Centre, First Affiliated Hospital of Xinjiang Medical University—Rongjiong Zheng, Fangrong Jie, Nan Li;
Department of Infectious Disease, Henan Provincial People's Hospital—Huiming Jin;
Infectious Disease Center, Affiliated Hospital of Logistics University of People's Armed Police Force—Hai Li, Qing Zhang, Xuequn Zheng;
Department of Infectious Disease, Fuzhou General Hospital of Nanjing Military Command—Shaoyang Wang, Taofa Lin.

FUNDING

This work was supported by the National Science and Technology Major Project (Grant Nos. 2018ZX10723203 and 2018ZX10302206), the Foundation for Innovative Research Groups of Hubei Provincial Natural Science Foundation (2018CFA031), Hubei Province's Outstanding Medical Academic Leader Program, Project of Hubei University of Medicine (FDFR201902, 2020XGFYZR05, and YC2019006), and Scientific and Technological Project of Shiyan City (20Y08).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.779744/full#supplementary-material

REFERENCES

1. Sarin SK, Choudhury A. Acute-on-chronic liver failure: terminology, mechanisms and management. *Nat Rev Gastroenterol Hepatol.* (2016) 13:131–49. doi: 10.1038/nrgastro.2015.219
2. Zhang S, Wang F, Zhang Z. Current advances in the elimination of hepatitis B in China by 2030. *Front Med.* (2017) 11:490–501. doi: 10.1007/s11684-017-0598-4
3. Xie GJ, Zhang HY, Chen Q, Liu HM, You JP, Yang S, et al. Changing etiologies and outcome of liver failure in Southwest China. *Virol J.* (2016) 13:89. doi: 10.1186/s12985-016-0536-0
4. Wiesner R, Edwards E, Freeman R, Harper A, Kim R, Kamath P, et al. Model for end-stage liver disease (MELD) and allocation of donor livers. *Gastroenterology.* (2003) 124:91–6. doi: 10.1053/gast.2003.50016
5. Mezzano G, Juanola A, Cardenas A, Mezey E, Hamilton JP, Pose E, et al. Global burden of disease: acute-on-chronic liver failure, a systematic review and meta-analysis. *Gut.* (2021) 2021:gutjnl-2020-322161. doi: 10.1136/gutjnl-2020-322161
6. Wu T, Li J, Shao L, Xin J, Jiang L, Zhou Q, et al. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure. *Gut.* (2018) 67:2181–91. doi: 10.1136/gutjnl-2017-314641
7. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology.* (2013) 144:1426–37. doi: 10.1053/j.gastro.2013.02.042
8. Shi Y, Yang Y, Hu Y, Wu W, Yang Q, Zheng M, et al. Acute-on-chronic liver failure precipitated by hepatic injury is distinct from that precipitated by extrahepatic insults. *Hepatology.* (2015) 62:232–42. doi: 10.1002/hep.27795
9. Li H, Chen LY, Zhang NN, Li ST, Zeng B, Pavesi M, et al. Characteristics, diagnosis and prognosis of acute-on-chronic liver failure in cirrhosis associated to hepatitis B. *Sci Rep.* (2016) 6:25487. doi: 10.1038/srep25487
10. Li J, Liang X, Jiang J, Yang L, Xin J, Shi D, et al. PBMC transcriptomics identifies immune-metabolism disorder during the development of HBV-ACLF. *Gut.* (2021) 2021:gutjnl-2020-323395. doi: 10.1136/gutjnl-2020-323395
11. Wiest R, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. *J Hepatol.* (2014) 60:197–209. doi: 10.1016/j.jhep.2013.07.044
12. Alexopoulou A, Agiasotelli D, Vasilieva LE, Dourakis SP. Bacterial translocation markers in liver cirrhosis. *Ann Gastroenterol.* (2017) 30:486–97. doi: 10.20524/aog.2017.0178
13. Trebicka J, Bork P, Krag A, Arumugam M. Utilizing the gut microbiome in decompensated cirrhosis and acute-on-chronic liver failure. *Nat Rev Gastroenterol Hepatol.* (2021) 18:167–80. doi: 10.1038/s41575-020-00376-3
14. Moreau R, Claria J, Aguilar F, Fenaille F, Lozano JJ, Junot C, et al. Blood metabolomics uncovers inflammation-associated mitochondrial dysfunction as a potential mechanism underlying ACLF. *J Hepatol.* (2020) 72:688–701. doi: 10.1016/j.jhep.2019.11.009
15. Arroyo V, Angeli P, Moreau R, Jalan R, Claria J, Trebicka J, et al. The systemic inflammation hypothesis: towards a new paradigm of acute

decompensation and multiorgan failure in cirrhosis. *J Hepatol.* (2021) 74:670–85. doi: 10.1016/j.jhep.2020.11.048
16. Casulleras M, Zhang IW, Lopez-Vicario C, Claria J. Leukocytes, systemic inflammation and immunopathology in acute-on-chronic liver failure. *Cells.* (2020) 9:122632. doi: 10.3390/cells9122632
17. Trebicka J, Fernandez J, Papp M, Caraceni P, Laleman W, Gambino C, et al. The PREDICT study uncovers three clinical courses of acutely decompensated cirrhosis that have distinct pathophysiology. *J Hepatol.* (2020) 73:842–54. doi: 10.1016/j.jhep.2020.06.013
18. Kim WR, Lake JR, Smith JM, Skeans MA, Schladt DP, Edwards EB, et al. OPTN/SRTR 2013 annual data report: liver. *Am J Transplant.* (2015) 15(Suppl.2):1–28. doi: 10.1111/ajt.13197
19. Israni AK, Zaun D, Hadley N, Rosendale JD, Schaffhausen C, McKinney W, et al. OPTN/SRTR 2018 annual data report: deceased organ donation. *Am J Transplant.* (2020) 20(Suppl.s1):509–41. doi: 10.1111/ajt.15678
20. Chen T, He Y, Liu X, Yan Z, Wang K, Liu H, et al. Nucleoside analogues improve the short-term and long-term prognosis of patients with hepatitis B virus-related acute-on-chronic liver failure. *Clin Exp Med.* (2011) 12:159–64. doi: 10.1007/s10238-011-0160-7
21. Sun LJ Yu JW, Zhao YH, Kang P, Li SC. Influential factors of prognosis in lamivudine treatment for patients with acute-on-chronic hepatitis B liver failure. *J Gastroenterol Hepatol.* (2010) 25:583–90. doi: 10.1111/j.1440-1746.2009.06089.x
22. Larsen FS, Schmidt LE, Bernsmeier C, Rasmussen A, Isoniemi H, Patel VC, et al. High-volume plasma exchange in patients with acute liver failure: an open randomised controlled trial. *J Hepatol.* (2016) 64:69–78. doi: 10.1016/j.jhep.2015.08.018
23. Nakamura T, Ushiyama C, Suzuki S, Shimada N, Ebihara I, Suzaki M, et al. Effect of plasma exchange on serum tissue inhibitor of metalloproteinase 1 and cytokine concentrations in patients with fulminant hepatitis. *Blood Purif.* (2000) 18:50–4. doi: 10.1159/000014407
24. Nakae H, Yonekawa T, Narita K, Endo S. Are proinflammatory cytokine concentrations reduced by plasma exchange in patients with severe acute hepatic failure? *Res Commun Mol Pathol Pharmacol.* (2001) 109:65–72.
25. Yue-Meng W, Yang LH, Yang JH, Xu Y, Yang J, Song GB. The effect of plasma exchange on entecavir-treated chronic hepatitis B patients with hepatic decompensation and acute-on-chronic liver failure. *Hepatol Int.* (2016) 10:462–9. doi: 10.1007/s12072-015-9667-4
26. Yu JW, Sun LJ, Zhao YH, Li SC. Prediction value of model for end-stage liver disease scoring system on prognosis in patients with acute-on-chronic hepatitis B liver failure after plasma exchange and lamivudine treatment. *J Gastroenterol Hepatol.* (2008) 23:1242–9. doi: 10.1111/j.1440-1746.2008.05484.x
27. Cheng YL, Chang CH, Chen WT, Tsai MH, Lee WC, Tu KH, et al. Prognostic factors and treatment effect of standard-volume plasma exchange for acute and acute-on-chronic liver failure: a single-center retrospective study. *Transfus Apher Sci.* (2018) 57:537–43. doi: 10.1016/j.transci.2018.05.030
28. Hung YM, Hung GC, Hsu PI, Hung SY, Chou KJ, Chung HM. Short-term survival advantage after plasma exchange in the treatment of acute on chronic liver failure or acute liver failure. *Clinical Intensive Care.* (2011) 15:93–9. doi: 10.3109/09563070400003714
29. Stahl K, Busch M, Fuge J, Schneider A, Manns MP, Seeliger B, et al. Therapeutic plasma exchange in acute on chronic liver failure. *J Clin Apher.* (2020) 35:316–27. doi: 10.1002/jca.21799
30. Guo X, Wu F, Guo W, Zhang J, Yang Y, Lu Y, et al. Comparison of plasma exchange, double plasma molecular adsorption system, and their combination in treating acute-on-chronic liver failure. *J Int Med Res.* (2020) 48:300060520932053. doi: 10.1177/0300060520932053
31. Tan EX, Wang MX, Pang J, Lee GH. Plasma exchange in patients with acute and acute-on-chronic liver failure: a systematic review. *World J Gastroenterol.* (2020) 26:219–45. doi: 10.3748/wjg.v26.i2.219
32. Ocskay K, Kanjo A, Gede N, Szakacs Z, Par G, Eross B, et al. Uncertainty in the impact of liver support systems in acute-on-chronic liver failure: a systematic review and network meta-analysis. *Ann Intens Care.* (2021) 11:10. doi: 10.1186/s13613-020-00795-0
33. Qiao L, Wang X, Deng G, Huang Y, Chen J, Meng Z, et al. Cohort profile: a multicentre prospective validation cohort of the Chinese Acute-on-Chronic Liver Failure (CATCH-LIFE) study. *BMJ Open.* (2021) 11:e037793. doi: 10.1136/bmjopen-2020-037793
34. Gu WY, Xu BY, Zheng X, Chen J, Wang XB, Huang Y, et al. Acute-on-chronic liver failure in China: rationale for developing a patient registry and baseline characteristics. *Am J Epidemiol.* (2018) 187:1829–39. doi: 10.1093/aje/kwy083
35. Malinchoc M, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology.* (2000) 31:864–71. doi: 10.1053/he.2000.5852
36. Jalan R, Yurdaydin C, Bajaj JS, Acharya SK, Arroyo V, Lin HC, et al. Toward an improved definition of acute-on-chronic liver failure. *Gastroenterology.* (2014) 147:4–10. doi: 10.1053/j.gastro.2014.05.005
37. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *ACCP/SCCM Consensus Conference Committee American College of Chest Physicians/Society of Critical Care Medicine Chest.* (1992) 101:1644–55. doi: 10.1378/chest.101.6.1644
38. Yang Z, Zhang Z, Cheng Q, Chen G, Li W, Ma K, et al. Plasma perfusion combined with plasma exchange in chronic hepatitis B-related acute-on-chronic liver failure patients. *Hepatol Int.* (2020) 14:491–502. doi: 10.1007/s12072-020-10053-x
39. Gerth HU, Pohlen M, Tholking G, Pavenstadt H, Brand M, Husing-Kabar A, et al. Molecular adsorbent recirculating system can reduce short-term mortality among patients with acute-on-chronic liver failure-a retrospective analysis. *Crit Care Med.* (2017) 45:1616–24. doi: 10.1097/CCM.0000000000002562
40. Wang FS, Fan JG, Zhang Z, Gao B, Wang HY. The global burden of liver disease: the major impact of China. *Hepatology.* (2014) 60:2099–108. doi: 10.1002/hep.27406
41. Xia Q, Dai X, Huang J, Xu X, Yang Q, Liu X, et al. A single-center experience of non-bioartificial liver support systems among Chinese patients with liver failure. *Int J Artif Organs.* (2014) 37:442–54. doi: 10.5301/ijao.5000341
42. Chen J-J, Huang J-R, Yang Q, Xu X-W, Liu X-L, Hao S-R, et al. Plasma exchange-centered artificial liver support system in hepatitis B virus-related acute-on-chronic liver failure: a nationwide prospective multicenter study in China. *Hepatobiliary Pancreatic Dis Int.* (2016) 15:275–81. doi: 10.1016/S1499-3872(16)60084-X
43. Yao J, Li S, Zhou L, Luo L, Yuan L, Duan Z, et al. Therapeutic effect of double plasma molecular adsorption system and sequential half-dose plasma exchange in patients with HBV-related acute-on-chronic liver failure. *J Clin Apher.* (2019) 34:392–8. doi: 10.1002/jca.21690
44. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. *Hepatol Int.* (2019) 13:353–90. doi: 10.1007/s12072-019-09946-3
45. Maiwall R, Bajpai M, Choudhury AK, Kumar A, Sharma MK, Duan Z, et al. Therapeutic plasma-exchange improves systemic inflammation and survival in acute-on-chronic liver failure: a propensity-score matched study from AARC. *Liver Int.* (2021) 41:1083–96. doi: 10.1111/liv.14806
46. Warkentin TE, Anderson JA. How i treat patients with a history of heparin-induced thrombocytopenia. *Blood.* (2016) 128:348–59. doi: 10.1182/blood-2016-01-635003
47. Krauel K, Preusse P, Warkentin TE, Trabhardt C, Brandt S, Jensch I, et al. Fibronectin modulates formation of PF4/heparin complexes and is a potential factor for reducing risk of developing HIT. *Blood.* (2019) 133:978–89. doi: 10.1182/blood-2018-05-850370

48. Stahl K, Hadem J, Schneider A, Manns MP, Wiesner O, Schmidt BMW, et al. Therapeutic plasma exchange in acute liver failure. *J Clin Apher.* (2019) 34:589–97. doi: 10.1002/jca.21737

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 18 November 2021
doi: 10.3389/fmed.2021.762291

Increased INR Values Predict Accelerating Deterioration and High Short-Term Mortality Among Patients Hospitalized With Cirrhosis or Advanced Fibrosis

Ying Wang[1,2†], Fuchen Dong[2,3†], Shuning Sun[2,4†], Xianbo Wang[2,5†], Xin Zheng[2,6†], Yan Huang[2,7†], Beiling Li[2,8†], Yanhang Gao[2,9], Zhiping Qian[2,10], Feng Liu[2,11], Xiaobo Lu[2,12], Junping Liu[2,13], Haotang Ren[2,14], Yubao Zheng[2,15], Huadong Yan[2,16], Guohong Deng[2,4], Liang Qiao[2,3], Yan Zhang[2,3], Wenyi Gu[2,3], Xiaomei Xiang[2,4], Yi Zhou[2,4], Baoyan Xu[2,4], Yixin Hou[2,5], Qun Zhang[2,5], Yan Xiong[2,6], Congcong Zou[2,6], Jun Chen[2,7], Zebing Huang[2,7], Xiuhua Jiang[2,8], Tingting Qi[2,8], Sen Luo[1,2], Yuanyuan Chen[1,2], Na Gao[2,9], Chunyan Liu[2,9], Wei Yuan[2,10], Xue Mei[2,10], Jing Li[2,11], Tao Li[2,11], Rongjiong Zheng[2,12], Xinyi Zhou[2,12], Weituo Zhang[2,17], Hai Li[2,3] and Zhongji Meng[1,2]**

[1] Department of Infectious Disease, Hubei Clinical Research Center for Precise Diagnosis and Treatment of Liver Cancer, Taihe Hospital, Hubei University of Medicine, Shiyan, China, [2] Chinese Chronic Liver Failure Consortium, China, [3] Department of Gastroenterology, School of Medicine, Ren Ji Hospital, Shanghai Jiao Tong University, Shanghai, China, [4] Department of Infectious Diseases, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China, [5] Center of Integrative Medicine, Beijing Ditan Hospital, Capital Medical University, Beijing, China, [6] Department of Infectious Diseases, Tongji Medical College, Institute of Infection and Immunology, Union Hospital, Huazhong University of Science and Technology, Wuhan, China, [7] Hunan Key Laboratory of Viral Hepatitis, Department of Infectious Diseases, Xiangya Hospital, Central South University, Changsha, China, [8] Hepatology Unit, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China, [9] Department of Hepatology, The First Hospital of Jilin University, Jilin, China, [10] Department of Liver Intensive Care Unit, Shanghai Public Health Clinical Centre, Fudan University, Shanghai, China, [11] Department of Infectious Diseases and Hepatology, The Second Hospital of Shandong University, Jinan, China, [12] Infectious Disease Center, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, China, [13] Department of Infectious Diseases, Henan Provincial People's Hospital, Henan, China, [14] State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Disease, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, [15] Department of Infectious Diseases, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, [16] Department of Infectious Diseases, Hwamei Hospital, Ningbo No. 2 Hospital, University of Chinese Academy of Sciences, Ningbo, China, [17] Clinical Research Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Background and Objective: An increase in the international normalized ratio (INR) is associated with increased mortality in patients with cirrhosis and other chronic liver diseases, while little is known about the quantitative relationship. This study aimed to investigate the quantitative relationship between the INR and short-term prognosis among patients hospitalized with cirrhosis or advanced fibrosis and to evaluate the role of the INR as a risk factor for short-term liver transplant (LT)-free mortality in these patients.

Patients and Methods: This study prospectively analyzed multicenter cohorts established by the Chinese Acute-on-Chronic Liver Failure (CATCH-LIFE) study. Cox regression was used to describe the relationship between the INR and independent risk factors for short-term LT-free mortality. Forest plots were used in the subgroup analysis. Generalized additive models (GAMs) and splines were used to illustrate the quantitative curve relationship between the INR and the outcome and inflection point on the curve.

OPEN ACCESS

Edited by:
Angel Lanas,
University of Zaragoza, Spain

Reviewed by:
Zhujun Cao,
Shanghai Jiao Tong University, China
Enqiang Chen,
Sichuan University, China
Qiao Yang,
Sir Run Run Shaw Hospital, China

***Correspondence:**
Zhongji Meng
zhongji.meng@163.com
Hai Li
haili_17@126.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 21 August 2021
Accepted: 18 October 2021
Published: 18 November 2021

Citation:
Wang Y, Dong F, Sun S, Wang X, Zheng X, Huang Y, Li B, Gao Y, Qian Z, Liu F, Lu X, Liu J, Ren H, Zheng Y, Yan H, Deng G, Qiao L, Zhang Y, Gu W, Xiang X, Zhou Y, Xu B, Hou Y, Zhang Q, Xiong Y, Zou C, Chen J, Huang Z, Jiang X, Qi T, Luo S, Chen Y, Gao N, Liu C, Yuan W, Mei X, Li J, Li T, Zheng R, Zhou X, Zhang W, Li H and Meng Z (2021) Increased INR Values Predict Accelerating Deterioration and High Short-Term Mortality Among Patients Hospitalized With Cirrhosis or Advanced Fibrosis. Front. Med. 8:762291. doi: 10.3389/fmed.2021.762291

Results: A total of 2,567 patients with cirrhosis and 924 patients with advanced fibrosis were included in the study. The 90-day LT-free mortality of patients with cirrhosis and advanced fibrosis was 16.7% (428/2,567) and 7.5% (69/924), respectively. In the multivariable Cox regression analysis, the increase in the INR was independently associated with the risk of 90-day LT-free mortality both in patients with cirrhosis (HR, 1.06; 95% CI, 1.04–1.07, $p < 0.001$) and in patients with advanced fibrosis (HR, 1.09; 95% CI, 1.06–1.12, $p < 0.001$). An INR of 1.6/1.7 was found to be the starting point of coagulation dysfunction with a rapid increase in mortality in patients with cirrhosis or in patients with advanced fibrosis, respectively. A 28-day LT-free mortality of 15% was associated with an INR value of 2.1 in both cirrhosis and advanced fibrosis patients.

Conclusions: This study was the first to quantitatively describe the relationship between the INR and short-term LT-free mortality in patients with cirrhosis or advanced fibrosis. The starting points of INR indicating the rapid increase in mortality and the unified cutoff value of coagulation failure in cirrhosis and advanced fibrosis, will help clinicians accurately recognize early disease deterioration.

Keywords: acute on chronic liver failure (ACLF), cirrhosis, advanced fibrosis, international normalized ratio (INR), short-term prognosis

INTRODUCTION

Cirrhosis and other chronic liver diseases are the main causes of death, affecting 1.5 billion people worldwide (1, 2) and accounting for 1.3 million deaths every year (3). Most chronic liver disease patients will remain in a stable state, while upon acute liver injury (ALI), they may progress to acute decompensation or even organ failure; the latter is defined as acute-on-chronic liver failure (ACLF) and is characterized by a high short-term mortality rate of over 50% (4, 5).

Most patients with liver cirrhosis or other chronic liver diseases hospitalized with ALI have coagulation disorders, which are associated with significantly prolonged prothrombin time (PT) and an increased international normalized ratio (INR) (6). As an indicator of severe liver injury, the INR has been included in the diagnostic criteria of ACLF by the European Association for The Study of the Liver (EASL) and the Asian-Pacific Association for the Study of the Liver (APASL) (7–9). The INR has also been incorporated in scoring systems such as the model for end-stage liver disease (MELD), MELD-Na, and chronic liver failure-sequential organ failure assessment (CLIF-SOFA) for assessing the severity of cirrhosis and liver failure (10–12).

However, the cutoff value of the INR for the diagnosis of ACLF has long been controversial in the East and the West (13). The APASL considers that an INR $\geq$ 1.5 and a total bilirubin (TB) $\geq$ 5 mg/dL in patients with cirrhosis and noncirrhotic chronic liver disease were important indicators for the diagnosis of ACLF. The INR cutoff value used by the APASL in the diagnostic criteria of ACLF is based on the definition of acute liver failure (9, 14–16). The EASL considers an INR $\geq$2.5 in patients with cirrhosis as the cutoff value for coagulation failure in the diagnostic criteria of ACLF (7). It is unclear whether coagulation failure in cirrhosis and advanced fibrosis can share the same cutoff value for the INR. Therefore, it is pivotal to explore the evidence-based cutoff values of the INR for coagulation failure in patients with cirrhosis and advanced fibrosis, and this is important to unify the thresholds of coagulation failure among the East and West.

This study was based on a large, multicenter, prospective cohort of patients in areas where hepatitis B virus (HBV) is highly endemic, and this study included both patients with cirrhosis and patients with advanced fibrosis (17, 18). This study aimed to investigate the quantitative relationship between the INR and the short-term (28 /90-day) LT-free mortality in patients with cirrhosis and advanced fibrosis separately and to provide evidence for establishing a reliable INR cutoff value for the diagnosis of coagulation failure.

PATIENTS AND METHODS

Patients

Patients with cirrhosis and other chronic liver diseases (3) hospitalized for acute decompensation (AD) (7) or acute liver injury (ALI) were enrolled in the Chinese Acute-on Chronic Liver Failure (CATCH-LIFE) study cohorts (NCT02457637 and NCT03641872) from 16 Chinese tertiary hospitals during the periods of January 2015–December 2016 and January 2018–December 2019 (17–19). Detailed inclusion and exclusion criteria have been published elsewhere (17–19). This study was approved by the Ethics Committee of Renji Hospital (the leading center of the CATCH-LIFE study), School of Medicine, Shanghai Jiao Tong University, Shanghai, China. Signed informed consent was obtained from all patients.

Cirrhosis was diagnosed based on computed tomography/magnetic resonance imaging, laboratory examination, and clinical symptoms (20). Cirrhosis without

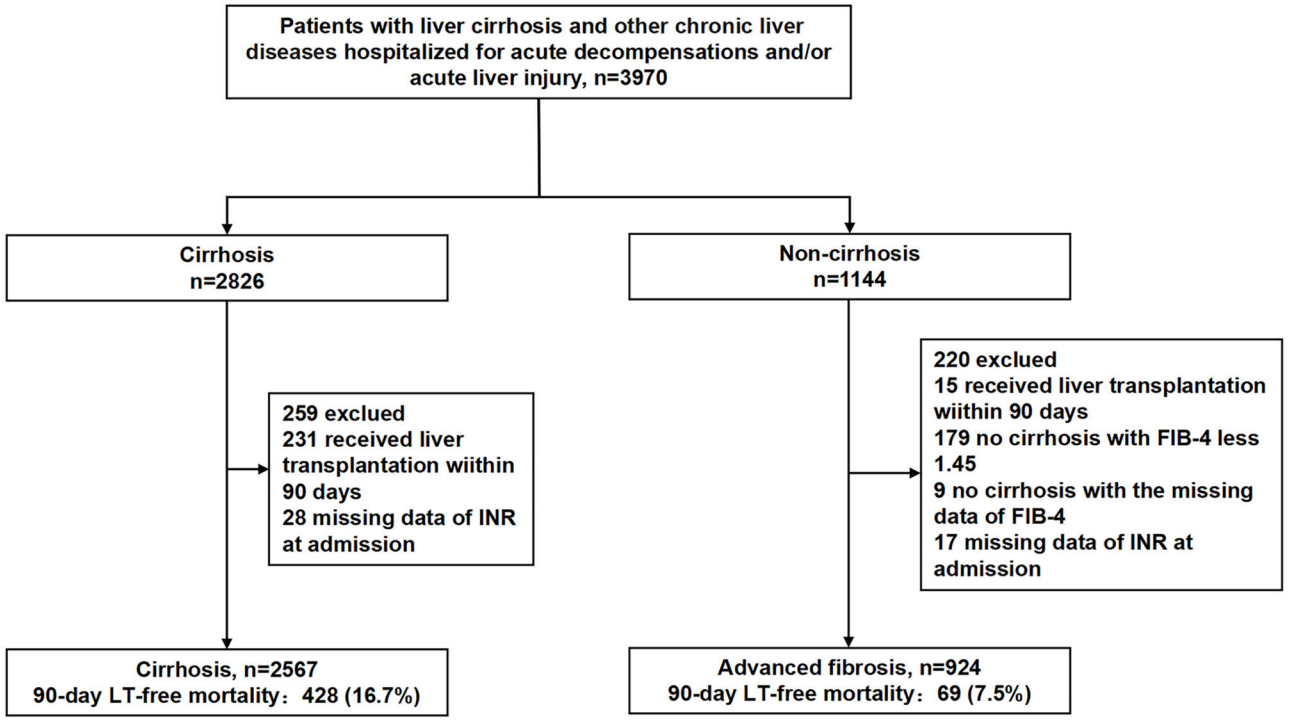


FIGURE 1 | Study participants. FIB-4, fibrosis-4 index; INR, international normalized ratio; LT, liver transplantation.

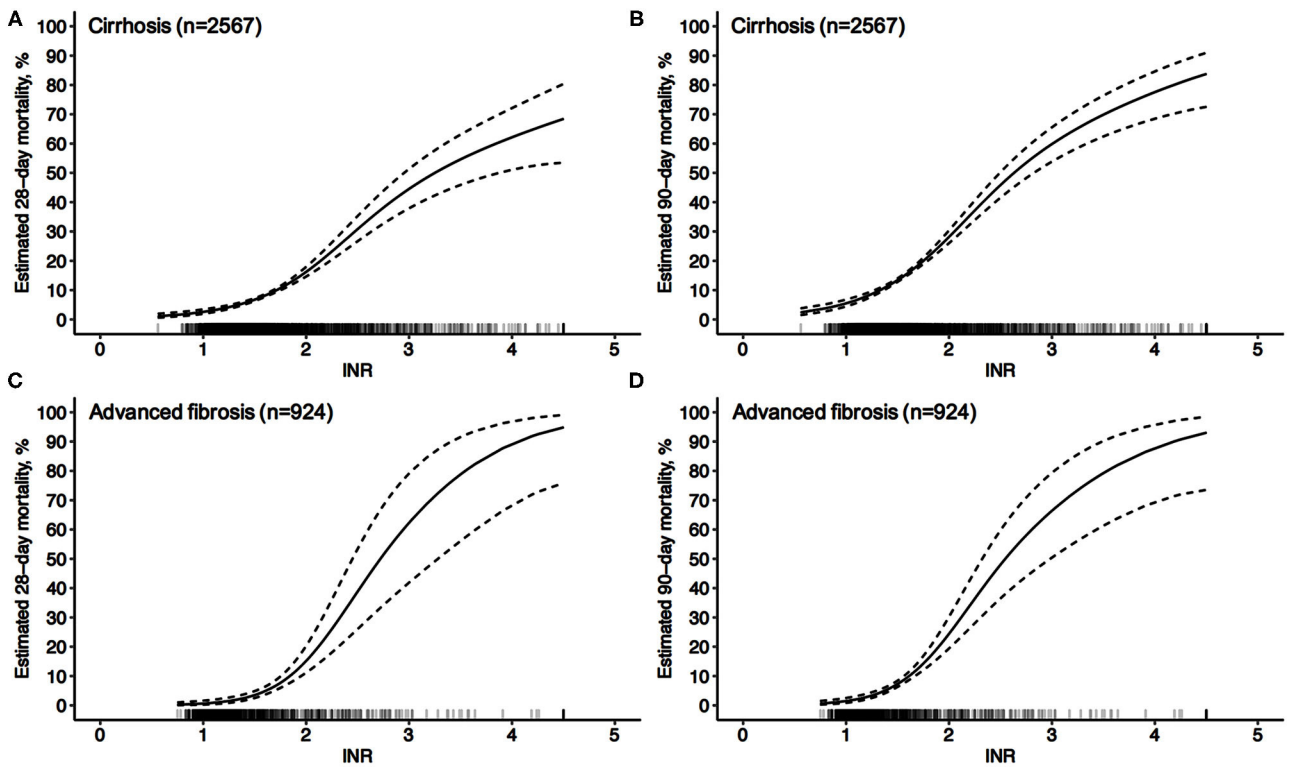


FIGURE 2 | Unadjusted probability of LT-free mortality by baseline INR in cirrhosis and advanced fibrosis. **(A,B)** 28-day **(A)** and 90-day **(B)** LT-free mortality by baseline INR in cirrhosis, **(C,D)** 28-day **(C)** and 90-day **(D)** LT-free mortality by baseline INR in advanced fibrosis.

prior AD was defined as cirrhotic patients who developed AD for the first time (7). Those with a prior history of AD were defined as cirrhotic patients with prior (one or more episodes) AD (7). Advanced fibrosis was defined as noncirrhotic patients who had a chronic liver disease history of at least 6 months and had FIB-4 scores over 1.45 (21).

Among the cirrhotic patients and the patients with advanced fibrosis, those who received liver transplant within 90 days, those with INR values missing values on admission, patients with FIB-4 $\leq$ 1.45 and patients with missing FIB-4 values in noncirrhotic patients were not included in the analysis (**Figure 1**; **Supplementary Figure 1**).

Data Collection

The following demographic and clinical information was collected on admission: age, sex, etiology of the liver disease, acute decompensated events, laboratory parameters, clinical symptoms involved in Child-Turcotte-Pugh [CTP] (22), and CLIF-SOFA scores. Details about the data collected in this study can be found elsewhere (17, 18).

Outcomes

The primary and secondary endpoints of the study were 90 and 28-day liver transplant (LT)-free mortality, respectively.

Statistical Analysis

Data are presented as medians and the first and third quartiles for continuous variables and as frequencies (%) for categorical variables at baseline. A multivariate Cox proportional hazard (COXPH) model was used to analyze the correlation between the INR and 90-day LT-free mortality. Important risk factors and potential confounding factors were adjusted, and these included age, sex, and etiology of underlying chronic liver disease, overt ascites, gastrointestinal bleeding, bacterial infection, hepatic encephalopathy (HE) grades, TB and creatinine. The risk of 90-day LT-free mortality was expressed by continuous variables as

TABLE 1 | Baseline characteristics of patients with cirrhosis based on different INR groups at admission.

Characteristics	INR < 1.2 *n* = 468	1.2 ≤ INR < 1.5 *n* = 852	1.5 ≤ INR < 2.0 *n* = 724	2.0 ≤ INR < 2.5 *n* = 275	INR ≥ 2.5 *n* = 248
Demographic					
Age	54 [47,62]	53 [45,61]	50 [44,58]	49 [42,57]	51 [42,59]
Gender	287 (61.3)	609 (71.5)	562 (77.6)	218 (79.3)	197 (79.4)
Etiology					
Alcoholic	55 (11.8)	117 (13.7)	88 (12.2)	34 (12.4)	23 (9.3)
HBV	238 (50.9)	528 (62.0)	531 (73.3)	212 (77.1)	203 (81.9)
AIH	93 (19.9)	90 (10.6)	48 (6.6)	6 (2.2)	10 (4.0)
Others	82 (17.5)	117 (13.7)	57 (7.9)	23 (8.4)	12 (4.8)
Acute decompensation					
HE					
Grade0	446 (95.3)	774 (90.8)	645 (89.1)	239 (86.9)	187 (75.4)
Grade1	6 (1.3)	33 (3.9)	35 (4.8)	16 (5.8)	13 (5.2)
Grade2	14 (3.0)	31 (3.6)	31 (4.3)	13 (4.7)	30 (12.1)
Grade3	1 (0.2)	11 (1.3)	10 (1.4)	4 (1.5)	13 (5.2)
Grade4	1 (0.2)	3 (0.4)	3 (0.4)	3 (1.1)	5 (2.0)
Infection	71 (15.2)	203 (23.8)	218 (30.1)	86 (31.3)	112 (45.2)
Ascites	215 (45.9)	502 (58.9)	483 (66.7)	205 (74.5)	187 (75.4)
GI bleeding	113 (24.1)	217 (25.5)	131 (18.1)	32 (11.6)	15 (6.0)
Laboratory tests					
TB	1.5 [0.9,3.1]	2.2 [1.2,5.4]	5.0 [2.5,13.5]	13.6 [6.7,22.6]	20.8 [13.0,29.3]
INR	1.1 [1.0,1.2]	1.3 [1.3,1.4]	1.7 [1.6,1.8]	2.2 [2.1,2.4]	3.0 [2.7,3.5]
Cr	0.8 [0.6,0.9]	0.8 [0.6,1.0]	0.8 [0.6,1.0]	0.8 [0.7,1.0]	0.9 [0.7,1.2]
Scores					
MELD	9 [7,12]	12 [10,16]	18 [15,23]	25 [22,28]	31 [28,34]
CLIF-SOFA	2 [1,3]	4 [3,5]	6 [5,7]	7 [6,8]	7 [7,9]
Child-Pugh	8 [6,9]	8 [7,1]	10 [9,11]	11 [10,12]	12 [11,13]
Outcome					
28-day LT-free mortality	10 (2.1)	29 (3.4)	44 (6.1)	55 (20.0)	98 (39.5)
90-day LT-free mortality	23 (4.9)	65 (7.6)	105 (14.5)	98 (35.6)	137 (55.2)

HBV, hepatitis B virus; AIH, autoimmune hepatitis; HE, hepatic encephalopathy; GI bleeding, gastrointestinal bleeding; TB, total bilirubin; INR, international normalized ratio; Cr, creatinine; MELD, model for end-stage liver disease; CLIF-SOFA, chronic liver failure-sequential organ failure assessment; LT, liver transplantation.

the risk ratio (HR), which was calculated by each unit of the INR. In patients with cirrhosis, the INR was categorized into fine levels (<1.2, 1.2–1.5, 1.5–2.0, 2–2.5, and ≥2.5), and patients with an INR < 1.2 were used as a reference. In advanced fibrosis, the INR was categorized into 4 levels (<1.5, 1.5–2.0, 2.0–2.5, and >2.5), and patients with an INR < 1.5 were used as a reference. Forest plots were used for subgroup analysis.

The nonlinear relationship between the INR and 90-day mortality was plotted as an "INR-mortality correlation curve". The estimated mortality rates corresponding to the INR values in the curves and the independent effect of INR on mortality were shown by the confounding factors adjusted generalized additive model (GAM) (23). Spline (24) was used as a connecting function to select the GAM and smoothing parameters to optimize the Akaike information criterion. The second derivative of the INR to mortality was used to describe the nonlinear relationship (to obtain the maximum acceleration peak). The maximum acceleration point on the GAM curve was defined as the starting point of the INR for disease deterioration. Meanwhile, the INR value corresponding to 15% LT-free mortality within 28 days was considered the clinical cutoff value for coagulation failure (the definition from the CANONIC study) (7). Statistical analyses were performed using R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria) and MATLAB 2016b. A two-sided $p < 0.05$ was considered statistically significant.

RESULTS

Characteristics of the Patients

As shown in **Figure 1**, 2,567 patients with cirrhosis and 924 patients with advanced fibrosis were ultimately included in the analysis. The 90-day LT-free mortality of cirrhosis and advanced fibrosis were 16.7% (428/2,567) and 7.5% (69/924), respectively. In **Figure 2**, we depict the uncorrected INR in relation to the 28/90-day LT-free mortality using GAM. In cirrhotic patients and advanced fibrosis, as the INR increased,

TABLE 2 | Baseline characteristics of patients with advanced fibrosis based on different INR groups at admission.

Characteristics	INR < 1.2 *n* = 384	1.2 ≤ INR < 1.5 *n* = 242	1.5 ≤ INR < 2.0 *n* = 165	2.0 ≤ INR < 2.5 *n* = 75	INR ≥ 2.5 *n* = 85
Demographic					
Age	44 [35,53]	41 [33,49]	43 [34,50]	40 [34,49]	39 [37,42]
Gender	263 (68.5)	194 (80.2)	134 (81.2)	58 (77.3)	47 (81.0)
Etiology					
Alcoholic	14 (3.6)	10 (4.1)	3 (1.8)	1 (1.3)	3 (5.2)
HBV	278 (72.4)	213 (88.0)	153 (92.7)	74 (98.7)	52 (89.7)
AIH	45 (11.7)	9 (3.7)	6 (3.6)	0 (0.0)	1 (1.7)
Others	47 (12.2)	10 (4.1)	3 (1.8)	0 (0.0)	2 (3.4)
Acute decompensation					
HE					
Grade0	382 (99.5)	239 (98.8)	160 (97.0)	70 (93.3)	37 (63.8)
Grade1	2 (0.5)	3 (1.2)	3 (1.8)	4 (5.3)	4 (6.9)
Grade2	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)	9 (15.5)
Grade3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (12.1)
Grade4	0 (0.0)	0 (0.0)	1 (0.6)	1 (1.3)	1 (1.7)
Infection	23 (6.0)	20 (8.3)	24 (14.5)	21 (28.0)	19 (32.8)
Ascites	12 (3.1)	19 (7.9)	36 (21.8)	26 (34.7)	26 (44.8)
GI bleeding	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Laboratory tests					
TB	2.3 [1.2,7.2]	5.8 [2.2,12.8]	12.1 [7.2,19.4]	16.4 [12.3,23.7]	21.8 [15.4,25.9]
INR	1.1 [1.0,1.1]	1.3 [1.3,1.4]	1.7 [1.6,1.8]	2.2 [2.1,2.4]	2.9 [2.7,3.6]
Cr	0.8 [0.6,0.9]	0.8 [0.6,0.9]	0.8 [0.6,0.9]	0.8 [0.6,0.9]	0.8 [0.7,1.0]
Scores					
MELD	10 [8,14]	16 [12,19]	22 [2,24]	26 [25,27]	35 [28,36]
CLIF-SOFA	2 [1,3]	4 [3,6]	7 [6,7]	7 [7,7]	8 [7,9]
Child-Pugh	6 [5,7]	7 [6,8]	8 [8,9]	10 [9,11]	12 [12,13]
Outcome					
28-day LT-free mortality	0 (0.0)	1 (0.4)	4 (2.4)	11 (14.7)	24 (41.4)
90-day LT-free mortality	0 (0.0)	5 (2.1)	12 (7.3)	23 (30.7)	29 (50.0)

HBV, hepatitis B virus; AIH, autoimmune hepatitis; HE, hepatic encephalopathy; GI bleeding, gastrointestinal bleeding; TB, total bilirubin; INR, international normalized ratio; Cr, creatinine; MELD, model for end-stage liver disease; CLIF-SOFA, chronic liver failure-sequential organ failure assessment; LT, liver transplantation.

TABLE 3 | The unadjusted and adjusted hazard ratios of INR for 90-day transplantation-free mortality in patients with cirrhosis and advanced fibrosis.

INR	*n*	Death (%)	HR, 95% CI, *P*-value	HR, 95% CI, *P*-value	HR, 95% CI, *P*-value	HR, 95% CI, *P*-value
Cirrhosis			**Unadjusted**	**Adjusted***	**Adjusted****	**Adjusted*****
Continuous	2,567	428 (16.7)	1.10 (1.09–1.11), <0.001	1.11 (1.10–1.12), <0.001	1.09 (1.08–1.10), <0.001	1.06 (1.04–1.07), <0.001
Categorical						
[0∼1.2)	468	23 (4.9)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
[1.2∼1.5)	852	65 (7.6)	1.49 (0.93–2.39), 0.102	1.53 (0.95–2.46), 0.082	1.34 (0.86–2.24), 0.18	1.27 (0.79–2.05), 0.324
[1.5∼2.0)	724	105 (14.5)	2.79 (1.77–4.38), <0.001	2.97 (1.88–4.67), <0.001	2.53 (1.60–3.99), <0.001	1.82 (1.14–2.90), 0.011
[2.0∼2.5)	275	98 (35.6)	7.94 (5.04–12.51), <0.001	8.99 (5.68–14.22), <0.001	7.39 (4.64–11.76), <0.001	3.78 (2.30–6.07), <0.001
[2.5, ∼)	248	137 (55.2)	13.57 (8.72–21.11), <0.001	15.70 (10.03–24.58), <0.001	10.69 (6.72–17.00), <0.001	4.45 (2.72–7.28), <0.001
Advanced fibrosis			**Unadjusted**	**Adjusted***	**Adjusted$^{\Delta}$**	**Adjusted$^{\Delta\Delta}$**
Continuous	924	69 (7.5)	1.14 (1.12–1.16), <0.001	1.14 (1.12–1.16), <0.001	1.11 (1.08–1.14), <0.001	1.09 (1.06–1.12), <0.001
Categorical						
[0∼1.5)	626	5 (0.8)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
[1.5∼2.0)	165	12 (7.3)	9.81 (3.46–27.85), <0.001	10.33 (3.60–29.65), <0.001	8.13 (2.81–23.53), <0.001	6.35 (2.15–18.71), <0.001
[2.0∼2.5)	75	23 (30.7)	43.20 (16.42–113.66), <0.001	51.28 (18.96–138.75), <0.001	34.03 (12.27–94.41), <0.001	22.40 (7.70–65.16), <0.001
[2.5, ∼)	58	29 (50)	72.04 (27.85–186.33), <0.001	75.75 (28.86–198.85), <0.001	35.50 (12.18–103.50), <0.001	23.84 (7.88–72.16), <0.001

Adjusted, adjusted age, gender, etiology; Adjusted**, adjusted age, gender, etiology, HE grades, ascites, infection, gastrointestinal bleeding; Adjusted***, adjusted age, gender, etiology, HE grades, ascites, infection, gastrointestinal bleeding, TB and Cr; Adjusted$^{\Delta}$, adjusted age, gender, etiology, HE grades, ascites, infection; Adjusted$^{\Delta\Delta}$, adjusted age, gender, etiology, HE grades, ascites, infection, TB and Cr.*

the disease severity (MELD score, CLIF-SOFA score, and Child Pugh score) and the corresponding short-term LT-free mortality increased (**Tables 1**, **2**).

INR Is an Independent Risk Factor for 90-Day LT-Free Mortality

As shown in **Table 3**, when the INR was used as a continuous variable, it was an independent risk factor for 90-day LT-free mortality in both cirrhotic and advanced fibrosis patients (**Table 3**). The analysis of the categorical variables showed that when the INR > 1.5, the elevated INR had a significantly greater impact on mortality than patients with an INR < 1.5 in both cirrhosis and advanced fibrosis patients. The INR was an independent risk factor for 90-day LT-free mortality without the interaction of most indicators except hepatic encephalopathy 3∼4, a phenomenon existed that was consistent in patients with cirrhosis and advanced fibrosis (**Figures 3**, **4**). The results of the 28-day univariate and multivariable analyses are also shown in **Supplementary Table 1**.

INR = 1.6 and 1.7 Are the Starting Points of INR for Acute Disease Deterioration in Cirrhosis and Advanced Fibrosis, Respectively

Through the peak of the second derivative, we found that the maximum acceleration point of the INR (INR = 1.6) corresponded to the most rapid increase in mortality on the GAM curve, which independently reflected the relationship between the INR and 90-day LT-free mortality (**Figures 5C,D**). The valley of the second derivative was when the INR = 2.6, which showed an INR of 1.6∼2.6 is the period with the most rapid increase in mortality. This suggests that an INR = 1.6 is the starting point of the INR for disease deterioration in cirrhosis. We further analyzed the relationship between the INR and 90-day LT-free mortality in patients with cirrhosis without prior AD and patients with cirrhosis with any prior AD. The trends of the two curves were similar, with starting points of INR = 1.7 and 1.5 for disease deterioration, respectively, which were similar to the values in patients with cirrhosis (**Figures 5E–H**).

In patients with advanced fibrosis, the peak INR value of the second derivative was 1.7, and the valley INR value was 2.7, which indicates that mortality rises the fastest when the INR is between 1.7 and 2.7. Therefore, an INR value of 1.7 can be used as the starting point of INR for disease deterioration (**Figures 5A,B**).

INR = 2.1 Was Associated With 28-Day LT-Free Mortality of 15% Both in Patients With Cirrhosis and in Patients With Advanced Fibrosis

EASL defines organ failure as a 28-day LT-free mortality of 15%; therefore, the quantitative analysis found that an INR = 2.1 corresponded to an LT-free mortality of 15% within 28 days after admission in cirrhotic patients (**Figure 6**). An INR = 2.1 can be used as the clinical cutoff value for coagulation failure in patients with liver cirrhosis, with the corresponding multivariable adjusted 28/90-day LT-free mortality of 15 and 25.1%, respectively (**Table 4**), and 447 (17.4%) patients in our study exceeded this clinical cutoff of INR for coagulation failure. Similarly, in patients with advanced fibrosis, an INR = 2.1 was also found to correspond to a 28-day LT-free mortality of 15%, and 116 (12.6%) patients in our study exceeded this threshold, with corresponding 28/90-day LT-free mortality of 15 and 26.3%,

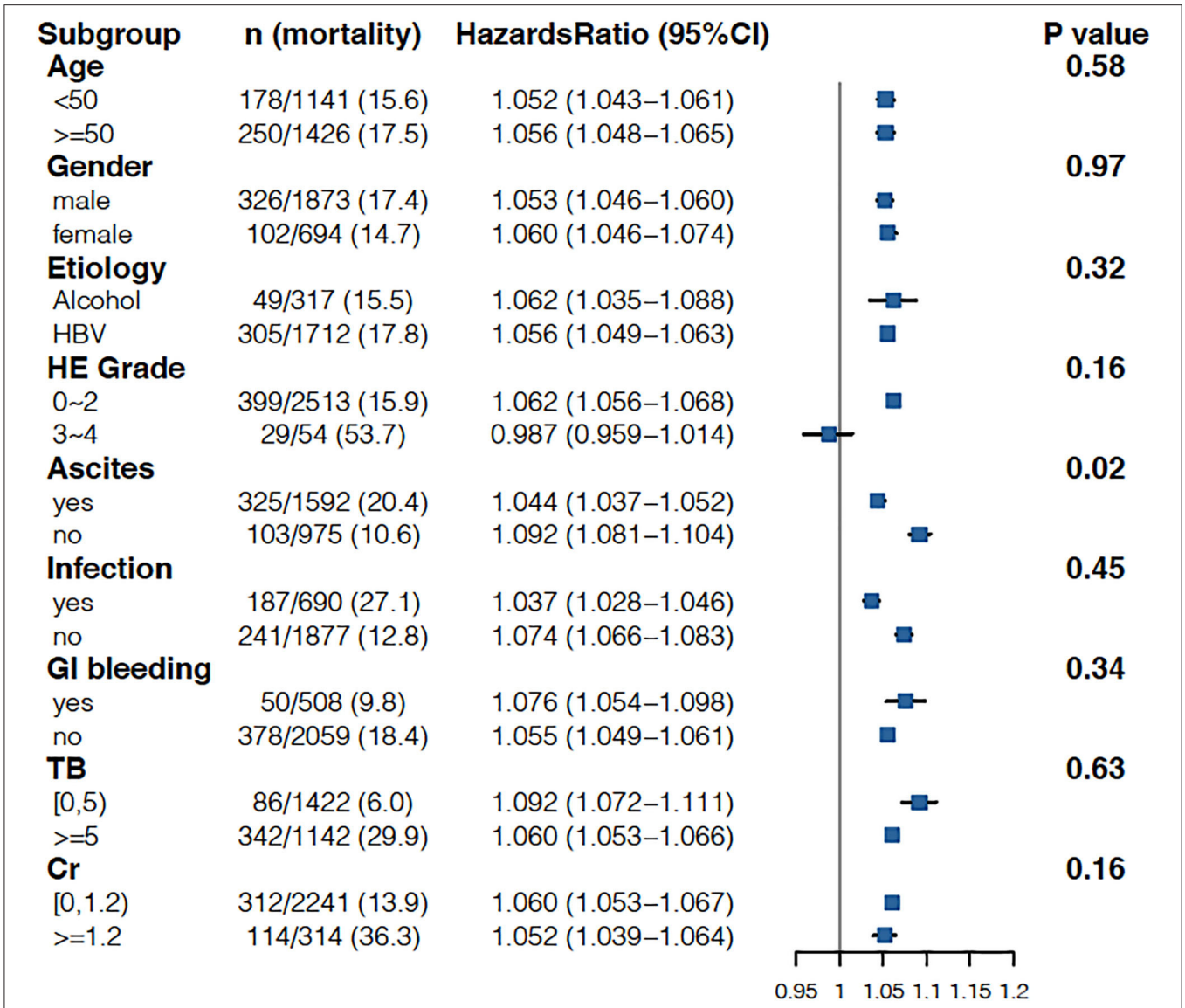


FIGURE 3 | Stratified analyses of adjusted risk of 90-day LT-free mortality in cirrhosis patients according to strata of baseline covariates INR. HBV, hepatitis B virus; HE, hepatic encephalopathy; GI bleeding, gastrointestinal bleeding; TB, total bilirubin; Cr, creatinine; INR, international normalized ratio.

respectively. Therefore, these values can be used as the clinical cutoff values of INR for coagulation failure.

The short-term LT-free mortality in patients with INR values between the starting point for disease deterioration and cutoff for coagulation failure was further analyzed. An INR of 1.6∼2.1 in the patients with cirrhosis was corresponded to 28/90-day LT-free mortality of 7.8 and 17.4%, respectively. Similarly, the INR value of 1.7∼2.1 in patients with advanced fibrosis was associated with a 28/90-day LT-free mortality of 3.3 and 7.8%, respectively (**Table 4**).

DISCUSSION

This study quantitatively described in detail the relationship between INR values and short-term LT-free mortality for the first time in patients with cirrhosis or advanced fibrosis through a multicenter, prospective cohort study. First, the results showed that the INR value was an independent risk factor for both 28- and 90-day LT-free mortality in patients with either cirrhosis or advanced liver fibrosis. In both patients with cirrhosis and in patients with advanced fibrosis, an INR $>$ 1.5 had a more significant impact on 90-day LT-free mortality than an INR $<$ 1.5. Second, we found for the first time the starting point of the INR that indicates acute disease deterioration (coagulation dysfunction) in a highly endemic area of HBV. Finally, when the INR $=$ 2.1, the 28-day LT-free mortality reached 15% in both patients with cirrhosis and patients with advanced fibrosis. Therefore, an INR $>$ 2.0 could be used as the cutoff of INR for the diagnosis of coagulation failure in either patients with cirrhosis or advanced fibrosis.

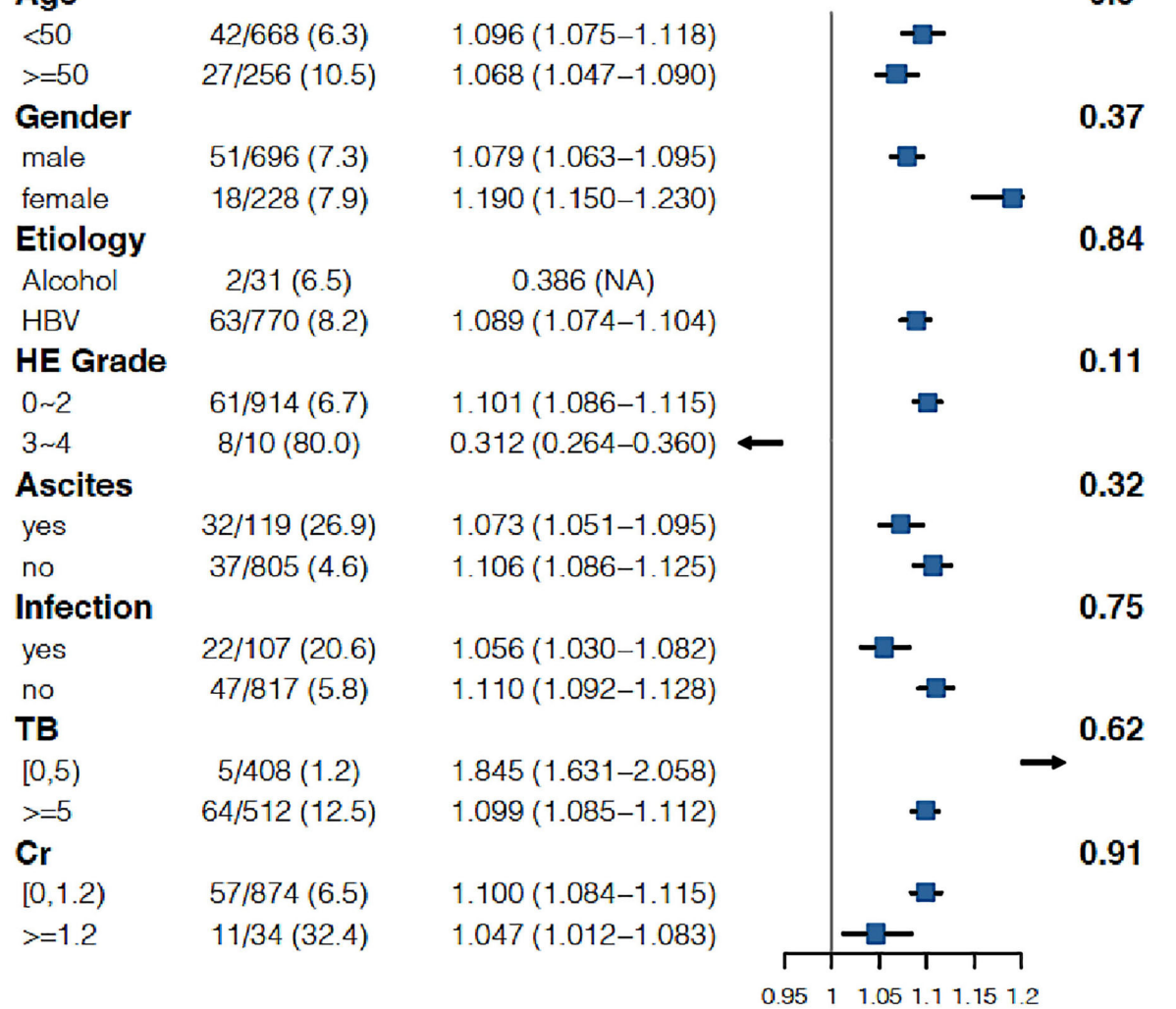


FIGURE 4 | Stratified analyses of adjusted risk of 90-day LT-free mortality in advanced fibrosis patients according to strata of baseline covariates INR. HBV, hepatitis B virus; HE, hepatic encephalopathy; GI bleeding, gastrointestinal bleeding; TB, total bilirubin; Cr, creatinine; INR, international normalized ratio.

In the diagnostic criteria of ACLF, the study population of the APASL included patients with cirrhosis and noncirrhotic chronic liver diseases, while the study population of the EASL included patients with cirrhosis only (7, 9, 25). To resolve this controversy, the World Gastroenterology Organization (WGO) proposed that significant hepatic fibrosis can be considered chronic hepatitis to help distinguish ACLF from acute liver failure (26–28). An FIB-4 > 1.45 was used to screen advanced fibrosis in noncirrhotic chronic liver diseases (21). In the present study, in patients with advanced fibrosis, the INR corresponding to a 28-day LT-free mortality of 15% was also 2.1, and this result suggested that coagulation failure in patients with advanced fibrosis shares the same INR cutoff value, which is also consistent with patients with cirrhosis. Furthermore, in patients with cirrhosis and advanced fibrosis who presented coagulation dysfunction before coagulation failure (INR = 16–2.1), the 28-day LT-free mortality was much lower than 15%, providing more evidence that INR > 2.0 could be used as the cutoff of INR for the diagnosis of coagulation failure in either patients with cirrhosis or advanced fibrosis.

In this study, the INR was not an independent risk factor for 90-day LT-free mortality in subgroups HE3–4. HE is one of the common complications of ALI or AD in patients with cirrhosis and other chronic liver diseases (29–31). The North American Liver Failure Alliance (NACSELD) identified HE grades 3–4 as an independent predictor of 30-day mortality in hospitalized patients with cirrhosis, with 30-day mortality for HE grades 3–4 as high as 38% (32, 33). Our previous study also demonstrated that the 28-day LT-free mortality rates were 34.5 and 55% for HE grades 3 and 4, respectively, among patients with cirrhosis and other chronic liver diseases (34). The results from the above studies and the results from our study demonstrated that grade

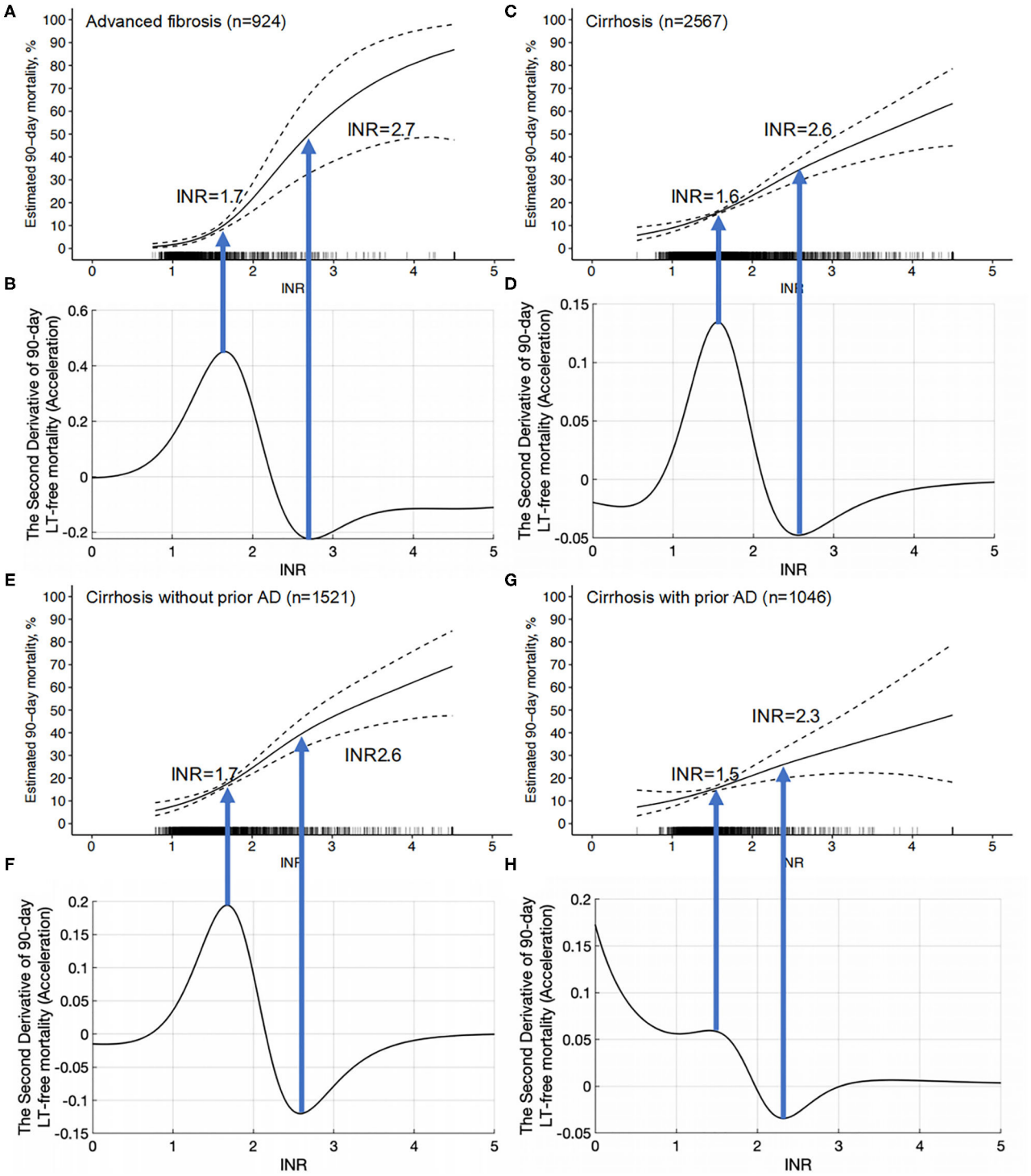


FIGURE 5 | The INR-mortality (90-day) correlation curves and their corresponding second derivative (acceleration) curves. **(A)** The INR-mortality (90-day) correlation curve of advanced fibrosis, **(B)** the second derivative (acceleration) of INR to mortality in advanced fibrosis, **(C)** the INR-mortality (90-day) correlation curve of cirrhosis, **(D)** the second derivative (acceleration) of INR to mortality in cirrhosis, **(E)** the INR-mortality (90-day) correlation curve of cirrhosis without prior AD, **(F)** the second derivative (acceleration) of INR to mortality in cirrhosis without prior AD, **(G)** the INR-mortality (90-day) correlation curve of cirrhosis with prior AD, and **(H)** the second derivative (acceleration) of INR to mortality in cirrhosis with prior AD.

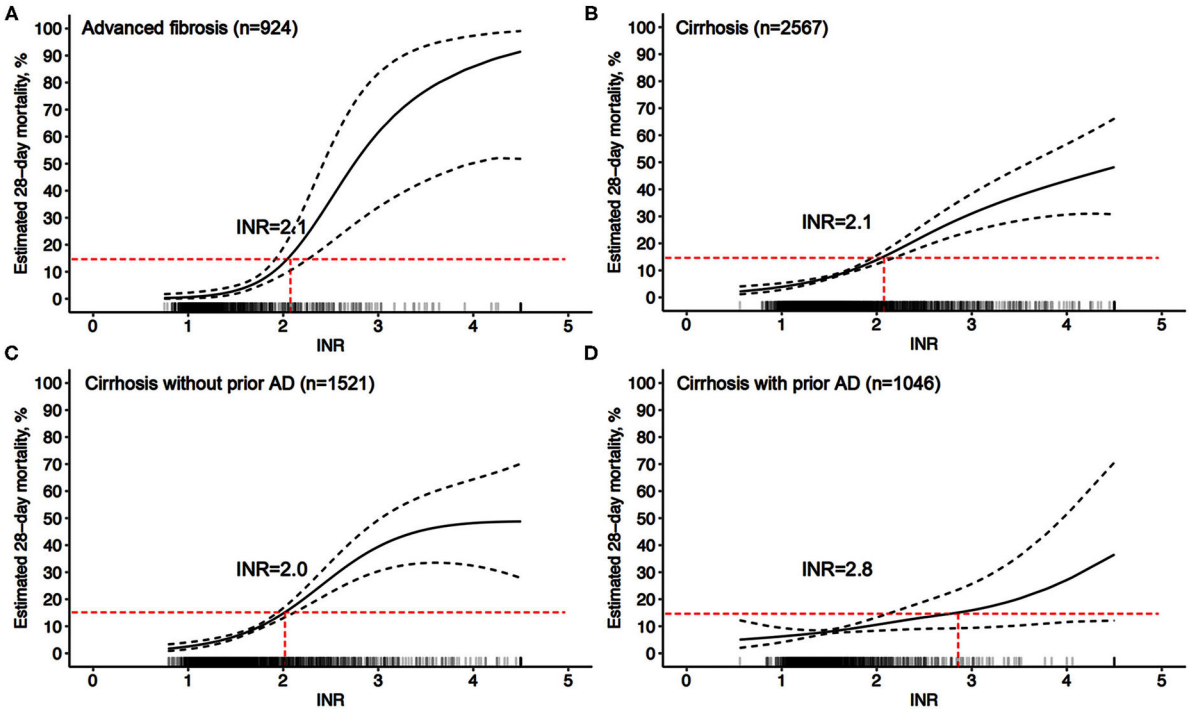


FIGURE 6 | Adjusted probability of INR-mortality (28-day) correlation curves. **(A)** adjusted (age, gender, etiology, HE grades, ascites, infection, TB, and Cr) probability of the INR-mortality (28-day) correlation curve in advanced fibrosis, **(B–D)** adjusted (age, gender, etiology, HE grades, ascites, infection, gastrointestinal bleeding, TB, and Cr) probability of the INR-mortality (28-day) correlation curve in cirrhosis **(B)**, in cirrhosis without prior AD **(C)**, and in cirrhosis with prior AD **(D)**.

TABLE 4 | Multivariable adjusted 28- and 90-day LT-free mortality based on the starting point of INR for disease deterioration and clinical INR cutoffs in cirrhosis and advanced fibrosis.

	Meaning of INR cutoff	Value of cutoff	Number of patients exceeding cutoff (%)	28-day LT-free mortality (%)	90-day LT-free mortality (%)
Cirrhosis	Peak of acceleration curve	1.6	40.2% (1033/2567)	8.5%	16.2%
	Valley of acceleration curve	2.6	8.4% (215/2567)	24.7%	34.7%
	Reaching 15% 28-day LT-free mortality (clinical cutoff)	2.1	17.4% (447/2567)	15%	25.1%
	Coagulation dysfunction without coagulation failure	1.6–2.1	22.8% (586/2567)	7.8%	17.4%
Advanced fibrosis	Peak of acceleration curve	1.7	22.3% (206/924)	5.6%	11.9%
	Valley of acceleration curve	2.7	5.6% (52/924)	46.5%	49.7%
	Reaching 15% 28-day LT-free mortality (clinical cutoff)	2.1	12.6% (116/924)	15%	26.3%
	Coagulation dysfunction without coagulation failure	1.7–2.1	9.7% (90/924)	3.3%	7.8%

3–4 HE plays a more important role than the INR in the short-term outcomes of patients with cirrhosis.

Our research has the following strengths. First, the data come from a high-quality large-scale prospective cohort in a high-endemic area of HBV (17, 18, 20). Second, we obtained for the first time the evidence-based starting point of the INR value that indicates acute disease deterioration both in patients with cirrhosis and in patients with advanced fibrosis, and this can be used as a warning sign of a rapid increase in mortality. The starting point of the INR is important for clinicians to timely identify the rapid increase in mortality caused by coagulation damage. Finally, the unified clinical cutoff of the INR for coagulation failure was found in patients with cirrhosis and advanced fibrosis.

This study had several limitations. First, the data in this study came from HBV high-endemic areas that were not representative of the global characteristics of patients with cirrhosis and other chronic liver diseases, but HBV accounts for 70% of the cases of cirrhosis and other chronic liver diseases and is the highlight of our cohort study. Second, our study only included

LT-free mortality as the outcome and did not include liver transplantation as a bad outcome. In most major studies, LT-free mortality was used as the only end point, which is consistent with our study (7, 35, 36). Finally, this was an observational study that did not investigate the effects of special treatments (such as artificial liver support systems, glucocorticoids, etc.) on the short-term adverse outcomes of patients with cirrhosis and advanced fibrosis. To date, no treatment except liver transplantation has been shown to significantly change the outcome of ACLF.

This was the first study to quantitatively describe the relationship between the INR and short-term LT-free mortality in patients with cirrhosis and advanced fibrosis. For the first time, we obtained the starting point of the INR that indicated a rapid increase in mortality. Patients with either cirrhosis or advanced fibrosis share the same clinical cutoff INR value for the diagnosis of coagulation failure.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethics Board of Shanghai Renji Hospital and Shiyan Taihe Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HL, SS, XW, XinZ, YaH, BL, ZM, YG, ZQ, FL, XL, JuL, HR, GD, YuZ, and HY contributed to the conception and design of the study. HL, XW, XinZ, YaH, ZM, YG, ZQ, FL, XL, JuL, GD, YuZ, HY, LQ, YaZ, WG, XX, YiZ, SS, BX, YiH, QZ, YX, CZ, JC, ZH, BL, XJ, TQ, SL, YC, NG, CL, WY, XM, JiL, TL, RZ, XinyiZ, and HR contributed to organization and data collection. YW wrote the first draft of the manuscript. FD and WZ performed the statistical analysis. ZM and HL performed critical revision of the manuscript for important intellectual content. All authors read and approved the final version of the submitted manuscript.

FUNDING

This research was supported by the National Science and Technology Major Project (2018ZX10723203, 2018ZX10302206, and 2017ZX10202202), National Natural Science Foundation of China (82070650, 81930061), Shanghai Hospital Development Commission (16CR1024B and SHDC2020CR1037B), Shanghai Municipal Education Commission–Guofeng Clinical Medicine and Shanghai Municipal Government Funding, National Key Research and Development Program of China (2017YFC0908100), the Foundation for Innovative Research Groups of Hubei Provincial Natural Science Foundation (2018CFA031), Hubei Province's Outstanding Medical Academic Leader Program, and Project of Hubei University of Medicine (FDFR201902, 2020XGFYZR05, 2021ESOF011, and YC2021012).

ACKNOWLEDGMENTS

We thank the following Chinese (Acute on) Chronic Liver Failure Consortium (Ch-CLIF. C) members and participants for the contributions to this study: Tongyu Wang, Dandan Wu, and Shan Yin: Department of Gastroenterology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University; YiH: Center of Integrative Medicine, Beijing Ditan Hospital, Capital Medical University; JX: Department of Infectious Diseases, Southwest Hospital, Third Military Medical University (Army Medical University); Yixiang Zheng: Department of Infectious Disease, Hunan Key Laboratory of Viral Hepatitis, Xiangya Hospital, Central South University; SL: Department of Infectious Diseases, Institute of Infection and Immunology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology; GZ and JC: Hepatology Unit, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University; Yannan Zhang: Department of Hepatology, First Hospital of Jilin University; QL and CW: Department of Infectious Disease, Taihe Hospital, Hubei University of Medicine; YS: Department of Infectious Disease, The First Hospital of Zhejiang University; Liujuan Ji: Department of Liver Intensive Care Unit, Shanghai Public Health Clinical Center, Fudan University; TL and JL: Department of Infectious Diseases and Hepatology, Second Hospital of Shandong University; RZ and XinyiZ: Liver Disease Center, First Affiliated Hospital of Xinjiang Medical University; Huiming Jin: Department of Infectious Disease, Henan Provincial People's Hospital; HL, Qing Zhang, and Xuequn Zheng: Infectious Disease Center, Affiliated Hospital of Logistics University of People's Armed Police Force; Shaoyang Wang and Taofa Lin: Department of Infectious Disease, Fuzhou General Hospital of Nanjing Military Command.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.762291/full#supplementary-material

REFERENCES

1. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* (2018) 392:1789–858. doi: 10.1016/S0140-6736(18)32279-7
2. Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. *J Hepatol.* (2019) 70:151–71. doi: 10.1016/j.jhep.2018.09.014
3. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* (2018) 392:1736–88. doi: 10.1016/S0140-6736(18)32203-7

4. Allen AM, Kim WR. Epidemiology and healthcare burden of acute-on-chronic liver failure. *Semin Liver Dis.* (2016) 36:123–6. doi: 10.1055/s-0036-1583201
5. Allen AM, Kim WR, Moriarty JP, Shah ND, Larson JJ, Kamath PS. Time trends in the health care burden and mortality of acute on chronic liver failure in the United States. *Hepatology (Baltimore, Md).* (2016) 64:2165–72. doi: 10.1002/hep.28812
6. Northup PG, Caldwell SH. Coagulation in liver disease: a guide for the clinician. *Clin Gastroenterol Hepatol.* (2013) 11:1064–74. doi: 10.1016/j.cgh.2013.02.026
7. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology.* (2013) 144:1426–37.e1–9. doi: 10.1053/j.gastro.2013.02.042
8. Mahmud N, Kaplan DE, Taddei TH, Goldberg DS. Incidence and mortality of acute-on-chronic liver failure using two definitions in patients with compensated cirrhosis. *Hepatology.* (2019) 69:2150–63. doi: 10.1002/hep.30494
9. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. *Hepatol Int.* (2019) 13:353–90. doi: 10.1007/s12072-019-09946-3
10. Sacleux SC, Samuel D. A critical review of MELD as a reliable tool for transplant prioritization. *Semin Liver Dis.* (2019) 39:403–13. doi: 10.1055/s-0039-1688750
11. Bolia R, Srivastava A, Yachha SK, Poddar U. Pediatric CLIF-SOFA score is the best predictor of 28-day mortality in children with decompensated chronic liver disease. *J Hepatol.* (2018) 68:449–55. doi: 10.1016/j.jhep.2017.10.001
12. Weil D, Levesque E, McPhail M, Cavallazzi R, Theocharidou E, Cholongitas E, et al. Prognosis of cirrhotic patients admitted to intensive care unit: a meta-analysis. *Ann Intens Care.* (2017) 7:33. doi: 10.1186/s13613-017-0249-6
13. Zaccherini G, Weiss E, Moreau R. Acute-on-chronic liver failure: definitions, pathophysiology and principles of treatment. *JHEP Rep Innov Hepatol.* (2021) 3:100176. doi: 10.1016/j.jhepr.2020.100176
14. Sarin SK, Kumar A, Almeida JA, Chawla YK, Fan ST, Garg H, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL). *Hepatol Int.* (2009) 3:269–82. doi: 10.1007/s12072-008-9106-x
15. Sarin SK, Kedarisetty CK, Abbas Z, Amarapurkar D, Bihari C, Chan AC, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) (2014). *Hepatol Int.* (2014) 8:453–71. doi: 10.1007/s12072-014-9580-2
16. Singh H, Pai CG. Defining acute-on-chronic liver failure: East, West or Middle ground? *World J Hepatol.* (2015) 7:2571–7. doi: 10.4254/wjh.v7.i25.2571
17. Qiao L, Wang X, Deng G, Huang Y, Chen J, Meng Z, et al. Cohort profile: a multicentre prospective validation cohort of the Chinese Acute-on-Chronic Liver Failure (CATCH-LIFE) study. *BMJ Open.* (2021) 11:e037793. doi: 10.1136/bmjopen-2020-037793
18. Gu WY, Xu BY, Zheng X, Chen J, Wang XB, Huang Y, et al. Acute-on-chronic liver failure in China: rationale for developing a patient registry and baseline characteristics. *Am J Epidemiol.* (2018) 187:1829–39. doi: 10.1093/aje/kwy083
19. Ouyang R LH, Xia J, Wang X, Zheng X, Huang Y, Meng Z, et al. Lower platelet counts were associated with 90-day adverse outcomes in acute-on-chronic liver disease patients. *Ann Palliat Med.* (2021) 10:9342–53. doi: 10.21037/apm-21-1019
20. Zhang Y, Xu BY, Wang XB, Zheng X, Huang Y, Chen J, et al. Prevalence and clinical significance of portal vein thrombosis in patients with cirrhosis and acute decompensation. *Clin Gastroenterol Hepatol.* (2020) 18:2564–72.e1. doi: 10.1016/j.cgh.2020.02.037
21. Qiao L, Tan W, Wang X, Zheng X, Huang Y, Li B, et al. Different effects of total bilirubin on 90-day mortality in hospitalized patients with cirrhosis and advanced fibrosis: a quantitative analysis. *Front Med.* (2021) 8:704452. doi: 10.3389/fmed.2021.704452
22. Child CG, Turcotte JG. Surgery and portal hypertension. *Major Probl Clin Surg.* (1964) 1:1–85.
23. SimonN. *Generalized Additive Models: An Introduction With R.* Chapman & Hall/CRC: Boca Raton, FL (2006).
24. Wood SN AN. GAMs with integrated model selection using penalized regression splines and applications to environmental modelling. *Ecol Model.* (2002) 157:157–77. doi: 10.1016/S0304-3800(02)00193-X
25. Arroyo V, Moreau R, Jalan R. Acute-on-chronic liver failure. *N Engl J Med.* (2020) 382:2137–45. doi: 10.1056/NEJMra1914900
26. Jalan R, Yurdaydin C, Bajaj JS, Acharya SK, Arroyo V, Lin HC, et al. Toward an improved definition of acute-on-chronic liver failure. *Gastroenterology.* (2014) 147:4–10. doi: 10.1053/j.gastro.2014.05.005
27. Duseja A, Singh SP. Toward a better definition of acute-on-chronic liver failure. *J Clin Exp Hepatol.* (2017) 7:262–5. doi: 10.1016/j.jceh.2017.05.002
28. Abbas Z, Shazi L. Pattern and profile of chronic liver disease in acute on chronic liver failure. *Hepatol Int.* (2015) 9:366–72. doi: 10.1007/s12072-015-9627-z
29. Wijdicks EF. Hepatic encephalopathy. *N Engl J Med.* (2016) 375:1660–70. doi: 10.1056/NEJMra1600561
30. Hadjihambi A, Arias N, Sheikh M, Jalan R. Hepatic encephalopathy: a critical current review. *Hepatol Int.* (2018) 12(Suppl. 1):135–47. doi: 10.1007/s12072-017-9812-3
31. Gow AG. Hepatic encephalopathy. *Vet Clin N Am Small Anim Pract.* (2017) 47:585–99. doi: 10.1016/j.cvsm.2016.11.008
32. Bajaj JS, O'Leary JG, Tandon P, Wong F, Garcia-Tsao G, Kamath PS, et al. Hepatic encephalopathy is associated with mortality in patients with cirrhosis independent of other extrahepatic organ failures. *Clin Gastroenterol Hepatol.* (2017) 15:565–74.e4. doi: 10.1016/j.cgh.2016.09.157
33. Ochoa-Sanchez R, Tamnanloo F, Rose CF. Hepatic encephalopathy: from metabolic to neurodegenerative. *Neurochem Res.* (2021) 46:2612–25. doi: 10.1007/s11064-021-03372-4
34. Long L, Li H, Deng G, Wang X, Lu S, Li B, et al. Impact of hepatic encephalopathy on clinical characteristics and adverse outcomes in prospective and multicenter cohorts of patients with acute-on-chronic liver diseases. *Front Med.* (2021) 8:709884. doi: 10.3389/fmed.2021.709884
35. O'Leary JG, Reddy KR, Garcia-Tsao G, Biggins SW, Wong F, Fallon MB, et al. NACSELD acute-on-chronic liver failure (NACSELD-ACLF) score predicts 30-day survival in hospitalized patients with cirrhosis. *Hepatology (Baltimore, Md).* (2018) 67:2367–74. doi: 10.1002/hep.29773
36. Wu T, Li J, Shao L, Xin J, Jiang L, Zhou Q, et al. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure. *Gut.* (2018) 67:2181–91. doi: 10.1136/gutjnl-2017-314641

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.

The reviewer ZC declared a shared parent affiliation with the authors HL, FD, LQ, YZha, WG, and WZ and the reviewer QY declared a shared parent affiliation with the author HR to the handling editor at the time of the review.

frontiers in Medicine

ORIGINAL RESEARCH
published: 22 November 2021
doi: 10.3389/fmed.2021.762504

Clinical Risk Score for Invasive Pulmonary Aspergillosis in Patients With Liver Failure: A Retrospective Study in Zhejiang

Xuan Zhang[1†], Sijia Shen[2†], Xiahong Dai[3†], Yunjiao Bi[4], Junjie Zhang[4], Yuhao Wu[4], Yishang Shi[4], Runan Wei[2] and Hainv Gao[3]*

[1] State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, National Medical Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China, [2] College of Medicine, Zhejiang University, Hangzhou, China, [3] Shulan (Hangzhou) Hospital Affiliated to Zhejiang Shuren University Shulan International Medical College, Hangzhou, China, [4] Shulan International Medical College, Zhejiang Shuren University, Hangzhou, China

OPEN ACCESS

Edited by:
Yu-Chen Fan,
Shandong University, China

Reviewed by:
Daxian Wu,
First Affiliated Hospital of Nanchang University, China
Yufeng Gao,
First Affiliated Hospital of Anhui Medical University, China

***Correspondence:**
Hainv Gao
gaohainv@163.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *22 August 2021*
Accepted: *11 October 2021*
Published: *22 November 2021*

Citation:
Zhang X, Shen S, Dai X, Bi Y, Zhang J, Wu Y, Shi Y, Wei R and Gao H (2021) Clinical Risk Score for Invasive Pulmonary Aspergillosis in Patients With Liver Failure: A Retrospective Study in Zhejiang. Front. Med. 8:762504. doi: 10.3389/fmed.2021.762504

Purpose: The mortality of invasive pulmonary aspergillosis (IPA) in patients with liver failure was high. However, the prophylactic treatment in those patients with a high-risk factor in IPA has not been researched.

Patients and methods: A multicenter, retrospective study was conducted in patients with liver failure. The study cohort of liver failure was randomly split into a training set for model development and the other served as the testing set for model verification. Multivariate analysis was performed to identify the risk factors of IPA. A weighted risk score for IPA was established. Anti-fungal treatment was prophylactically used in patients with medium and high IPA risk to evaluate the effect.

Results: In total, 1,722 patients with liver failure were enrolled. Fifty-seven patients who received prophylactic treatment were excluded from the risk factor system study. About 1,665 patients were randomly split at a ratio of 2:1 into two datasets. Diabetes, glucocorticoids, plasma exchange, and hepatorenal syndrome (HRS) were risk factors in IPA in patients with liver failure, with weighted risk scores of 4, 7, 2, and 3, respectively. In the validation set and test set, the patients with risk scores of $\leq$3 presented low incidences of IPA at 4 and 2.7%. Patients with risk scores of 4–5 had an IPA incidence of 7.6% and 10.1%, and could be considered as a medium-risk group ($p < 0.01$ vs. the group with scores of $\leq$3), whereas those with risk scores of >5 manifested a significantly higher IPA incidence of 21.2 and 12.7%, who were considered a high-risk group ($p < 0.01$ vs. the groups with scores of 4–5 and >5, respectively). The IPA risk scores in the training set and the testing set were also analyzed by the ROC with an area under the ROC of 0.7152 and 0.6912. In this study, 57 patients received antifungal prophylaxis; the incidence of IPA was 1.8%, which was significantly lower after prophylactic antifungal therapy ($p < 0.001$).

Conclusions: A weighted risk score for patients with liver failure, complicated with IPA, was established and confirmed in the testing cohort. Voriconazole prophylactic treatment to patients with liver failure with medium and high IPA risk can effectively prevent Aspergillus infection.

Keywords: liver failure, invasive pulmonary aspergillosis, prophylaxis, voriconazole, risk score

INTRODUCTION

Invasive pulmonary aspergillosis (IPA) is a devastating infectious disease in patients with liver failure; the mortality rate exceeds 80% (1, 2). Early diagnosis and treatment of IPA are particularly important. However, clinical diagnosis of IPA in liver failure is a huge challenge. The traditional G test and the GM test can diagnose IPA early in patients with allogeneic hematopoietic stem cell transplantation, but they lack sensitivity in patients with liver failure (3). Invasive diagnostic procedures are often not feasible due to prolonged prothrombin time and thrombocytopenia. So, at present, the clinical diagnosis of liver failure complicated with IPA infection depends more on symptom-triggered pulmonary CT screening. However, due to the lack of specificity in clinical symptoms and imaging of liver failure complicated with IPA infection, the study found that the positive predictive value of this method was 61% and the negative predictive value was 92% (4). Moreover, previous studies have shown that patients with IPA were diagnosed when the CLIF-SOFA lung score is >1 and had the worst prognosis, poor antifungal treatment effect, and the highest mortality (5). These studies demonstrated that early diagnosis and early treatment are the key factors to improve the prognosis of patients with liver failure, complicated with IPA infection, but it was difficult to achieve.

In view of the disastrous consequences of IPA in patients with liver failure, the risk factor in IPA has been researched extensively, such as hemodialysis and prior antibiotics use (3), prolonged and high-dose corticosteroid therapy (6, 7), and recent history of neutropenia (8, 9). The prophylactic antifungal treatment, which was based on the risk factors, had been evaluated in multiple clinical trials in different diseases. Rijnders et al. showed that prophylactic inhalation of liposomal amphotericin B significantly reduced the incidence of IPA in patients during prolonged neutropenia (10). The China Assessment of Antifungal Therapy in Hematological Diseases (CAESAR) study showed antifungal prophylaxis was beneficial in patients with hematological malignancies with an intermediate and high risk of invasive fungal disease (11). A clinical trial at Mayo Clinic (12) researched lung transplant recipients who receive prolonged and mostly lifelong azole antifungal prophylaxis; none of the patients developed disseminated invasive aspergillosis. However, the prophylactic treatment in patients with liver failure with a risk factor for IPA has not been researched.

In this study, we established a weighted risk score for IPA that accurately discriminated a cohort of patients with liver failure with low, intermediate, and high risks of IPA. Then, the efficacy of antifungal prophylaxis with voriconazole or caspofungin was evaluated in patients with different IPA risks in order to reduce mortality in this population.

MATERIALS AND METHODS

Study Design

A multicenter, retrospective observational study was carried out in patients with liver failure admitted to two tertiary hospitals in Zhejiang province from December 2008 to July 2021: one is Shulan Hangzhou Hospital; the other is the First Affiliated Hospital, School of Medicine, Zhejiang University. The collected data included baseline characteristics, type of liver failure, clinical features, underlying disease, complication, antifungal treatment, treatment-related potential risk factors of IPA, and the prognosis. Because the study was retrospective and the data were analyzed anonymously, the need for consent was waived.

Enrollment Criteria

Liver failure was defined according to the Diagnosis and Treatment Guideline for Liver Failure in China (2018) (13). The definition of IPA was used by the European Organization for Research and Treatment of Cancer (EORTC) consensus (14), which was diagnosed if the following two criteria were fulfilled: (1) positive culture of Aspergillus spp. from sputum; (2) presence of one of the following three signs on computed tomography: dense, well-circumscribed lesions with or without a halo sign, air-crescent sign, or cavity.

Statistical Analysis

The study cohort of liver failure was randomly split at a ratio of 2:1 into two sets, of which one served as the training set for model development and the other served as the testing set for model verification. The characteristics of patients were compared between two datasets using Student's *t*-tests or Kruskal-Wallis tests for continuous variables where appropriate and χ^2 or Fisher exact tests for categorical variables. We first, by using univariate analysis with $p < 0.10$, identified the risk factors that were individually complicated with probable IPA. The factors that demonstrated an individual association were demonstrated the multivariate logistic regression with the stepwise criteria of 0.05. Points were assigned for the variables and were weighted approximately by the corresponding regression β-coefficients. Receiver operator curves (ROC) were calculated to assess the discrimination capacity of the risk score. Once the model was determined, it was tested in the test set to confirm its performance in predicting the IPA incidence. All statistical analyses were performed using SPSS version 18.0 (SPSS, Chicago, IL, USA).

RESULTS

Study Populations and Incidence of IPA

In this study, 1,722 patients with liver failure were enrolled. Fifty-seven patients who received voriconazole or caspofungin prophylactic treatment were excluded from the risk factor system study. Of the remaining 1,665 patients, the patients with IPA were 111, the overall incidence of IPA was 6.7%, and the mortality of IPA was 85.6%. Acute (subacute) liver failure, acute-on-chronic (subacute-on-chronic) liver failure, and chronic liver failure were 198, 902, and 565 cases, and the IPA incidences were 7.1, 7.1, and 5.9%, respectively. There was no significant difference between any two groups for IPA incidence.

About 1,665 liver failure patients were randomly split at a ratio of 2:1 into two datasets, 1,155 patients were enrolled in the training set, and 510 patients were in the testing set.

The characteristics of patients in training and testing datasets were similar in all aspects, as shown in **Table 1**.

Risk Scores Associated With IPA in the Training Dataset

The variables of the training set that was associated with IPA incidence, including diabetes, corticosteroid, plasma exchange treatment, and INR, were significant in the multivariate logistic regression, and weighted points were assigned (**Table 2**).

Based on the multivariate logistic regression analysis, IPA risk scores ranged from 0 to 16 (**Table 3**) and were calculated in the training set. The distribution of the risk scores and the cumulative incidence of IPA in liver failure patients were shown in **Table 4**. The patients with risk scores of $\leq$3 presented low incidences of IPA at 4%. Patients with risk scores of 4–5 had an IPA incidence of 7% and could be considered as a medium-risk group ($p < 0.01$ vs. the group with scores of $\leq$3), whereas those with risk scores of $>$5 manifested a significantly higher IPA incidence of 21.2%, who were considered as a high-risk group ($p < 0.01$ vs. the groups with scores of 4–5 and $>$5, respectively).

The ROC curve was used to identify the ability of the IPA risk scores, and the area under the curve was 0.6912, which is shown in **Figure 1**.

Risk Scores Associated With IPA in the Testing Dataset

The IPA risk score was used to assess the patients with liver failure in the testing dataset. The incidence of IPA was 2.7% when the risk scores of IPA were less than or equal to 3. As the risk scores reached 4–5, the IPA incidence increased to 7% ($p <0.01$ vs. the group with scores of $\leq$3), whereas the IPA incidence was 12.7% in the patients with risk scores of $>$5 ($p < 0.01$ vs. the groups with scores of 4–5 and $>$5, respectively).

The IPA risk scores in the testing set were also analyzed by the ROC with an area under the ROC of 0.7152, as shown in **Figure 2**.

Impact of Antifungal Prophylaxis in Patients With Different Risk Scores

In this study, 57 patients received antifungal prophylaxis, who had significantly more seriously impaired liver function than patients without prophylaxis ($p < 0.001$). Meanwhile, the risk scores were significantly higher in the population with antifungal prophylaxis ($p < 0.001$) (**Table 5**). However, the incidence of IPA was 1.8%, which was significantly lower after prophylactic antifungal therapy ($p < 0.001$).

DISCUSSION

Patients with end-stage liver diseases are now considered additional risk factors of IPA, along with allogeneic bone marrow transplantation (15). The prevalence of IPA in HBV-related ACLF patients has been reported to be 5–8.3%. The short-term mortality observed in these patients ranged from 73.5 to 100% (1, 5, 7). In our study, the incidence of IPA was 6.7%, and the mortality was 85.6%, which was consistent with previous reports. It is not difficult to find that the IPA rate in patients with liver failure is not high, but, once it occurs, the mortality is very high. Hence, numerous studies have reported lots of IPA risk factors of liver failure (1, 2, 7, 16, 17) in order to provide a way for clinical early diagnosis and early treatment.

The previous study showed antibiotic use was an independent factor associated with the occurrence of IPA in patients with liver failure (2), and the patients were prone to many types of infections caused by opportunistic pathogens, including Aspergillus spp. after antibiotics use. However, our results showed there was no significant difference in antibiotic use between the patients with IPA and those without. Instead, diabetes, INR, plasma exchange, and steroid use were proved to be significant risk factors for IPA. Diabetes and steroid use indirectly reflect the immune status, which was easier to understand that they are the risk factors of IPA in patients with liver failure. But, in this study, we, for the first time, confirmed that the plasma exchange therapy was the risk factor of IPA in liver failure; this risk score is 2, which belongs to the low-level risk factor. This means that patients with severe liver failure treated with plasma exchange received steroid treatment or had diabetes at the same time, which will increase the risk of IPA.

Over the past three decades, plasma exchange has been employed to treat liver failure. Due to the lack of randomized controlled studies, the effect of plasma exchange on liver failure has always been controversial (18). Until Larsen et al. (19) published the first randomized control trial of plasma exchange in patients with acute liver failure in 2016, then, plasmapheresis was added to the European guidelines (20) as Level I, Grade 1 recommendation in management of acute liver failure. Its proposed mechanism is the removal of plasma cytokines and drivers of systemic inflammatory cascade by plasma exchange. But why does plasma exchange increase the risk of Aspergillus infection? We speculate whether plasma exchange removes some cytokines at the same time, which mediate the inflow of macrophage and might limit the degree of local tissue destruction of Aspergillus infection (21).

Despite so many studies on risk factors, there is no study that has considered an early prophylactic treatment for high-risk patients. There may be two reasons that hinder the measures to be taken for clinical prophylactic treatment. First, the risk factors found in different studies are inconsistent, there is no further verification of these risk factors, and there is no distinction between low-risk, medium-risk, and high-risk factors. So, it is difficult for clinical doctors to judge all of these risk factors and

TABLE 1 | Characteristics of patients with liver failure in the training and testing sets.

Characteristic			Training set (n = 1,155)	Testing set (n = 510)	P value
Sex	Male		887 (76.8%)	391 (76.7%)	0.9499
	Female		268 (23.2%)	119 (22.3%)	
Age	Mean (S.D.)		49.96 (13.61)	50.63 (13.22)	0.2606
Diagnosis	ALF[a]		98 (8.5%)	44 (8.6%)	0.0604
	SALF[b]		35 (3.0%)	20 (3.9%)	
	ACLF[c]		649 (56.2%)	252 (49.4%)	
	CLF[d]		370 (32.0%)	193 (37.8%)	
Etieology	Hepatitis B		774 (67.0%)	323 (63.3%)	0.4003
	Hepatitis E		9 (0.8%)	6 (1.2%)	
	Alcohol		67 (5.8%)	34 (6.7%)	
	Drug		77 (6.7%)	30 (5.9%)	
	Autoimmunity		27 (2.3%)	15 (2.9%)	
	Hepatolenticular degeneration		8 (0.7%)	2 (0.4%)	
	Schistosome		7 (0.6%)	4 (0.8%)	
	Cryptogenic		58 (5.0%)	39 (7.6%)	
	Malignancy		52 (4.5%)	22 (4.3%)	
	Two or more factors		53 (4.6%)	27 (5.3%)	
	Other		19 (1.6%)	5 (1.0%)	
underlaying diseases	Diabetes	Yes	119 (10.3%)	56 (11.0%)	0.7423
		No	1,036 (89.7%)	454 (89.0%)	
	Malignancy	Yes	151 (13.1%)	63 (13.3%)	0.7447
		No	1,004 (86.9%)	447 (87.6%)	
Complications	Neutropenia	Yes	22 (1.9%)	14 (2.7%)	0.3660
		No	1,133 (98.1%)	496 (97.3%)	
	Gastrointestinal bleeding	Yes	160 (13.9%)	84 (16.5%)	0.1878
		No	995 (86.1%)	426 (83.5%)	
	HE[e]	Yes	504 (43.6%)	221 (43.3%)	0.9511
		No	651 (56.4%)	289 (56.7%)	
	HRS[f]	Yes	187 (16.2%)	85 (16.7%)	0.8647
		No	968 (83.8%)	425 (83.3%)	
Liver function	TBil[g]	Median	419.00	436.00	0.1583
		Min, Max	170.00, 3,015.00	171.00,990.00	
	INR[h]	Median	2.58	2.64	0.8341
		Min, Max	1.50,12.30	1.51,6.52	
Treatment	Antibiotic usage[i]	Yes	822 (71.2%)	372 (72.9%)	0.4958
		No	333 (28.8%)	138 (27.1%)	
	Steroid exposure[j]	Yes	88 (7.6%)	35 (6.9%)	0.6583
		No	1,067 (92.4%)	475 (93.1%)	
	Plasma exchange	Yes	631 (54.6%)	274 (53.7%)	0.7726
		No	524 (45.4%)	236 (46.3%)	
Prognosis	Liver transplantion		196 (17.0%)	89 (17.5%)	0.8617
	Recovery		318 (27.5%)	139 (27.3%)	
	Demise		220 (19.0%)	107 (21.0%)	
	Transfer to another hospital		58 (5.0%)	26 (5.1%)	
	Give up treatment		363 (31.4%)	149 (29.2%)	
IPA		Yes	81 (7.0%)	30 (5.9%)	0.6222
		No	1,074 (92.9%)	480 (94.1%)	

[a]ALF, acute liver failure; [b]SALF, subacute liver failure; [c]ACLF, acute-on-chronic liver failure; [d]CLF, chronic liver failure; [e]HE, hepatic encephalopathy; [f]HRS, hepatorenal syndrome; [g]TBil, total bilirubin; [h]INR, international normalized ratio; [i]antibiotic usage, antimicrobial agent use for ≥5 days; [j]steroid exposure, steroid treatment for ≥7 days, maximum dosage (equivalent methylprednisolone) ≥40 mg/day.

TABLE 2 | Multivariate logistic regression analysis of risk factors associated with invasive pulmonary aspergillosis (IPA) development in the training dataset.

Variable	Univariate logistic regression				Multivariate logistic regression				
	Coefficients	Standard error	Walds	*p*-value	Coefficients	Weight of score	Standard error	Walds	*p*-value
Gender	0.3531	0.3031	1.165	0.244					
Age category	0.2551	0.2329	1.095	0.273					
Diabetes	0.5945	0.3203	1.856	0.0634	0.8256	4	0.3309	2.495	0.01258
Cancer	−0.4315	0.4061	1.063	0.288					
Antibiotic	0.4198	0.2815	1.491	0.136					
Corticosteroid	1.4576	0.2897	5.032	$p < 0.01$	1.5561	7	0.2974	5.232	p<0.01
Granulocytopenia	−0.5996	1.027	0.584	0.559					
Artifital liver treatment	0.4629	0.2423	1.91	0.0561	0.4286	2	0.249	1.722	0.03513
Gastrointestinal bleeding	0.3733	0.3000	1.245	0.213					
HRS	0.635	0.3576	3.123	0.10176					
HE	0.06876	0.23377	0.294	0.769					
Tbil	0.6228	0.3405	3.589	0.1012					
INR	0.7214	0.2442	2.954	0.00314	0.7128	3	0.2504	2.847	0.00441
Etiology	0.2851	0.2361	1.208	0.227					

HRS, hepatorenal syndrome; HE, hepatic encephalopathy; Tbil (≥ 430 vs. < 430) , total bilirubin; INR (≥ 2.6 vs. < 2.6), international normalized ratio, etiology (HBV vs. others).

TABLE 3 | Risk scores for IPA.

Factor	Variables	Weight of score
Diabetes	No	0
	YES	4
Corticosteroid	No	0
	YES	7
Plasma exchange	No	0
	YES	2
INR	No	0
	YES	3

TABLE 4 | Distribution of risk scores vs. the cumulative incidence of IPA in the training and testing datasets.

Risk scores	Patients (*n*)	IPA episodes (*n*)	IPA incidence (%)
Training set			
≤3	649	26	4.0%
4∼5*	330	25	7.6%
>5**	137	29	21.2%
Testing set			
≤3	296	8	2.7%
4–5#	139	14	10.1%
>5##	71	9	12.7%

**$p < 0.01$ vs. the group with scores of ≤ 3; **$p < 0.01$ vs. the groups with scores of 4–5 and >5, respectively; #$p < 0.01$ vs. the group with scores of ≤ 3; ##$p < 0.01$ vs. the groups with scores of 4–5 and >5, respectively.*

TABLE 5 | The characteristics of patients with or without antifungal prophylaxis and impact of antifungal prophylaxis in patients with different risk scores.

Characteristics		No prophylaxis		Prophylaxis		*P* value
		n	%	*n*	%	
Sex						
	Male	1,269	76.9%	45	78.9%	0.8357
	Female	382	23.1%	12	21.1%	
Age						
	≤50	845	51.2%	24	42.1%	0.2252
	>50	806	48.8%	33	57.9%	
Corticosteroid						
	No	1,531	92.7%	54	94.7%	0.7943
	Yes	120	7.3%	3	5.3%	
Diabetes						
	No	1,477	89.5%	49	86.0%	0.5335
	Yes	174	10.5%	8	14.0%	
Plasma exchange treatment						
	No	749	45.4%	27	47.4%	0.8704
	Yes	902	54.6%	30	52.6%	
INR[a]						
	<2.6	829	50.2%	14	24.6%	<0.001
	≥2.6	822	49.8%	43	75.4%	
Risk scores (means ± sd)		3.5±2.8		4.2±2.6		<0.001
and IPA incidence (%)	≤3	945	3.6%	27	3.7%	
	4–5	469	11.4%	22	0	
	>5	237	18.3%	8	0	
IPA[b]						
	No	1,540	93.3%	56	98.2%	<0.001
	Yes	111	6.7%	1	1.8%	

[a]INR, international normalized ratio; [b]IPA, invasive pulmonary aspergillosis.

take steps. In fact, almost all liver failure will have 1–2 risk factors above mentioned, but it is not impossible to give prophylactic antifungal therapy to all the patients with liver failure; second, it is difficult to determine which prophylactic method has to be taken. If voriconazole prophylactic treatment is taken in line

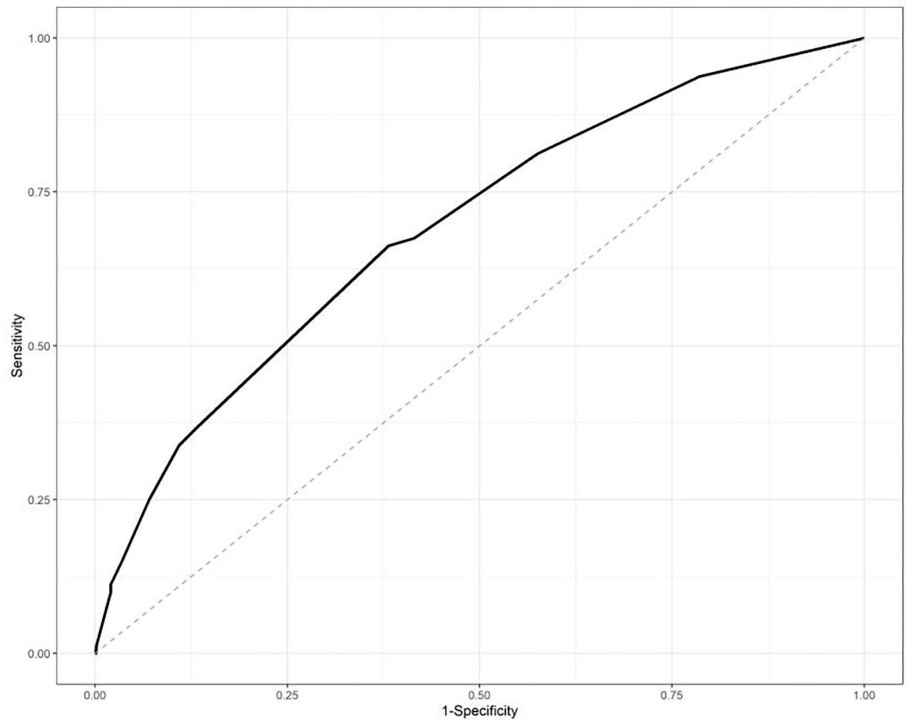


FIGURE 1 | Receiver-operator curve (ROC) analysis of the risk scores in the training datasets. The area under the curve was 0.6912.

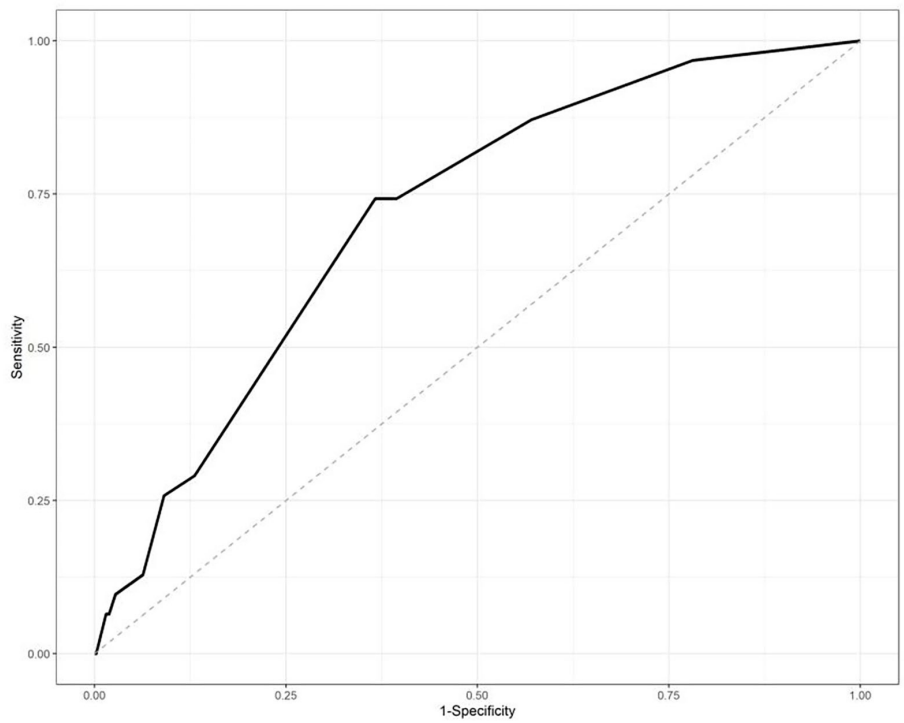


FIGURE 2 | Receiver-operator curve (ROC) analysis of the risk scores in the testing datasets. The area under the curve was 0.7152.

with HSCT (11), how much dosage of voriconazole needs to be used in patients with liver failure, because of the hepatotoxicity of voriconazole, whether its prophylactic use will be harmful to the liver of patients with liver failure. Different from creatinine clearance, the pharmacokinetics of drugs metabolized from the liver with different disease severities is not a simple linear relationship, and there are too many concerns about voriconazole metabolism and human CYP2C19 gene polymorphism at the same time (22, 23).

In this study, we conducted a multicenter-based study to build up a risk score system for IPA in patients with liver failure. We confirmed the discriminative performance of the IPA risk score system in both the training and testing sets. The effective concentration range of voriconazole is between 1 and 5 ug/ml. As long as the concentration is monitored, the risk can be avoided. Prevention failed in one patient among 27 low-risk patients, who were prevented with caspofungin. However, what we need to further study is the difference between caspofungin and voriconazole in the prevention of Aspergillus infection.

There are several limitations to our study. First, we can only, according to the results of sputum culture and pulmonary CT, diagnose IPA in patients with liver failure due to their coagulation function and platelet status, which will not lead to a precise diagnosis; second, this study is a retrospective analysis, which could lead to the unbalanced distribution of confounding factors when we evaluate the efficacy of the prophylactic treatment. Some factors may affect the results, such as the duration of the disease, the severity of the disease, or the dosage of the prophylactic antifungal drug. Third, we included liver failure caused by different causes, and they have different pathogenesis, which will affect the incidence rate of IPA. Finally, the sample size was insufficient to compare different prophylactic treatment effects in different subgroups.

CONCLUSION

To the best of our knowledge, this is the first report for developing an IPA risk score system based on a large patient population with liver failure. We established a weighted risk score for IPA that could reliably discriminate the incidence of IPA. The precise risk assessment of IPA may provide a chance for risk based antifungal treatment in patients with liver failure. For the first time, we tried to give voriconazole prophylactic treatment to patients with liver failure with medium and high IPA risk and found that it can effectively prevent Aspergillus infection. Furthermore, we need to expand the sample size and design a multicenter prospective study to further verify the effect of preventive antifungal therapy in high-risk groups and explore the best dose of preventive therapy in patients with liver failure.

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 Shulan Hangzhou Hospital. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HG designed and conceptualized the study. XZ, SS, and XD designed the table to collect and analyze the data. YB, YW, YS, and JZ provided clinical data. XZ, SS, and HG wrote the manuscript. SS helped to revise the manuscript. All the authors have read and approved the final manuscript.

FUNDING

This work was supported by the Zhejiang Basic Public Welfare Research Program (No. LGF21H030012) and the Zhejiang Non-public Medical Specialty Program.

ACKNOWLEDGMENTS

Jie Wu was involved in the development of the methodology and participated in the statistical analysis.

REFERENCES

1. Chen J, Yang Q, Huang J, Li L. Risk factors for invasive pulmonary aspergillosis and hospital mortality in acute-on-chronic liver failure patients: a retrospective-cohort study. *Int J Med Sci.* (2013) 10:1625–31. doi: 10.7150/ijms.6824
2. Zhang X, Yang M, Hu J, Zhao H, Li L. Epidemiology of invasive pulmonary aspergillosis in patients with liver failure: clinical presentation, risk factors, and outcomes. *J Int Med Res.* (2018) 46:819–27. doi: 10.1177/0300060517729907
3. Verma N, Singh S, Taneja S, Duseja A, Singh V, Dhiman RK, et al. Invasive fungal infections amongst patients with acute-on-chronic liver failure at high risk for fungal infections. *Liver Int.* (2019) 39:503–13. doi: 10.1111/liv.13981
4. Blot SI, Taccone FS, Van den Abeele AM, Bulpa P, Meersseman W, Brusselaers N, et al. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. *Am J Respir Crit Care Med.* (2012) 186:56–64. doi: 10.1164/rccm.201111-1978OC
5. Gao J, Zhang Q, Wu Y, Li Y, Qi T. Zhu C, et al. Improving survival of acute-on-chronic liver failure patients complicated with invasive pulmonary aspergillosis. *Sci Rep.* (2018) 8:876. doi: 10.1038/s41598-018-19320-2
6. Lionakis MS, Kontoyiannis DP. Glucocorticoids and invasive fungal infections. *Lancet.* (2003) 362:1828–38. doi: 10.1016/S0140-6736:14904-5
7. Wu Z, Ling Z, Shao F, Sheng J, Li L. Invasive pulmonary aspergillosis in patients with acute-on-chronic liver failure. *J Int Med Res.* (2012) 40:1958–65. doi: 10.1177/030006051204000537
8. Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, et al. Revision and update of the consensus definitions of invasive fungal disease from the european organization for research and treatment of cancer and the mycoses study group education and research consortium. *Clin Infect Dis.* (2020) 71:1367–76. doi: 10.1093/cid/ciz1008

9. Kousha M, Tadi R, Soubani AO. Pulmonary aspergillosis: a clinical review. *Eur Respir Rev*. (2011) 20:156–74. doi: 10.1183/09059180.00001011
10. Rijnders BJ, Cornelissen JJ, Slobbe L, Becker MJ, Doorduijn JK, Hop WC, et al. Aerosolized liposomal amphotericin B for the prevention of invasive pulmonary aspergillosis during prolonged neutropenia: a randomized, placebo-controlled trial. *Clin Infect Dis.* (2008) 46:1401–8. doi: 10.1086/586739
11. Wang L, Wang Y, Hu J, Sun Y, Huang H. Chen J, et al. Clinical risk score for invasive fungal diseases in patients with hematological malignancies undergoing chemotherapy: China Assessment of Antifungal Therapy in Hematological Diseases (CAESAR) study. *Front Med.* (2019) 13:365–77. doi: 10.1007/s11684-018-0641-0
12. Chong PP, Kennedy CC, Hathcock MA, Kremers WK, Razonable RR. Epidemiology of invasive fungal infections in lung transplant recipients on long-term azole antifungal prophylaxis. *Clin Transplant.* (2015) 29:311–8. doi: 10.1111/ctr.12516
13. Liver Failure and Artificial Liver Group, Chinese Society of Infectious Diseases, Chinese Medical Association; Severe Liver Diseases and Artificial Liver Group, Chinese Society of Hepatology. Chinese Medical Association. Diagnositic and treatment guidelines for liver failure. *Chinese Journal of Infectious Diseases.* (2019) 37:1–9. doi: 10.3760/cma.j.issn.1000-6680.2019.01.001
14. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European organization for research and treatment of cancer/invasive fungal infections cooperative group and the national institute of allergy and infectious diseases mycoses study group (EORTC/MSG) Consensus Group. *Clin Infect Dis.* (2008) 46:1813–21. doi: 10.1086/588660
15. Koulenti D, Garnacho-Montero J, Blot S. Approach to invasive pulmonary aspergillosis in critically ill patients. *Curr Opin Infect Dis.* (2014) 27:174–83. doi: 10.1097/QCO.0000000000000043
16. Wang W, Zhao CY, Zhou JY, Wang YD, Shen C, Zhou DF, et al. Invasive pulmonary aspergillosis in patients with HBV-related liver failure. *Eur J Clin Microbiol Infect Dis.* (2011) 30:661–7. doi: 10.1007/s10096-010-1137-2
17. Lahmer T, Brandl A, Rasch S, Baires GB, Schmid RM, Huber W, et al. Prevalence and outcome of invasive pulmonary aspergillosis in critically ill patients with liver cirrhosis: an observational study. *Sci Rep.* (2019) 9:11919. doi: 10.1038/s41598-019-48183-4
18. Tan EX, Wang MX, Pang J, Lee GH. Plasma exchange in patients with acute and acute-on-chronic liver failure: a systematic review. *World J Gastroenterol.* (2020) 26:219–45. doi: 10.3748/wjg.v26.i2.219
19. Larsen FS, Schmidt LE, Bernsmeier C, Rasmussen A, Isoniemi H, Patel VC, et al. High-volume plasma exchange in patients with acute liver failure: an open randomised controlled trial. *J Hepatol.* (2016) 64:69–78. doi: 10.1016/j.jhep.2015.08.018
20. Wendon J, Cordoba J, Dhawan A, Larsen FS, Manns M, Samuel D, et al. EASL Clinical Practical Guidelines on the management of acute (fulminant) liver failure. *J Hepatol.* (2017) 66:1047–81. doi: 10.1016/j.jhep.2016.12.003
21. Caglar K, Kalkanci A, Fidan I, Aydogan S, Hizel K, Dizbay M, et al. Investigation of interleukin-10, tumor necrosis factor-alpha and interferon-gamma expression in experimental model of pulmonary aspergillosis. *Mikrobiyol Bul.* (2011) 45:344–52.
22. Lin XB Li ZW, Yan M, Zhang BK, Liang W, Wang F, et al. Population pharmacokinetics of voriconazole and CYP2C19 polymorphisms for optimizing dosing regimens in renal transplant recipients. *Br J Clin Pharmacol.* (2018) 84:1587–97. doi: 10.1111/bcp.13595
23. Tanaka R, Fujioka T, Suzuki Y, Iwao M, Itoh H. A prospective study on the usefulness of initial voriconazole dose adjustment based on CYP2C19 gene polymorphism analysis. *Chemotherapy*. (2020) 65:59–64. doi: 10.1159/000509970

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.

frontiers in Medicine

ORIGINAL RESEARCH
published: 06 December 2021
doi: 10.3389/fmed.2021.762296

CD200R Combined Neutrophil-Lymphocyte Ratio Predict 90-Day Mortality in HBV-Related Acute-On-Chronic Liver Failure

Yuxin Li[1†], Yaxian Kong[2†], Ke Shi[1], Yunyi Huang[1], Qun Zhang[1], Bingbing Zhu[1], Hui Zeng[3] and Xianbo Wang[1*]*

[1] Center of Integrative Medicine, Beijing Ditan Hospital, Capital Medical University, Beijing, China, [2] Beijing Key Laboratory of Emerging Infectious Diseases, Institute of Infectious Diseases, Beijing Ditan Hospital, Capital Medical University, Beijing, China, [3] Biomedical Innovation Center, Beijing Shijitan Hospital, Capital Medical University, Beijing, China

OPEN ACCESS

Edited by:
Yu-Chen Fan, Shandong University, China

Reviewed by:
Huiying Rao, Peking University People's Hospital, China
Xiaoning Wang, Shanghai University of Traditional Chinese Medicine, China

***Correspondence:**
Hui Zeng
zenghui@ccmu.edu.cn
Xianbo Wang
wangxb@ccmu.edu.cn

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *21 August 2021*
Accepted: *17 November 2021*
Published: *06 December 2021*

Citation:
Li Y, Kong Y, Shi K, Huang Y, Zhang Q, Zhu B, Zeng H and Wang X (2021) CD200R Combined Neutrophil-Lymphocyte Ratio Predict 90-Day Mortality in HBV-Related Acute-On-Chronic Liver Failure. Front. Med. 8:762296. doi: 10.3389/fmed.2021.762296

Background: Survival of acute-on-chronic liver failure (ACLF) cannot be properly predicted based on clinical characteristics.

Aims: This study aimed to develop a predictive model to evaluating the prognosis for hepatitis B virus-related ACLF (HBV-ACLF) based on specific laboratory and immune indicators.

Methods: Baseline laboratory results were obtained and immune indicators were detected by flow cytometry. A predictive model, which estimates the prognosis at 90-day follow-up, was developed using data from a prospective study on 45 patients hospitalized of HBV-ACLF from June 2016 to April 2018 at the Beijing Ditan Hospital, Capital Medical University. The prognostic values of the predictive factors were determined by the area under the receiver operating characteristic (AUROC) curves.

Results: Six factors exhibited statistical differences between the survival and non-survival groups: proportions of $CD4^+T_N$, $CD4^+T_{EM}$, $CD8^+T_N$, $CD8^+T_{EM}$, $CD200R^+CD4^+$T cells and neutrophil-lymphocyte ratio (NLR). CD200R combined with the NLR had an AUROC of 0.916, which was significantly higher than the AUROC values of $CD200R^+CD4^+$T cells (0.868), NLR (0.761), model for end-stage liver disease (MELD) (0.840), MELD-Na (0.870), Child-Turcotte-Pugh (CTP) (0.580), or chronic liver failure-consortium ACLF (CLIF-C ACLF) score(0.840). At the cut-off point of−3.87, matching the maximum Youden index determined by ROC analysis, the positive predictive and negative predictive values for the mortality were 0.86 and 0.97, respectively.

Conclusions: The 90-day prediction model based on baseline levels of $CD200R^+CD4^+$T cells and NLR offers potential predictive value for the mortality of HBV-ACLF.

Keywords: ACLF, CD200R, HBV, prognosis, T cell, NLR

INTRODUCTION

Acute-on-chronic liver failure (ACLF) is an acute deterioration of pre-existing chronic liver diseases, manifesting as jaundice, coagulopathy and complicated within 4 weeks by ascites and/or hepatic encephalopathy, ultimately resulting in high short-term mortality (1–3). In particular, hepatitis B virus (HBV) infections account for most cases in the Asia-Pacific region because of their high prevalence (1).

In patients with chronic hepatitis B (CHB), persistent exposure to antigens often leads to depressed T cell function, characterized by the multiple expression of coinhibitory molecules (4–6). The upregulation of inhibitory receptors, such as programmed cell death protein-1 (PD-1), cytotoxic T-lymphocyte associated protein-4 (CTLA-4), and 2B4 (CD244) (7–9), has been confirmed to be related to the dysfunction of HBV-specific $CD8^+$ T cells in chronic infection. Hepatitis B virus-related ACLF (HBV-ACLF) is a more complicated disease with excessive inflammation and immune dysfunction. Multiple factors, particularly host immunity, are involved in the pathogenesis of HBV-ACLF (10–12). T cell dysfunction and increased expression of coinhibitory molecules are also involved in the pathogenesis of HBV-ACLF.

CD200 is a cell surface glycoprotein that functions by engaging the CD200 receptor on cells of the myeloid and lymphoid lineages to transmit signals affecting responses in multiple physiological systems (13, 14). CD200 expression has been reported to affect cancer growth, autoimmune and allergic disorders, infection, transplantation, bone development and homeostasis, and reproductive biology (15–17). However, its role in the pathogenesis of HBV-ACLF remains to be explored. Neutrophil-lymphocyte ratio (NLR) has been confirmed as a potential short-term prognostic indicator for patients with HBV-ACLF (18). In this study, we investigated the correlation between CD200R along with NLR and the prognosis of HBV-ACLF and whether CD200R combined NLR could be used to predict prognosis. These findings may contribute to a better understanding of the pathogenesis of HBV-ACLF, which could be helpful in clinical decision-making and the development of novel therapeutic methods.

PATIENTS AND METHODS

Patients

A total of 45 patients with HBV-ACLF and 169 patients with CHB were enrolled between June 2016 and April 2018 at the Beijing Ditan Hospital, Capital Medical University. The inclusion criteria for CHB was set according to the Chinese guideline of prevention and treatment for CHB (2015 version) (19): (1) 18 years or older, (2) HBsAg positive status for at least 6 months. The inclusion criteria for HBV-ACLF patients were set according to the consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) for ACLF (2014) (20): (1) age 18 years or older, (2) HBsAg positive status for at least 6 months, (3) serum bilirubin $\geq$ 5 mg/dL (85 μmol/L) and coagulopathy (international normalized ratio (INR) $\geq$ 1.5 or prothrombin activity (PTA) $<$ 40%) complicated within 4 weeks by clinical ascites and/or encephalopathy. The key exclusion criteria included pregnancy, mental illness, decompensated liver cirrhosis, hepatocellular carcinoma, liver transplants, immune

TABLE 1 | Characteristics of the subjects included in the study.

Variables	HC *n* = 50	CHB *n* = 169	ACLF *n* = 45	*p*-value
Demographics				
Age (year)	34.5 (29.0,43.0)	33.0 (28.0,42.0)	44.5 ± 12.8	<0.001
Male/Female,%	27/23	99/70	36/9	0.040
Laboratory data				
WBC (10^9/L)	6.1 ± 12	5.4 ± 1.3	4.9 (4.0,6.7)	0.004
NC (10^9/L)	3.6 ± 1.0	3.0 ± 1.0	3.0 (1.9,4.2)	0.006
LC (10^9/L)	2.0 ± 0.5	1.8 (1.5,2.2)	1.4 ± 0.6	<0.001
NLR	1.9 ± 0.7	1.6 (1.2,2.0)	2.4 (1.6,3.6)	<0.001
ALT (U/L)	13.5 (10.0,25)	31.1 (23.0,200.6)	355.5 (130.8,832.9)	<0.001
AST (U/L)	17.1 (15.3,21.4)	24.0 (18.9,94.7)	217.9 (94.9,453.0)	<0.001
ALB (g/L)	47.9 (46.7,49.9)	47.0 (42.9,49.1)	32.9 ± 4.8	<0.001
GLO (g/L)	22.3 (21.1,25.0)	26.7 (24.2,29.4)	27.7 ± 6.7	<0.001
TBIL (μmol/L)	11.6 (9.4,12.9)	13.8 (10.3,21.2)	232.7 (148.9,325.8)	<0.001
PTA (%)	/	94.3 ± 13.2	35.9 ± 10.5	<0.001
INR	/	1.1 (1.0,1.1)	2.1 (1.8,2.5)	<0.001
logHBVDNA log (IU/mL)	/	6.1 (3.4,8.1)	5.2 ± 1.7	0.160

HC, healthy control; CHB, chronic hepatitis B; ACLF, acute-on-chronic liver failure; WBC, white blood cell; LC, lymphocyte count; NC, neutrophil count; NLR, neutrophil-lymphocytes ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; GLO, globulin; TBIL, total bilirubin; PTA, prothrombin activity; INR, international normalized ratio; p values were calculated by χ^2 for categorical variables and Kruskal-Wallis test or unpaired t test for continuous variables.

regulatory treatment within 6 months, co-infection with hepatitis A, C, D, E, or liver diseases caused by other reasons. In addition, patients with CHB who meet the criteria of serum bilirubin ≥ 5 mg/dL (85 μmol/L) and coagulopathy (INR ≥ 1.5 or PTA < 40%) was excluded from CHB group. Patients with HBV-ACLF were followed up for 90 days. Fifty healthy control (HC) subjects were also recruited for the study. The present study was approved by the Ethics Committee of the Beijing Ditan Hospital, Capital Medical University, China. Written informed consent was obtained from all the patients before their participation.

Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

Peripheral blood samples were collected from all the subjects. PBMCs were isolated by Ficoll-Paque PLUS (GE Healthcare Biosciences AB, Uppsala, Sweden) density gradient centrifugation.

Cell Surface Staining

PBMCs were incubated with directly conjugated antibodies for 30 min at 4°C. The cells were washed before flow cytometric analysis. The antibodies used were anti-human CD3-BV786, CD4-APC-Fire750, CD8-BV510, CD45RA-AF700 (Becton, Dickinson, and Company [BD], Franklin Lakes, NJ, USA), and CD200R APC and CCR7-BV421 (BioLegend, San Diego, CA, USA). Additionally, 7-AAD (BD, Franklin Lakes, NJ, USA) was used to exclude non-viable cells.

Multiparameter Flow Cytometry

Cells stained with fluorescent antibodies were acquired with an LSR Fortessa flow cytometer (BD Biosciences) and analyzed with FlowJo software (Tree Star, Ashland, OR, USA).

Statistical Analysis

Statistical analysis was performed using GraphPad 6 (GraphPad Software, La Jolla, CA, USA) or SPSS 23.0 (IBM Corporation, New York, NY, USA). Clinical and demographic characteristics were summarized as the mean ± standard deviation, median and 25th and 75th percentiles for continuous variables, and frequency and percentage for categorical variables. The Kolmogorov–Smirnov test was used to assess the normality of the sample data distribution. Fisher's exact test or χ^2 test was used to compare the categorical variables. A one-way ANOVA test or Kruskal–Wallis test was performed to compare two more independent samples. The accuracy of prognosis was evaluated using the receiver operating characteristic (ROC) curve, and the area under the ROC (AUROC) curve was calculated. For all analyses, a p value of < 0.05 was considered statistically significant.

RESULTS

Patient Characteristics and Baselines

The demographics and characteristics of the subjects are shown in **Table 1**. The HBV-ACLF group displayed expected differences in liver function and coagulation function parameters compared with the CHB and HC groups. Higher levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), NLR and INR were observed in patients with HBV-ACLF. Notably, the increase in NLR was mainly caused by a decrease in lymphocyte count. Additionally, PTA and albumin levels were significantly lower ($p < 0.001$) than those in patients with CHB.

TABLE 2 | Characteristics of the survival and non-survival groups in patients with HBV-ACLF.

Variables	Survival $n = 34$	Non-survival $n = 7$	p-value
Demographics			
Age (year)	44.0 (30.8,58.5)	47.0 (46.0,50.0)	0.859
Male/Female, %	25/9	7/0	0.315
Laboratory data			
WBC (10^9/L)	4.7 (3.9,6.5)	5.4 (5.2,13.1)	0.079
NC (10^9/L)	3.1 ± 1.3	6.4 ± 4.5	<0.001
LC (10^9/L)	1.4 ± 0.6	1.2 ± 0.4	0.352
NLR	2.3 (1.4,2.7)	5.2 ± 3.3	0.031
PLT (10^9/L)	79.0 (61.6,109.4)	125.7 ± 63.2	0.077
ALT (U/L)	344.2 (127.5,784.5)	739.6 ± 839.1	0.905
AST (U/L)	217.6 (87.2,472.4)	364.7 ± 276.4	0.552
ALB (g/L)	32.6 ± 4.6	33.2 ± 4.8	0.748
GLO (g/L)	28.0 ± 6.8	25.0 ± 7.5	0.312
TBIL (μmol/L)	238.9 ± 119.6	251.0 (204.2,295.0)	0.444
Cr (μmol/L)	64.0 ± 13.6	63.3 (53.0,78.0)	0.832
Na (mmol/L)	135.0 (135.0,135.0)	135.0 (135.0,135.0)	0.690
PTA (%)	38.8 ± 9.6	26.4 ± 8.1	0.003
INR	2.0 (1.8,2.3)	3.1 ± 1.3	0.003
logHBVDNA log (IU/mL)	5.0 ± 1.5	6.9 (3.5,7.3)	0.158

CHB, chronic hepatitis B; ACLF, acute-on-chronic liver failure; WBC, white blood cell; LC, lymphocyte count; NC, neutrophil count; NLR, neutrophil-lymphocytes ratio; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; GLO, globulin; TBIL, total bilirubin; Cr, creatinine; Na, sodium; PTA, prothrombin activity; INR, international normalized ratio; p values were calculated by χ^2 or Fisher exact test for categorical variables and unpaired t test or Mann-Whitney test for continuous variables.

In total, 41 patients were included for the analysis, 4 out of 45 patients were excluded for liver transplantation. The differences between the survival and non-survival groups are shown in **Table 2**. Higher levels of neutrophil count and INR and lower level of PTA were observed in the non-survival group, indicating that infection and worse coagulation function may contribute to the adverse outcomes.

Peripheral Frequencies of T Cell Subsets in Patients With HBV-ACLF

First, we investigated the differentiation status of peripheral T cells in patients with HBV-ACLF, including naïve T cells (T_N, $CCR7^+CD45RA^+$), central memory T cells (T_{CM}, $CCR7^+CD45RA^-$), effector memory T cells (T_{EM}, $CCR7^-CD45RA^-$), and terminally differentiated effector cells (T_{EMRA}, $CCR7^-CD45RA^+$) (**Figure 1A**). As shown in **Figure 1B**, in $CD4^+$ T cell subsets, the proportion of T_N and T_{CM} cells in patients with HBV-ACLF was significantly lower than that in the HC group, and the proportion of T_{EMRA} cells was significantly higher than that in the HC group. Similar

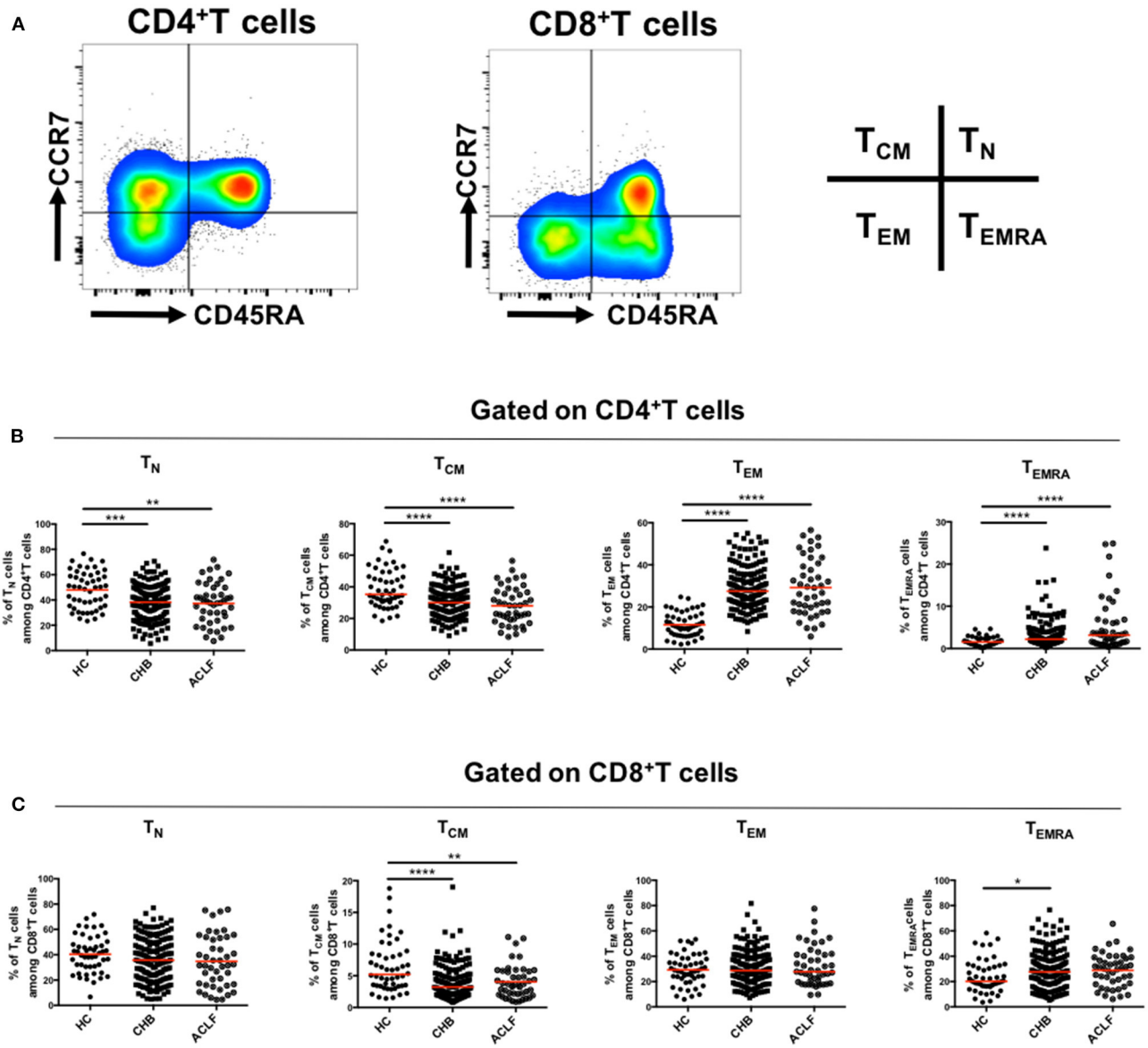


FIGURE 1 | Differentiation of circulating T cells. Representative flow data gated on $CD4^+$ and $CD8^+$ T cells **(A)** and scatter dot plots of the percentage of T_N, T_{CM}, T_{EM}, and T_{EMRA} subsets gated on $CD4^+$ **(B)** and $CD8^+$T cells **(C)** in different groups. A one-way ANOVA or Kruskal–Wallis test was used to analyze statistical differences. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

results were observed in $CD8^+$ T_{CM} cells and $CD8^+$ T_{EMRA} cells (**Figure 1C**). However, the number of $CD8^+$ T_{EM} cells did not increase as in $CD4^+$T cells. Additionally, no differences were observed between patients with HBV-ACLF and CHB in these subsets.

The proportions of $CD200R^+$T cells in both $CD4^+$ (**Figure 2A**) and $CD8^+$ (**Figure 2B**) T cells were increased in patients with HBV-ACLF and CHB compared to the HC group. Moreover, the proportion of $CD200R^+CD8^+$T cells in patients with HBV-ACLF was significantly higher than that in patients with CHB.

CD200R Expression Levels on Different Subsets of Circulating T Cells

To further determine the upregulation of CD200R in patients with HBV-ACLF, we investigated whether CD200R was differentially expressed on each differentiation subset of T cells. As shown in **Figure 3A**, CD200R expression on each differentiation subset was dramatically increased in patients with HBV-ACLF compared with that in HCs on $CD4^+$ T cells, along with a more significant increase in the proportions of $CD8^+$ T_{CM} and $CD8^+$ T_{EM} cells (**Figure 3B**). This indicated that elevated $CD200R^+$ frequency was caused not only by variation in the

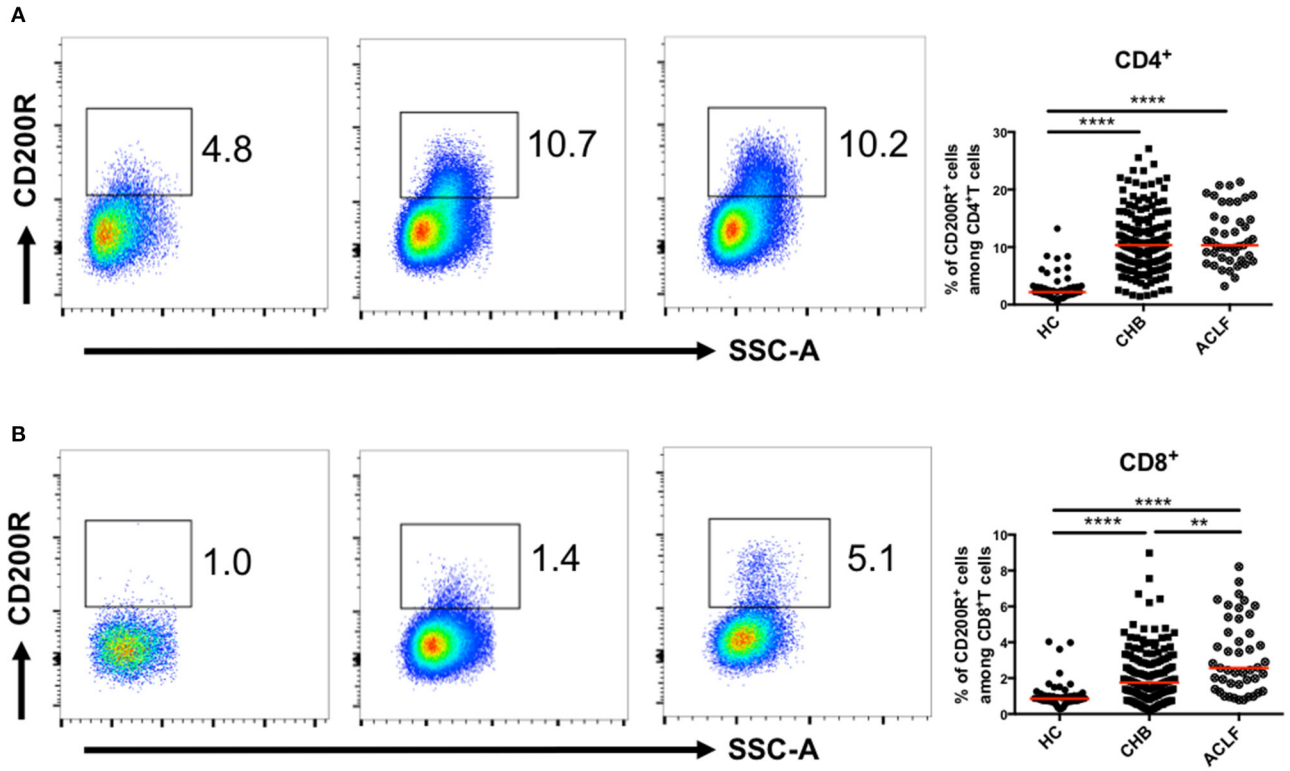


FIGURE 2 | CD200R$^+$T cells in patients with ACLF and CHB. Representative dot plots of CD200R$^+$CD4$^+$ **(A)** and CD200R$^+$CD8$^+$ **(B)** T cell frequency for each group. A Kruskal–Wallis test was used to analyze statistical differences. $^{**}p < 0.01$, $^{****}p < 0.0001$.

proportion of T cell subsets, but also the expression on each differentiation subset.

Decreased Frequency of Circulating CD200R$^+$CD4$^+$T Cells Was Associated With a Poor Survival Rate for HBV-ACLF

To further investigate the correlation between the indicators and prognosis of HBV-ACLF, we compared the frequencies of the subsets in each group. A lower percentage of CD200R$^+$CD4$^+$T cells was observed in the non-survival group than in the survival group ($p = 0.0013$) (**Figure 4A**). Consistent with the findings of our previous study, the NLR was significantly higher in the non-survival group than in the survival group ($p = 0.0309$) (**Figure 4B**). Additionally, the proportion of CD4$^+$ and CD8$^+$ T_N cells increased significantly in the non-survival group, accompanied by a decreased proportion of CD4$^+$ and CD8$^+$ T_{EM} subsets (**Supplementary Figure 1**). Thus, it is suggested that the dysfunction of T_{EM} subsets may not only play a role in the pathogenesis of ACLF but also affect adverse outcomes of ACLF.

CD200R Combined With the NLR Could Predict HBV-ACLF Prognosis

The relationship between baseline CD200R$^+$CD4$^+$T cell frequency and prognosis for patients with HBV-ACLF was determined at the 90d follow-up. As shown in **Figure 5**, the baseline level of CD200R$^+$CD4$^+$T cells yielded an area under the receiver operating characteristic [AUROC (95% CI)] [0.868 (0.733–1.000)] curve that predicted 90 d mortality rate vs. that of NLR, model for end-stage liver disease (MELD), MELD-Na, Child-Turcotte-Pugh (CTP), and chronic liver failure-consortium ACLF (CLIF-C ACLF) score [0.761 (0.538–0.983), 0.840 (0.672–1.000), 0.870 (0.702–1.000), 0.580 (0.322–0.838), and 0.840 (0.684–0.996), respectively]. A combination of CD200R$^+$CD4$^+$T cells and NLR was used to predict mortality in patients with HBV-ACLF. It was observed that the combination (AUROC [95% CI] [0.916 (0.782–1.000)] predicted 90 d mortality better than that of CD200R$^+$CD4$^+$T cells alone. At the cut-off point of−3.87, which matched the maximum Youden index determined by ROC analysis, the positive predictive and negative predictive values for mortality were 0.86 and 0.97, respectively.

Discussion

In the present study, we investigated the frequencies of CD200R$^+$ and the differentiation status of T cells in patients with HBV-ACLF and their possible role in predicting prognosis. With chronic HBV infection, the T lymphocyte response plays an important role in host immunity. Elevated expression of

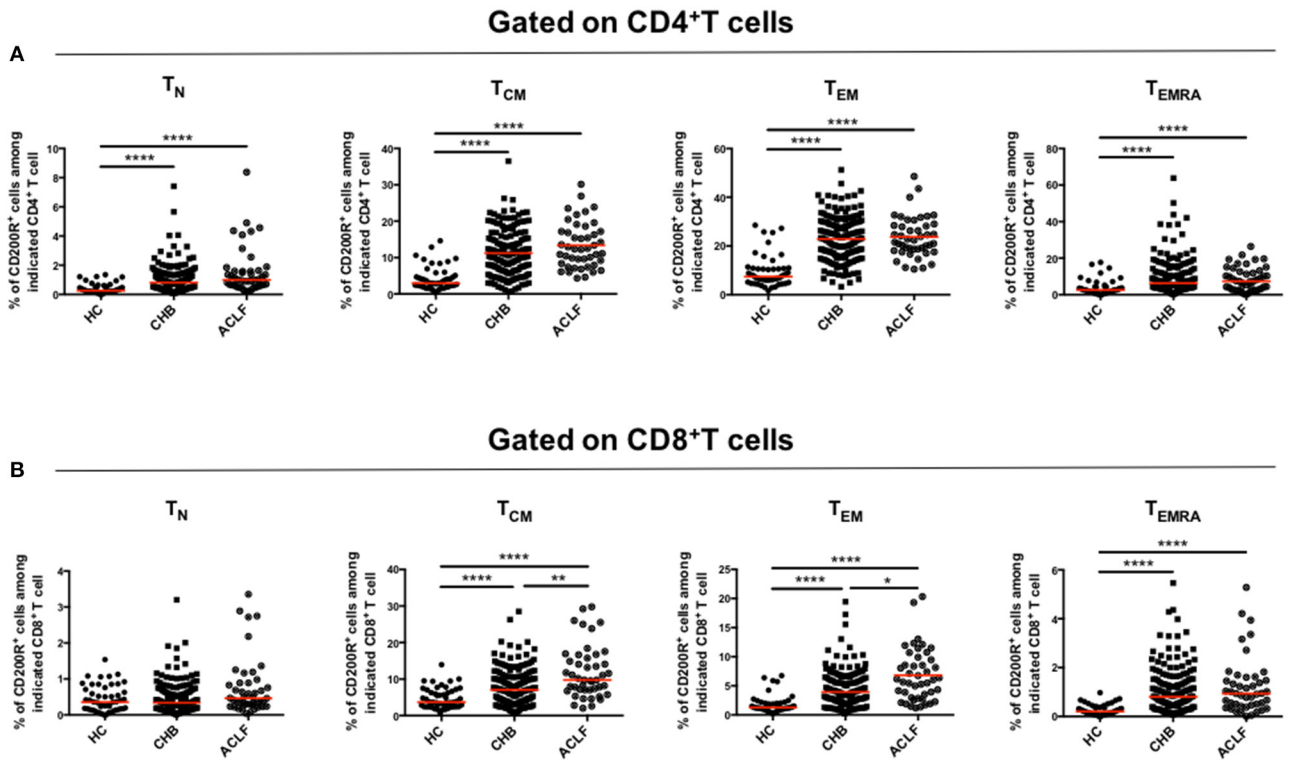


FIGURE 3 | CD200R is significantly upregulated on T_{CM} and T_{EM} cells in patients with ACLF. Scatter dot plots of CD200R expression in each group among four subsets (T_N, T_{CM}, T_{EM}, and T_{EMRA}) gated on $CD4^+$T cells **(A)** and $CD8^+$T cells **(B)**. A one-way ANOVA or Kruskal–Wallis test was used to analyze statistical differences. $^{**}p < 0.01$, $^{****}p < 0.0001$.

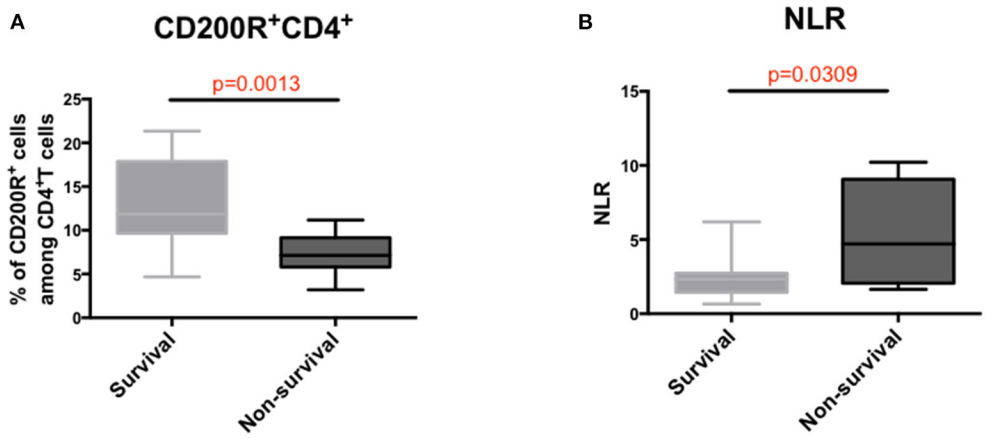


FIGURE 4 | Analysis of prognostic factors in patients with ACLF. **(A)** Comparison of the proportions of $CD200R^+CD4^+$T cells in ACLF survival and non-survival groups. **(B)** Comparison of NLR levels between survival and non-survival groups with ACLF. A Mann–Whitney test was used to analyze statistical differences.

coinhibitory receptors on T cells was also found in patients with CHB and acute liver injury and was correlated with disease progression in HBV infection (21, 22). Although increased CD200R expression was found in both $CD4^+$ and $CD8^+$T cells in patients with HBV-ACLF and CHB, only the percentage of $CD200R^+CD8^+$T cells was further upregulated in patients with HBV-ACLF. Further analysis revealed that upregulation of CD200R occurred in each differentiation subset. Moreover, although the percentage of $CD8^+T_{CM}$ cells was decreased as that of $CD4^+T_{CM}$ cells, the level of $CD8^+T_{EM}$ cells was not upregulated, as in $CD4^+$T cells. This suggests that further dysfunction of T cells, especially defective

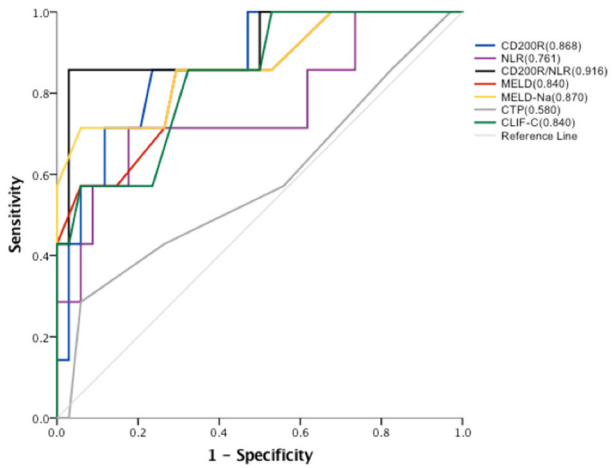


FIGURE 5 | ROC curve of each factor and areas under ROC curves. Proportions of baseline CD200R$^+$CD4$^+$T cells, NLR, CD200R/NLR, MELD, MELD-Na, CTP and CLIF-C scores in predicting 90 d mortality in patients with HBV-ACLF.

CD8$^+$T cell function, may contribute to the pathogenesis of HBV-ACLF.

Interestingly, in contrast to the increase in peripheral frequencies of CD200R$^+$T cells in patients with HBV-ACLF and CHB compared with HCs, CD200R$^+$CD4$^+$T cells in the non-survival HBV-ACLF group were decreased. The 90 d prognosis analysis of patients with HBV-ACLF but without liver transplantation showed that the proportion of CD200R$^+$CD4$^+$T cells in the HBV-ACLF non-survival group was lower than that in the survival group. Notably, the opposite tendencies were observed in T_N cells, demonstrating statistical differences between the survival and non-survival groups. Compared with the healthy control group, the proportion of CD4$^+$$T_N$ cells in patients with HBV-ACLF decreased and differentiated into effector T cells, whereas the proportion of effector memory CD8$^+$T cells did not change significantly. In summary, the above results suggest that T cells might play different roles in the pathogenesis and prognosis of HBV-ACLF.

Previous studies have found that CD200 and CD200R can provide negative regulatory signals, change the response threshold of myeloid cells to stimulation signals, reduce the activity of myeloid cells, and maintain immune homeostasis (23–25). Multiple effects on T cells have been reported in interactions between CD200/CD200R, including the shift from a Th1 cytokine profile to a Th2 cytokine profile (26) and the inhibition of the CTL response (27). Ren Y et al. (28) revealed that the CD200/CD200R interaction could reduce the differentiation of CD4$^+$T cells into Th17 cells in rheumatoid arthritis, downregulate Th17 chemotaxis mediated by chemokine receptor 6, and reduce the inflammatory response. Additionally, the CD200/CD200R interaction could indirectly regulate T cell function through macrophages or dendritic cells (29, 30). Therefore, the decrease in the proportion of CD200R$^+$CD4$^+$T cells could possibly promote the inflammatory response and aggravate tissue damage, affecting the prognosis of patients with HBV-ACLF. To further verify this hypothesis, additional functional experiments must be conducted, and the identification of the interaction between CD200R and other coinhibitory molecules should be the focus of future studies.

Our study findings also showed that the baseline percentage of CD200R$^+$CD4$^+$T cells was a potential predictive marker for 90 d mortality in patients with HBV-ACLF. NLR has been proved to be a predictor of the prognosis in patients with HBV-ACLF (18). A combination of CD200R and NLR provided a better prediction of 90 d mortality than CD200R alone. This further illustrates the potential importance of CD200R in the prognosis of HBV-ACLF.

In summary, during the development of HBV-ACLF, the role of T cells in promoting or suppressing inflammation may shift with variations in regulatory factors. CD200R, as a potential predictor and possible mechanism of HBV-ACLF pathogenesis, is worthy of further study. However, there were some limitations to our study. The number of cases was limited, and subjects were recruited from a single center; thus, further validation and mechanistic research need to be performed in the future.

CONCLUSION

Overall, CD200R combined NLR offers potential predictive value regarding the mortality of HBV-ACLF, and the findings could contribute to the elucidation of the pathogenesis of HBV-ACLF.

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 Beijing Ditan Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XW and HZ were responsible for the conception, design of the study, revised, and commented on the draft. YL and YK performed the analysis and interpretation of the data. KS, YH, QZ, and BZ participated in the data collection and follow-up of patients. YL drafted the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by National Natural Science Foundation of China (81774234 and 81804009)

and Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support (ZYLX201707).

ACKNOWLEDGMENTS

We thank all the patients participated in this research.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.762296/full#supplementary-material

Supplementary Figure 1 | Analysis of prognostic factors in patients with ACLF. Comparison of T cell differentiation between survival and non-survival groups with ACLF. Mann–Whitney or unpaired *t* test was used to analyze statistical differences.

REFERENCES

1. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian pacific association for the study of the liver (APASL): an update. *Hepatol Int.* (2019) 13:353–90. doi: 10.1007/s12072-019-09946-3
2. Hernaez R, Solà E, Moreau R, Ginès P. Acute-on-chronic liver failure: an update. *Gut.* (2017) 66:541–53. doi: 10.1136/gutjnl-2016-312670
3. Gustot T, Fernandez J, Garcia E, Morando F, Caraceni P, Alessandria C, et al. Clinical course of acute-on-chronic liver failure syndrome and effects on prognosis. *Hepatology.* (2015) 62:243–52. doi: 10.1002/hep.27849
4. Park JJ, Wong DK, Wahed AS, Lee WM, Feld JJ, Terrault N, et al. Hepatitis B Virus-specific and global T-Cell dysfunction in chronic hepatitis B. *Gastroenterology.* (2016) 150:684–95. doi: 10.1053/j.gastro.2015.11.050
5. Yang F, Yu X, Zhou C, Mao R, Zhu M, Zhu H, et al. Hepatitis B e antigen induces the expansion of monocytic myeloid-derived suppressor cells to dampen T-cell function in chronic hepatitis B virus infection. *PLoS Pathog.* (2019) 15:e1007690. doi: 10.1371/journal.ppat.1007690
6. Ye B, Liu X, Li X, Kong H, Tian L, Chen Y, et al. T-cell exhaustion in chronic hepatitis B infection: current knowledge and clinical significance. *Cell Death Dis.* (2015) 6:e1694. doi: 10.1038/cddis.2015.42
7. Bengsch B, Martin B, Thimme R. Restoration of HBV-specific CD8+ T cell function by PD-1 blockade in inactive carrier patients is linked to T cell differentiation. *J Hepatol.* (2014) 61:1212–19. doi: 10.1016/j.jhep.2014.07.005
8. Schurich A, Khanna P, Lopes AR, Han KJ, Peppa D, Micco L, et al. Role of the coinhibitory receptor cytotoxic T lymphocyte antigen-4 on apoptosis-Prone CD8 T cells in persistent hepatitis B virus infection. *Hepatology.* (2011) 3:1494–503. doi: 10.1002/hep.24249
9. Raziorrouh B, Schraut W, Gerlach T, Nowack D, Grüner NH, Ulsenheimer A, et al. The immunoregulatory role of CD244 in chronic hepatitis B infection and its inhibitory potential on virus-specific CD8+ T-cell function. *Hepatology.* (2010) 52:1934–47. doi: 10.1002/hep.23936
10. Zeng Y, Li Y, Xu Z, Gan W, Lu L, Huang X, et al. Myeloid-derived suppressor cells expansion is closely associated with disease severity and progression in HBV-related acute-on-chronic liver failure. *J Med Virol.* (2019) 91:1510–18. doi: 10.1002/jmv.25466
11. Yang J, Yi P, Wei L, Xu Z, Chen Y, Tang L, et al. Phenotypes and clinical significance of circulating CD4(+)CD25(+) regulatory T cells (Tregs) in patients with acute-on-chronic liver failure (ACLF). *J Transl Med.* (2012) 10:193. doi: 10.1186/1479-5876-10-193
12. Dong X, Gong Y, Zeng H, Hao Y, Wang X, Hou J, et al. Imbalance between circulating CD4+ regulatory T and conventional T lymphocytes in patients with HBV-related acute-on-chronic liver failure. *Liver Int.* (2013) 33:1517–26. doi: 10.1111/liv.12248
13. Barclay AN, Wright GJ, Brooke G, Brown MH. CD200 and membrane protein interactions in the control of myeloid cells. *Trends Immunol.* (2002) 23:285–90. doi: 10.1016/S1471-4906(02)02223-8
14. Wright GJ, Puklavec MJ, Willis AC, Hoek RM, Sedgwick JD, Brown MH, et al. Lymphoid/neuronal cell surface OX2 glycoprotein recognizes a novel receptor on macrophages implicated in the control of their function. *Immunity.* (2000) 13:233–42. doi: 10.1016/S1074-7613(00)00023-6
15. Moreaux J, Veyrune JL, Reme T, De Vos J, Klein B. CD200: a putative therapeutic target in cancer. *Bioche Biophys Res Commun.* (2008) 366:117–22. doi: 10.1016/j.bbrc.2007.11.103
16. Estep RD, Rawlings SD, Li H, Manoharan M, Blaine ET, O'Connor MA, et al. The rhesus rhadinovirus CD200 homologue affects immune responses and viral loads during in vivo infection. *J Virol.* (2014) 88:10635–54. doi: 10.1128/JVI.01276-14
17. Manich G, Recasens M, Valente T, Almolda B, González B, Castellano B. Role of the CD200-CD200R axis during homeostasis and neuroinflammation. *Neuroscience.* (2019) 405:118–36. doi: 10.1016/j.neuroscience.2018.10.030
18. Liu H, Zhang H, Wan G, Sang Y, Chang Y, Wang X, et al. Neutrophil-lymphocyte ratio: a novel predictor for short-term prognosis in acute-on- chronic hepatitis B liver failure. *J Viral Hepat.* (2014) 21:499–507. doi: 10.1111/jvh.12160
19. Chinese Society of Hepatology, Chinese Medical Association, Chinese Society of Infectious Diseases, Chinese Medical Association, Hou JL, Lai W. The guideline of prevention and treatment for chronic hepatitis B: a 2015 update. *Zhonghua Gan Zang Bing Za Zhi.* (2015)23: 888–905. doi: 10.3760/cma.j.issn.1007-3418.2015.12.002
20. Sarin SK, Kedarisetty CK, Abbas Z, Amarapurkar D, Bihari C, Chan AC, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian pacific association for the study of the liver (APASL) 2014. *Hepatol Int.* (2014) 8:453–71. doi: 10.1007/s12072-014-9580-2
21. Wu W, Shi Y, Li J, Chen F, Chen Z, Zheng M. Tim-3 expression on peripheral T cell subsets correlates with disease progression in hepatitis B infection. *Virol J.* (2011) 8:113. doi: 10.1186/1743-422X-8-113
22. Xu P, Chen YJ, Chen H, Zhu XY, Song HF, Cao LJ. The expression of programmed death-1 in circulating CD4+ and CD8+ T cells during hepatitis B virus infection progression and its correlation with clinical baseline characteristics. *Gut Liver.* (2014) 8:186–95. doi: 10.5009/gnl.2014.8.2.186
23. Jenmalm MC, Cherwinski H, Bowman EP, Phillips JH, Sedgwick JD. Regulation of myeloid cell function through the CD200 receptor. *J Immunol.* (2006) 176:191–9. doi: 10.4049/jimmunol.176.1.191
24. Gorczynski RM, Yu K, Clark D. Receptor engagement on cells expressing a ligand for the tolerance-inducing molecule OX2 induces an immunoregulatory population that inhibits alloreactivity in vitro and in vivo. *J Immunol.* (2000) 165:4854–60. doi: 10.4049/jimmunol.165.9.4854
25. Kotwica-Mojzych K, Jodłowska-Jedrych B, Mojzych M. CD200:CD200R interactions and their importance in immunoregulation. *Int J Mol Sci.* (2021) 22:1602. doi: 10.3390/ijms22041602
26. Gorczynski L, Chen Z, Hu J, Kai Y, Lei J, Ramakrishna V, et al. Evidence that an OX-2-positive cell can inhibit the stimulation of type 1 cytokine production by bone marrow-derived B7-1 (and B7-2)-positive dendritic cells. *J Immunol.* (1999) 162:774–81.
27. Boudakov I, Liu J, Fan N, Gulay P, Wong K, Gorczynski RM. Mice lacking CD200R1 show absence of suppression in LPS-induced TNF and MLC responses by CD200. *Transplantation.* (2007) 84:251–7. doi: 10.1097/01.tp.0000269795.04592.cc
28. Ren Y, Yang B, Yin Y, Leng X, Jiang Y, Zhang L, et al. Aberrant CD200/CD200R1 expression and its potential role in Th17 cell differentiation, chemotaxis and osteoclastogenesis in rheumatoid arthritis. *Rheumatology.* (2015) 54:712–21. doi: 10.1093/rheumatology/keu362
29. Gorczynski R, Khatri I, Lee L, Boudakov I. An interaction between CD200 and monoclonal antibody agonists to CD200R2 development of dendritic cells that preferentially induce of CD4+25+T regulatory cells. *J Immunol.* (2008) 180:5946–55. doi: 10.4049/jimmunol.180.9.5946
30. Koning N, van Eijk M, Pouwels W, Brouwer MS, Voehringer D, Huitinga I, et al. Expression of the inhibitory CD200 receptor is associated

with alternative macrophage activation. *J Innate Immun.* (2010) 2:195–200. doi: 10.1159/000252803

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership