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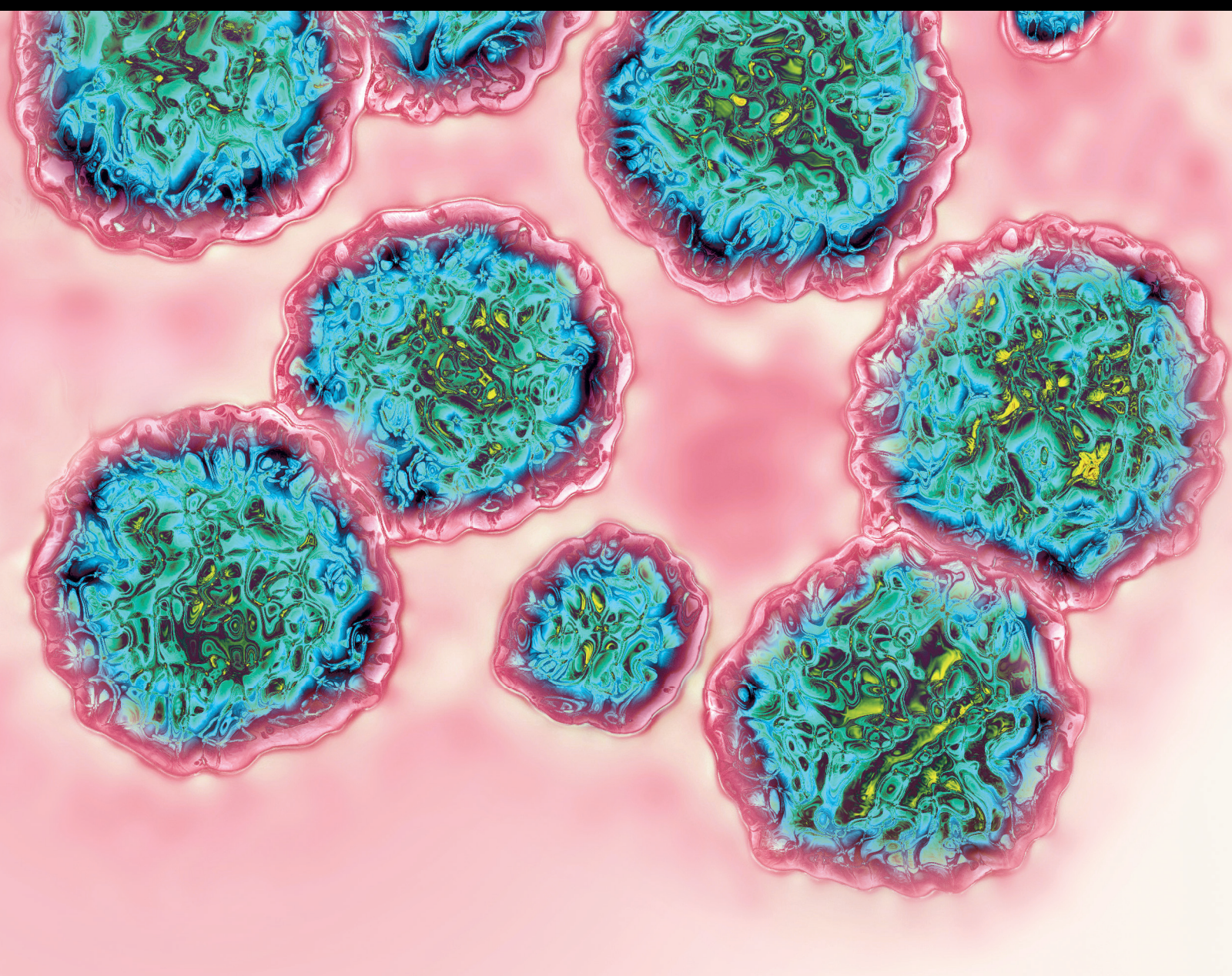
Acute-on-Chronic Liver Failure: From Basic Research to Clinical Applications

Lead Guest Editor: En-Qiang Chen
Guest Editors: Tetsuro Shimakami, Yu-Chen Fan, and Paolo Angeli



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Editorial Board

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Editorial

Acute-on-Chronic Liver Failure: From Basic Research to Clinical Applications

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Acute-on-chronic liver failure (ACLF) is a clinical syndrome of acute hepatic decompensation observed in patients with preexisting chronic liver disease (CLD) characterized by one or more extrahepatic organ failures with a significantly increased risk of death [1]. It is well known that the etiology of ACLF would be a precipitating event on a preexisting liver condition. However, no matter the etiology of preexisting chronic liver damage, ACLF patients are always with ascites, jaundice, portal hypertension with variceal bleeding, and/or hepatic encephalopathy [2]. Though the exact pathogenesis of ACLF is not fully elucidated in past decades, there is also significant progress in its basic and clinical research. There are still huge differences in the definitions of ACLF between the West and East [3]. As far as the precipitating events, in the West, acute alcohol injury and bacterial infections are the most common. In the East, this is true also for India and Korea, while in China reactivation of chronic hepatitis B is the most common.

ACLF can develop in patients with any underlying cirrhosis/CLD [1, 4]. According to our estimation, ACLF may be still a common severe liver disease in the coming decade. How to prevent the occurrence and development of ACLF and how to effectively treat and predict the prognosis of ACLF patients are still embarrassing problems that we have to face in real-life clinical practice. Nowadays, besides the prevention of precipitating factors leading to acute hepatic decompensation, the effective management of ACLF should also include active supportive care and earliest initiation of specific therapies against cytokine storm [5]. For ACLF patients with particularly serious condition, artificial liver support system and possible liver transplantation should be also considered.

This special issue encompasses basic and clinical studies as well as review article focusing on ACLF. It includes 6 research articles, 1 clinical study, and 1 review article describing the advance of the pathogenesis and clinical management of ACLF, and all of them are summarized as follows.

According to the review article titled "Systemic Inflammation and Acute-on-Chronic Liver Failure: Too Much, Not Enough," dysbalanced immune function is central to ACLF's pathogenesis and outcome with an initial excessive systemic inflammatory response that drives organ failure and mortality. And the immunoexhaustion/immunoparalysis in later course prevails predisposing the patient to secondary infectious events and reescalation in end-organ dysfunction and mortality. In this review, W. Laleman et al. also mention that the management of ACLF patients is still poorly defined at present. However, with understanding of the pathophysiology of ACLF gradually deepened, potential therapeutic targets emerge that warrant further study such as restoring or substituting albumin via plasma exchange or via albumin dialysis and evaluating usefulness of TLR4 antagonists, modulators of gut dysbiosis, and FXR-agonists.

The research article titled "Management Strategies and Outcomes for Hyponatremia in Cirrhosis in the Hyponatremia Registry" aims to assess the treatment practices and effectiveness in cirrhotic patients with hyponatremia (HN) in the HN Registry. It is well-known that dilutional HN is a frequent consequence of severe portal hypertension in patients with cirrhosis and ACLF. In this research study, S.

Sigal et al. have included 595 cirrhotic patients with HN and compared the characteristics, treatments, and outcomes between patients with HN at admission and during hospitalization. They found that patients with HN at admission have a lower [Na] and shorter length of stay (LOS) than those who develop HN; and the treatment approaches for HN are variable and frequently ineffective. However, success is greatest with hypertonic saline and tolvaptan; and relapse of HN is associated with increased LOS. The findings from the study have given us a deeper understanding of the effectiveness of treatment strategies and impact on LOS for hospitalized cirrhotic patients with HN.

The research article titled "The Correlation between miR-122 and Lipoprotein Lipase Expression in Chronic Hepatitis C Patients" aims to analyze the relationship between viral load, lipid profile, IFNγ, and the expression of miR-122 and LPL in the liver and PBMCs. As we know, miR-122 is the foremost liver-related micro-RNA [6], and it is reported that chronic hepatitis C (CHC) patients have very elevated serum miR-122 levels in the range of most patients with severe hepatic injury leading to acute liver failure [7]. In this research article, M. Sidorkiewicz et al. have enrolled 17 chronic hepatitis C patients with matching sera, PBMCs, and liver tissue specimen and found that liver (not PBMCs) miR-122 expression is positively correlated with HCV RNA load and IFNγ and reversely with LPL expression in CHC patients. Importantly, the results of the study firstly reveal the reverse correlation of miR-122 and LPL expression in liver. And the findings from the study would help us to have a deeper understanding of miR-122 in the occurrence and development of ACLF from CHC patients in future.

The research article titled "Serum Metabonomics Analysis of Liver Failure Treated by Nonbioartificial Liver Support Systems" aims to analyze the small molecular metabolic compounds of nonbioartificial liver for treatment of hepatic failure; and a total of 52 patients who meet the standard of artificial liver treatment for liver failure are enrolled. In this research article, significant changes in metabolic compounds of liver failure in the treatment before and after artificial liver are screened by using Ultra-Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS), and related metabolic pathways are analyzed by MetaboAnalyst. Corresponding results suggest that artificial liver treatment could have significant effects on fatty acids and primary bile acid synthesis pathways; and the changes of fatty acid, primary bile acid synthesis pathway, and phenylalanine metabolic pathway are also significantly different among different artificial liver patterns of chronic liver failure. The findings from the study have somehow enriched our knowledge of the mechanism of Nonbioartificial Liver Support Systems in therapy of liver failure patients.

The research article titled "Transcriptome Analysis of Porcine PBMCs Reveals the Immune Cascade Response and Gene Ontology Terms Related to Cell Death and Fibrosis in the Progression of Liver Failure" aims to gain a deeper understanding of the transcriptional response of PBMCs following ALF. In this basic research article, Y. Zhang et al. have found that the dramatic PBMC transcriptome changes triggered by ALF progression are predominantly related to immune responses. And the enriched GO terms related to cell death, fibrosis, and so on, as indicated by PBMC transcriptome analysis, seem to be useful in elucidating potential key gene sets in the progression of ALF. As the authors have mentioned, a better understanding of these gene sets might be of preventive or therapeutic interest.

The clinical study titled "Good Tolerance of Citrate Accumulation due to Plasma Exchange among Patients with Acute-on-Chronic Liver Failure: A Prospective, Observational Study" is conducted among ACLF patients who plan to receive heparin anticoagulation during PE-centered therapy without filtration and dialysis. In this clinical research article, Y. Ma et al. have enrolled 54 patients, and they report that hypocalcemia (ionized calcium) and mild alkalosis are the main metabolic alterations. Additionally, no symptom associated with hypocalcemia occurred. To some extent, this study has suggested that citrate accumulation is well tolerated by ACLF patients who receive PE-centered therapy without filtration and dialysis. However, large sample size studies are required to confirm the present findings.

The research article titled "Alpha-Fetoprotein as a Predictive Marker for Patients with Hepatitis B-Related Acute-on-Chronic Liver Failure" aims to evaluate the prognostic effect of serum AFP on the prediction of HBV-ACLF outcomes. The results are basically consistent with previous similar studies. Recently, the role of stratified AFP is also concerned, and its power for predicting short-term survival of HBV-ACLF patients is superior to the absolute level of serum AFP [8]. For example, the Combining AFP quartiles with low serum sodium and high INR may better predict poor outcome in HBV-ACLF patients [8]. Previous reports have suggested that higher serum AFP levels could reflect a better regeneration ability of injured liver in patients [9, 10], and this may explain why high serum AFP level could predict a good outcome of ACLF patients.

The research article titled "Bacterial Infection and Predictors of Mortality in Patients with Autoimmune Liver Disease-Associated Acute-on-Chronic Liver Failure" aims to investigate bacterial infection and predictors of mortality among autoimmune liver disease-associated ACLF. In this retrospectively study, 53 ACLF patients and 53 patients without ACLF have been analyzed. Patients with bacterial infection have displayed a more severe condition than those without infection, and elevated CRP is found to be an accurate marker for diagnosing bacterial infection in autoimmune liver disease-associated ACLF patients. In fact, bacterial infection is an important precipitating factor of ACLF in one-third of ACLF, irrespective of the etiology of preexisting CLD [11, 12]. And the occurrence of bacterial infection is usually thought to be caused by systemic translocation of microbes from gut-derived organisms, impaired hepatic clearance mechanisms, and an ability of circulating immune cells to combat infectious cues [11, 13].

Conflicts of Interest

Prof. Paolo Angeli has been served as a Consultant, Advisory Board, or Speaker for Ferring Pharmaceuticals A/S (2018), Grifols (2018), Sequana Medical AG (2014-2018), Biovie

(2016-2018), Gilead (Italy)(2016), Bhering (2014-2018), and Kedrion (2016) and also received grants from Grifols (2018), Gilead (Italy)(2016) and Bhering (2014-2018).

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References

[1] R. Moreau, R. Jalan, P. Gines et al., "Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis," *Gastroenterology*, vol. 144, no. 7, pp. 1426.e9–1437.e9, 2013.

[2] W. Bernal, R. Jalan, A. Quaglia, K. Simpson, J. Wendon, and A. Burroughs, "Acute-on-chronic liver failure," *The Lancet*, vol. 386, no. 10003, pp. 1576–1587, 2015.

[3] H. Singh and C. G. Pai, "Defining acute-on-chronic liver failure: East, West or Middle ground?" *World Journal of Hepatology*, vol. 7, no. 25, pp. 2571–2577, 2015.

[4] S. K. Sarin, C. K. Kedarisetty, Z. Abbas et al., "Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) 2014," *Hepatology International*, vol. 8, no. 4, pp. 453–471, 2014.

[5] J. Clària, V. Arroyo, and R. Moreau, "The acute-on-chronic liver failure syndrome, or when the innate immune system goes astray," *The Journal of Immunology*, vol. 197, no. 10, pp. 3755–3761, 2016.

[6] W. J. Wang, R. T. Lai, J. Lu et al., "Correlation between circulating miR-122 and prognosis of chronic HBV-related liver failure," *Journal of Digestive Diseases*, vol. 17, no. 5, pp. 334–339, 2016.

[7] P. H. Dubin, H. Yuan, R. K. Devine et al., "Micro-RNA-122 levels in acute liver failure and chronic hepatitis C," *Journal of Medical Virology*, vol. 86, no. 9, pp. 1507–1514, 2014.

[8] G. Huang, Y. Xie, P. Zhu et al., "Stratified alpha-fetoprotein pattern accurately predicts mortality in patients with acute-on-chronic hepatitis B liver failure," *Expert Review of Gastroenterology & Hepatology*, vol. 12, no. 3, pp. 295–302, 2018.

[9] Y. Tao, M. Wang, E. Chen, and H. Tang, "Liver Regeneration: Analysis of the Main Relevant Signaling Molecules," *Mediators of Inflammation*, vol. 2017, Article ID 4256352, 9 pages, 2017.

[10] F. V. Schiodt, G. Ostapowicz, N. Murray et al., "Alpha-fetoprotein and prognosis in acute liver failure," *Liver Transplantation*, vol. 12, no. 12, pp. 1776–1781, 2006.

[11] L. Yang, T. Wu, J. Li, and J. Li, "Bacterial Infections in Acute-on-Chronic Liver Failure," *Seminars in Liver Disease*, vol. 38, no. 02, pp. 121–133, 2018.

[12] J. Fernández, J. Acevedo, R. Wiest et al., "Bacterial and fungal infections in acute-on-chronic liver failure: prevalence, characteristics and impact on prognosis," *Gut*, vol. 67, no. 10, pp. 1870–1880, 2018.

[13] T. Zhang, K. Sun, Y. Wang, L. Huang, R. Lang, and W. Jiang, "Disruption of the gut-liver axis in the pathogenesis of acute-on-chronic liver failure," *European Journal of Gastroenterology & Hepatology*, vol. 30, no. 2, pp. 130–135, 2018.

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Research Article

Management Strategies and Outcomes for Hyponatremia in Cirrhosis in the Hyponatremia Registry

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Aim. Treatment practices and effectiveness in cirrhotic patients with hyponatremia (HN) in the HN Registry were assessed. *Methods*. Characteristics, treatments, and outcomes were compared between patients with HN at admission and during hospitalization. For HN at admission, serum sodium concentration [Na] response was analyzed until correction to > 130 mmol/L, switch to secondary therapy, or discharge or death with sodium ≤ 130 mmol/L. *Results*. Patients with HN at admission had a lower [Na] and shorter length of stay (LOS) than those who developed HN ($P < 0.001$). Most common initial treatments were isotonic saline (NS, 36%), fluid restriction (FR, 33%), and no specific therapy (NST, 20%). Baseline [Na] was higher in patients treated with NST, FR, or NS versus hypertonic saline (HS) and tolvaptan (Tol) ($P < 0.05$). Treatment success occurred in 39%, 39%, 52%, 78%, and 81% of patients with NST, FR, NS, HS, and Tol, respectively. Relapse occurred in 55% after correction and was associated with increased LOS (9 versus 6 days, $P < 0.001$). 34% admitted with HN were discharged with HN corrected. *Conclusions*. Treatment approaches for HN were variable and frequently ineffective. Success was greatest with HS and Tol. Relapse of HN is associated with increased LOS.

1. Introduction

Dilutional hyponatremia (HN) is a frequent consequence of severe portal hypertension in cirrhosis. It is the result of severe vasodilation, leading to increased arginine vasopressin (AVP) release and consequent water retention [1, 2]. HN is especially common in the hospitalized patient [3] and is associated with severe ascites, hepatic encephalopathy (HE), and impaired renal function. In a retrospective study of 20,000 patients, HN was predictive of worsening disease, mortality, a higher 30-day readmission rate [4], and a 1.74-day increase in average hospital length of stay (LOS).

Management options for HN include discontinuation of diuretics, fluid restriction (FR), and administration of isotonic saline (NS), hypertonic saline (HS), or a vasopressin-receptor antagonist (or "vaptan"). FR is usually the first treatment used but is limited by patient adherence. Administration of NS and HS is problematic as they exacerbate fluid overload and ascites. Vaptans block the actions of AVP at vasopressin-2 receptors in cells of the renal collecting duct and provide a targeted approach to treatment in patients with inappropriately elevated AVP levels [5, 6]. There are currently two FDA-approved vaptans: conivaptan (Cumberland Pharmaceuticals, Inc., Nashville, Tennessee, USA) is a dual vasopressin-1A/2-receptor antagonist available for IV use, and tolvaptan (TO; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) is an oral selective vasopressin-2-receptor antagonist [5, 6]. Both are indicated for euvolemic and hypervolemic HN. Cirrhosis was initially an approved indication for TO but was subsequently removed due to the development of hepatocellular injury during an investigational study of its use in autosomal dominant polycystic kidney disease [7].

The effectiveness of treatment strategies and impact on LOS for hospitalized cirrhotic patients with HN have not been previously reported. The HN Registry (NCT01240668) is an observational, multicenter, real-world study of patients

hospitalized with euvolemic or hypervolemic HN. The objectives were to obtain clinical characteristics of patients and assess treatment practices, effectiveness, and resource utilization using LOS as a surrogate. The results of the entire population have previously been published [8]. In that report, the management and response of all patients with HN regardless of underlying condition were reported with only mention of the percentage of patients with cirrhosis. This analysis specifically assessed the subpopulation of patients with cirrhosis.

2. Materials and Methods

2.1. Study Design. Data from cirrhotic patients enrolled in the HN Registry [8] without concomitant nephrotic proteinuria, severe cardiomyopathy (ejection fraction <50%), or severe azotemia (creatinine ≥3.0 mg/dL) were entered in a database that included clinical characteristics, laboratory results, volume of fluid intake and output over each 24-hour period (if available), amount of FR, treatment with IV NS and HS, diuretics, medications used to treat HN, paracentesis, and LOS [8]. Severity of ascites and hepatic encephalopathy (HE) were recorded, and Child-Pugh scores, MELD, and MELD-Na scores were calculated [9]. Patients were classified as having HN diagnosed at the time of hospital admission versus hospital-acquired HN and categorized as mild ($[Na^+]$ > 125–130 mmol/L), moderate (120–125 mmol/L), and severe (< 120 mmol/L).

Initial treatment for HN was recorded. FR was based on an order by the treating physician. NS treatment was defined as administration of > 500 ml NS over a 24-hour period. No specified therapy (NST) was defined as observation for ≥2 days without a specific treatment. Patients who received NS, HS, or TO alone or in conjunction with FR were combined. A 1-day gap of no therapy between 2 treatment episodes constituted the end of initial treatment except for patients receiving TO in which case a 1-day gap was permitted. The study was exclusively observational, and treatment was solely determined by the treating physician.

Response to therapy for patients admitted with HN was assessed daily. On each day, patients were categorized based on HN severity and achievement of a treatment endpoint (correction to $[Na^+]$ > 130 mmol/L, increase in $[Na^+]$ ≥ 5 mmol/L from baseline, switch to another therapy, discharge with persistent HN, or death or transfer to hospice with persistent HN). The first $[Na^+]$ obtained on the day after treatment was discontinued and was then used as the end-of-therapy value. Patients in whom $[Na^+]$ corrected to > 130 mmol/L with initial therapy were assessed for relapse of HN during the subsequent hospitalization. Patients who were switched by the treating physician to a different treatment were assessed and reclassified based on second treatment provided in the same manner as that for the initial therapy.

At hospital discharge, final $[Na^+]$ and disposition (discharge home with corrected or persistent HN, and mortality [hospital death or transfer to hospice]) were recorded, and LOS was determined. For patients in whom discharge was delayed due to nonmedical reasons, the additional days were not included in LOS if documented as such in the clinical record.

2.2. Statistical Analysis. Clinical characteristics, initial and final $[Na^+]$, hospital mortality, and median LOS of patients with HN at hospital admission versus those who developed HN during hospitalization were compared. The relationship between HN severity and the various clinical parameters, LOS, and hospital mortality were assessed for patients admitted with HN.

Characteristics were compared among the various treatment groups. Cumulative endpoint outcomes were recorded for Days 1–5 and final outcomes at the end of primary treatment. The percentage of patients with initial [Na] < 125 in whom the level increased by ≥ 5 mmol/L on Days 2 and 3 and at the end of therapy was assessed. For patients with moderate or severe HN, the percentage of patients with an increase in $[Na^+]$ ≥ 5 mmol/L was assessed on Days 2 and 3 and end of treatment. A similar analysis was performed for patients who received a secondary therapy. For patients in whom $[Na^+]$ corrected to >130 mmol/L with initial therapy, characteristics and LOS were compared between patients who did and did not experience a relapse.

Descriptive statistics for continuous variables consisted of median number of observations and interquartile range (IQR). Frequency counts and percentages were obtained for categorical variables. Statistical comparisons of continuous variables were performed using nonparametric tests such as the Wilcoxon rank-sum test. Comparisons of categorical variables were performed using chi-square tests for association. Statistical significance for the tests was defined at the 5% level ($P < 0.05$).

2.3. Internal Review Board Approval. Approval was sought from the local research ethics review board at each site using either informed consent or a waiver of consent.

3. Results

3.1. Patient Characteristics. Of the 3087 patients who satisfied the inclusion and exclusion criteria, 650 (21%) had cirrhosis and 595 met the criteria for the current analysis. Baseline characteristics are presented in Table 1. HN was associated with advanced liver disease and severe portal hypertension (Table 2). HN was present in 518 patients (87%) on admission and developed during hospitalization in 77 (13%). Patients with HN on admission had lower initial $[Na^+]$, higher blood urea nitrogen (BUN), and MELD-Na score ($P < 0.05$; Table 2). More than half of the patients had large-volume ascites (Supplemental Table 1). Patients with moderate (25%; $P < 0.05$) or severe HN (28%; $P < 0.05$) more commonly had overt HE than those with mild HN (17%).

3.2. Initial HN Treatment. The most common initial therapies were NS (36%), FR (33%), NST (20%), TO (5%), and HS (2%; Supplemental Table 2). A variety of other therapies (e.g., salt tablets and conivaptan) and combinations were administered to 22 patients (4%). Initial $[Na^+]$ in the NST group was higher than in the other groups ($P < 0.05$). Initial $[Na^+]$ in the FR

Table 1: Baseline demographic characteristics.

	All Patients[i] (N = 3,087)	Cirrhosis (n = 630)
Age distribution, n (%)[a]		
≤50 y	479 (16)	190 (30)
51–64 y	937 (30)	339 (54)
65–74 y	587 (19)	81 (13)
≥75 y	1,084 (35)	20 (3)
Men, n (%)[b]	1,558 (51)	419 (67)
Race distribution: US only, n (%)[a]		
White	1,927 (74)	455 (72)
African-American	309 (12)	58 (9)
Asian	57 (2)	13 (2)
Other	154 (6)	53 (9)
Unknown	149 (6)	51 (8)
Mean initial [Na+] ± SD, mEq/L[c]	123.6 ± 5.5	124.1 ± 5.0
Mean initial BUN ± SD, mg/dL[a]	20.8 ± 16.8	25.5 ± 18.8
Mean initial creatinine ± SD, mg/dL[d]	1.1 ± 0.73	1.28 ± 0.85
Initial BUN:creatinine ratio[a]	19.4 ± 9.4	19.8 ± 8.6
Prior HN, n (%)[a,e]		
Yes	909 (29)	240 (38)
No	1,176 (38)	178 (28)
Unknown	1,001 (32)	212 (34)
HN at admission, n (%)[f]		
Yes	2,532 (82)	549 (87)
No	531 (17)	81 (13)
Unknown	24 (1)	0 (0)
Primary physician specialty, n (%)		
Nephrologist	104 (3)	8 (1)
Endocrinologist	108 (4)	0
Cardiologist	321 (10)	7 (1)
Hepatologist	260 (8)	246 (39)
Oncologist	111 (4)	11 (2)
Generalist	1,844 (60)	315 (50)
Other	338 (11)	43 (7)
HN subspecialist consulted, n (%)[g,h]		
No	1989 (64)	501 (80)
Yes	1,096 (36)	129 (21)

Abbreviations: BUN, blood urea nitrogen; CHF, congestive heart failure; HN, hyponatremia; [Na^+], sodium concentration; SD, standard deviation; SIADH, syndrome of inappropriate antidiuretic hormone secretion.

[a]SIADH vs CHF and cirrhosis, and CHF vs cirrhosis: $P < 0.001$.

[b]SIADH vs CHF: $P = 0.79$; and SIADH and CHF vs cirrhosis: $P < 0.001$.

[c]SIADH vs CHF and cirrhosis: $P < 0.001$; CHF vs cirrhosis: $P = 0.01$.

[d]SIADH vs CHF and cirrhosis: $P < 0.001$; and CHF vs cirrhosis: $P = 0.05$.

[e]HN during previous hospital admission in prior 12 months.

[f]Data missing for 24 patients in All, 19 in SIADH, and 4 in CHF populations; SIADH vs CHF: $P = 0.04$; SIADH vs cirrhosis: $P = 0.001$; and CHF vs cirrhosis: $P < 0.001$.

[g]SIADH vs CHF and cirrhosis: $P < 0.001$; and CHF vs cirrhosis: $P = 0.01$.

[h]HN specialist defined as nephrologist or endocrinologist.

[i]Includes 171 patients without a diagnosis of SIADH, cirrhosis, or CHF.

Table 2: Clinical characteristics of cirrhosis patients admitted with HN subdivided by hyponatremia severity.

	Total N = 518	$[Na^+]$, mmol/L <120 n = 106	≥120–≤125 n = 202	>125–≤130 n = 210
Median age, y	56	54	56	57
Male/female, n	345/173	73/33	130/72	142/68
BUN, mg/dL	20.0 (19.0)	18.0 (20.5)	21.0 (20.0)	19.0 (17.0)
Cr, mg/dL	1.0 (0.6)	1.0 (0.7)	1.1 (0.7)	1.0 (0.6)
BUN:Cr ratio	18.9 (10.4)	19.0 (12.0)	19.8 (11.6)	18.0 (9.1)
Alb, g/dL	2.5 (0.8)	2.6 (1.1)	2.5 (0.8)	2.4 (0.6)
Tbili, μmol/L	4.3 (7.4)	4.3 (7.2)	4.5 (6.9)	4.3 (7.6)
INR, s	1.7 (0.6)	1.6 (0.5)	1.7 (0.7)	1.7 (0.7)
Severe ascites, n (%)	284 (55)	53 (50)	124 (61)	107 (51)
Severe HE, n (%)[a]	116 (22)	30 (28)	51 (25)	35 (17)
C-P score	11.0 (3.0)	10.5 (3.0)	11.0 (3.0)	10.0 (3.0)
MELD score	20.2 (9.7)	18.7 (9.0)	20.8 (8.0)	20.2 (10.4)
MELD-Na score	27.3 (6.3)	26.7 (5.6)	28.0 (5.0)	26.3 (7.6)

[a] $P < 0.01$.

Values for blood urea nitrogen (BUN), creatinine (Cr), BUN:Cr ratio, albumin (Alb), total bilirubin (Tbili), international normalized ratio (INR), and Child-Pugh (CP), Model for End-Stage Liver Disease (MELD), and MELD-NA scores are median (interquartile range). HE, hepatic encephalopathy; HN, hyponatremia.

group was higher than in the HS and TO groups (both $P < 0.05$). $[Na^+]$ increased at greater rates in the FR versus NST group ($P = 0.03$), NS versus NST and FR groups ($P < 0.05$), and HS and TO versus NST and FR groups ($P < 0.05$ for all). Median length of treatment for NST, FR, and TO was 3 days and 2 days for NS and HS. It is important to note that the goal of this observational study was to demonstrate the current state of treatment management of hypervolemic HN in various real-world hospital settings. The duration of therapy and the treatment choice were determined by the treating physician as indicated in the clinical chart.

Figure 1 presents the response to initial treatment by various HN categories at Days 1–5. The percentages of patients with moderate or severe HN were significantly higher in the HS (82%) and TO (78%) groups than in the NS (65%) and FR (63%) groups, which, in turn, were higher than in the NST group (32%; all $P < 0.05$). Patients in the HS or TO groups more frequently improved into more less severe HN or treatment success categories than in those treated with NST, FR, or NS.

Table 3 presents the percentages of patients with moderate or severe HN in which $[Na^+]$ increased by ≥ 5 mmol/L at Days 2 and 3 and at final outcome. In the NST and FR groups, 27% of patients achieved this endpoint at Day 2 and 33% and 36%, respectively, at Day 3. Higher percentages achieved this endpoint in the NS versus NST and FR groups at Day 2 ($P = 0.08$ and $= 0.02$, respectively) and Day 3 ($P = 0.08$ and < 0.01). There was a more rapid response in the HS versus NST and FR groups (Days 2 and 3, $P < 0.03$ for both) and TO versus NST and FR groups (Day 2 [$P = 0.15$ and 0.07, respectively] and Day 3 [$P < 0.01$ for both]). The percentages of patients with treatment success were significantly higher in the TO versus NST, FR, and NS groups, and HS versus FR group (all $P < 0.05$).

Of patients admitted to the hospital with HN, 151 (29%) were not receiving diuretic therapy prior to hospital admission. Among these patients, 34 (23%) received ≥ 1 dose of a diuretic during initial HN therapy. Of 367 patients (71%) who received diuretics prior to hospital admission, 287 (78%) received ≥ 1 dose of a diuretic during initial HN therapy.

3.3. Secondary HN Treatment. A second therapy was provided to 275 patients. The secondary HN treatments based on initial therapy, and the characteristics and outcomes by secondary treatment group are presented in Supplemental Tables 4 and 5. Sodium levels prior to the secondary therapy and response rates are presented in Table 3. In general, the sodium response was similar for a specific therapy regardless of whether it was administered as initial or secondary therapy.

3.4. HN Relapse and Final Outcomes. Of the 110 patients who corrected with initial therapy, 61 (55%) experienced a relapse of HN during the subsequent hospitalization (Table 4). Characteristics, initial and final $[Na^+]$ levels, and LOS until HN correction were comparable between patients who did and did not relapse. The LOS after HN correction (6 versus 2 days) and total LOS (9 versus 6 days) were higher in patients who relapsed versus those patients whose [Na] remained above 130 ($P < 0.05$).

For patients admitted with HN, final median $[Na^+]$ was 129 (IQR 7) mmol/L. Of these patients, 174 (34%) were discharged alive with corrected HN, 292 (56%) were discharged with persistent HN (mild: 203 [39%]; moderate: 84 [16%]; and severe: 5 [1%]), and 49 (10%) died during hospitalization or were discharged to hospice. For patients who developed HN during hospitalization, final median $[Na^+]$ was 130 (IQR 6) mmol/L. Of these patients, 25 (33%) were discharged alive with corrected HN, 39 (51%) were discharged with persistent

Table 3: Patients with [Na^+] ≥5 mmol/L in response to initial and secondary therapy[a].

	Day 2 Response	Day 3 response	Final response
Initial therapy, n (%)			
NST	9 (27)	11 (33)	13 (39)
FR	29 (27)	38 (36)	42 (39)
NS	54 (45)	61 (51)	62 (52)
HS	7 (78)	7 (78)	7 (78)
TO	10 (48)	15 (71)	17 (81)
Secondary therapy, n (%)			
NST	5 (36)	5 (36)	5 (36)
FR	15 (31)	18 (37)	18 (37)
NS	9 (29)	10 (32)	10 (32)
HS	10 (100)	10 (100)	10 (100)
TO	15 (58)	17 (65)	18 (68)

[a]Patients with initial moderate or severe hyponatremia.
Initial therapy P <0.05 for *Day 2 response*: no specific therapy (NST) vs hypertonic saline (HS), and fluid restriction (FR) vs HS and isotonic saline (NS); *Day 3 response:* NST vs HS and tolvaptan (TO); FR vs HS, NS, and TO: *final response:* NST vs TO, FR vs HS and TO, and NS vs TO.

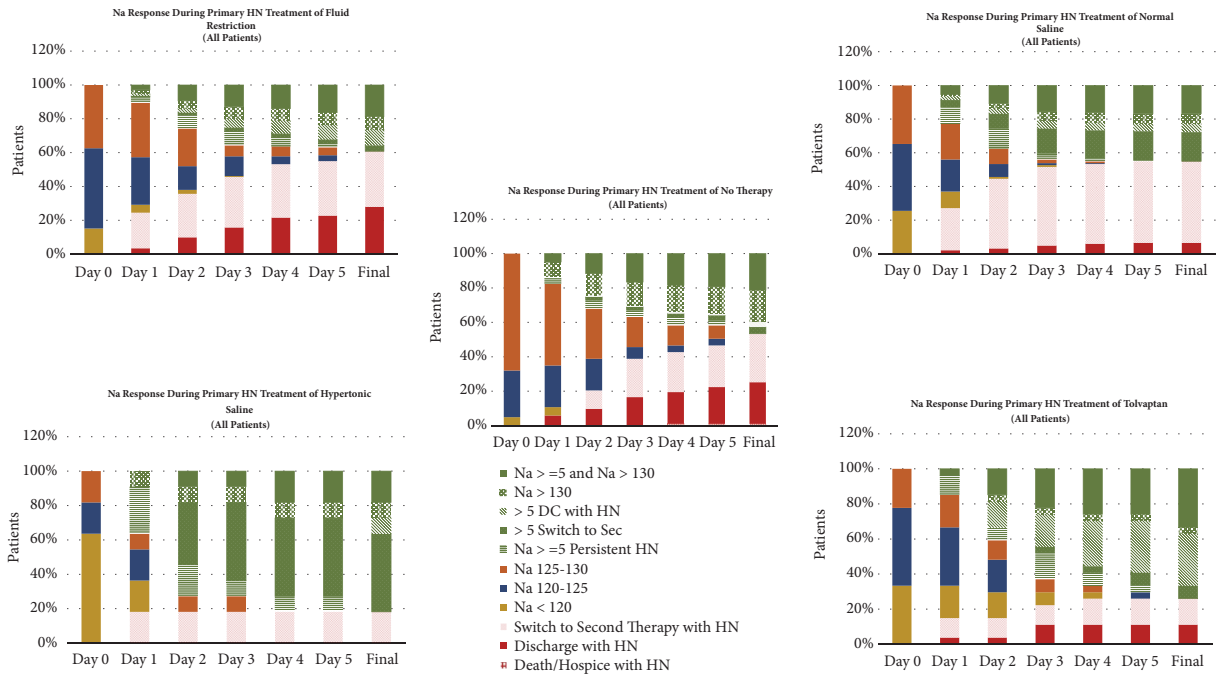

Figure 1: Response to primary hyponatremia (HN) treatment by various HN categories. [Na^+], sodium concentration; FR, fluid restriction; NS, isotonic saline; NST, no specified therapy; HS, hypertonic saline; TO, tolvaptan; DC, discontinuation.

HN (mild: 31 [40%]; moderate: 8 [10%]; and severe: 0), and 12 (16%) died during hospitalization or were discharged to hospice.

Median (IQR) LOS values for patients admitted with HN and those who developed HN during hospitalization were 6 (5) and 9 (8), respectively (P < 0.05). The distribution of LOS for patients admitted with HN versus those who developed HN during hospitalization is presented in Figure 2. Of patients admitted with HN compared with those who developed HN during hospitalization, 90% versus 75% were discharged by 14 days. Hospital mortality was numerically greater in patients who developed HN during hospitalization than in those admitted with HN but not statistically significant (16% versus 10%; P = 0.25).

4. Discussion

This analysis of the HN Registry—the largest observational study to specifically examine HN in the hospital setting—produced several important findings. There was a

Table 4: Patient characteristics and LOS for patients admitted with HN who corrected with initial HN therapy.

	Relapse (n = 61)	No relapse (n = 49)
$[Na^+]$, mmol/L	128.0 (4.0)	127.0 (5.0)
$[Na^+]$ at time of correction, mmol/L[a]	132.0 (2.0)	132.0 (2.0)
BUN, mg/dL	17.0 (17.0)	16.0 (16.0)
Cr, mg/dL	1.0 (0.5)	1.0 (0.9)
BUN:Cr ratio	19.1 (9.6)	17.4 (11.4)
Tbili, μmol/L	4.8 (7.3)	4.3 (6.7)
INR, s	1.8 (0.8)	1.8 (0.5)
Alb, g/doll	2.6 (0.9)	2.8 (0.7)
MELD score at correction	20.9 (6.6)	21.9 (11.3)
LOS, d[a]	9 (6.0)	6 (5.0)
LOS until correction, d	3 (2.0)	3 (1.0)
LOS after correction, d[a]	6 (5.0)	2 (3.0)
Death/ hospice, n (%)	5 (8)	5 (10)

[a] < 0.05.

Values for sodium concentration ($[Na^+]$), $[Na^+]$ at time of correction, blood urea nitrogen (BUN), creatinine (Cr), BUN:CR ratio, total bilirubin (Tbili), albumin (Alb), international normalized ratio (INR), Model for End-Stage Liver Disease (MELD) score at correction, length of stay (LOS), LOS until correction, and LOS after correction are median (interquartile range). HN, hyponatremia.

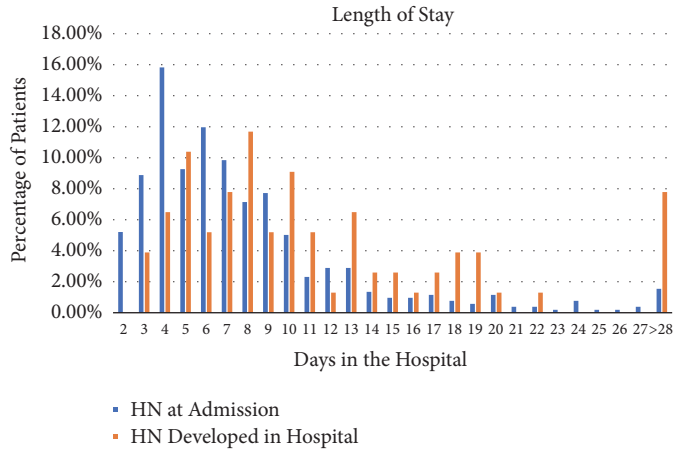

Figure 2: The distribution of length of stay (LOS) for patients with hyponatremia (HN) at admission versus those who developed HN during hospitalization.

strong association between HN and advanced cirrhosis with severe portal hypertension as has been previously reported [3]. Large-volume ascites and overt HE were noted in 54% and 22% of patients admitted with HN, respectively. There was not a relationship between the presence of severe ascites and the severity of HN ($p = 0.216$). However, the prevalence of overt HE was related to HN severity.

Treatment approaches were highly variable and frequently ineffective [7]. Twenty percent of patients received NST, and only 34% of the cirrhotic patients admitted to the hospital with HN were discharged with corrected HN. Although FR is recommended in treatment guidelines as initial therapy [10], this is the first study to report outcomes in patients treated with FR in real life practice. The rate of increase in $[Na^+]$ with FR treatment was of limited efficacy and comparable to NST. Of patients with moderate and severe HN, $[Na^+]$ increased by ≥ 5 mmol/L in only 27% and 36% at Days 2 and 3, respectively. These results are in accordance with previous studies that showed a limited or no response with FR [11, 12].

Although not recommended, treatment with NS was the most common initial therapy [13]. Ascites is frequently the reason for hospitalization, and NS can exacerbate its severity and increase the need for invasive procedures, such as paracentesis. It may also exacerbate HN through "a desalination process" in which increased AVP levels lead to water retention and excretion of hypertonic urine [14]. Patients receiving NS had lower $[Na^+]$ than those receiving FR. Although the rate of increase in $[Na^+]$ was greater, its effectiveness was limited: of patients with moderate or severe HN, $[Na^+]$ only increased by ≥ 5 mmol/L in 45% and 51% of patients at Days 2 and 3, respectively.

Correction of HN was most effective with HS and TO. HS is recommended for severe symptomatic HN and was used

as initial therapy in 2% of patients [15]. [Na^+] was lowest in patients receiving HS, and 82% had either moderate or severe HN. There are currently no reports on the response to HS in cirrhosis. In this study, HS led to a rapid increase in [Na^+]—an increase ≥ 5 mmol/L by Day 2 in 78% of patients.

TO was used as primary therapy in 5% of patients. The severity of HN was similar to those receiving HS, and 78% patients had either moderate or severe HN. As with HS, [Na^+] rapidly increased but at a slightly more gradual rate. At Day 2, 48% of patients had an increase ≥ 5 mmol/L. However, the percentage at Day 3 was comparable to HS (71% versus 78%; $P = 1.00$). This response rate was comparable to that observed in the cirrhosis population from the SALT1/SALT2 trials in which 40% and 70% of patients treated with TO had an increase ≥ 5 mmol/L at Days 2 and 3, respectively (data on file).

HN is frequent in patients with large-volume ascites [3]. In addition to the nonosmotic release of AVP, the renin-aldosterone system is activated, leading to increased renal sodium reabsorption. Diuretics are the mainstay of treatment of fluid overload but can exacerbate HN by decreasing intravascular volume (leading to increased AVP release) and only blocking sodium reabsorption, leaving continued free-water absorption unopposed [16]. Guidelines recommend tapering and then discontinuing diuretics if HN persists despite FR. However, diuretics were discontinued in only 17% of patients with HN at admission and were initiated in 7% of patients.

HN is associated with increased LOS in hospitalized patients [17–19]. In this study the impact of HN relapse in LOS after initial sodium correction was especially striking. Despite comparable initial Na level, level at correction, and time to correction, LOS was 4 days longer in those in whom HN recurred.

The results of this study are limited by its observational nature and broad definitions. FR was defined only by the physician orders indicated in the chart. NS and diuretic administration were also broadly defined. Albumin administration which is increasingly being used for the treatment of HN and infectious complications were not assessed [20]. In addition, Na levels have recently been reported to increase in response to treatment with midodrine and octreotide in a noncontrolled study [21]. However, the goal of this study was to evaluate treatment practices and outcomes in the real-world setting with the most commonly used approaches. The frequent administration of ineffective and/or nonstandardized therapy that frequently includes NS is not consistent with treatment guidelines. Correction of only 34% of patients at discharge suggests that most physicians do not view correction of HN as a meaningful clinical endpoint. Correction of HN is less important than demonstration of a beneficial impact on clinical endpoints. A question that invariably arises is whether HN is a direct participant in the pathophysiologic process and directly contributes to poor outcomes and increased LOS, or whether it is only a marker of end-stage disease. Preliminary evidence supports a contributory role for HN in hepatic encephalopathy [22]. Finally, treatment of HN with TO has also been shown to shorten LOS in patients with heart failure, syndrome of inappropriate diuretic hormone, and cancer [23, 24].

Determination of the impact of the treatment of HN in patients with cirrhosis will first require standardization of its management with effective therapy. The initial use of FR should be reevaluated, while NS administration should be avoided. Although HS is effective, it is limited by its deleterious impact on fluid overload and need for close monitoring, which frequently requires an intensive-care setting. In addition, the development of tense ascites due to HS administration can aggravate the severity of portal hypertension [23, 24]. Early treatment with TO offers an effective approach that should allow comprehensive assessment of the importance of HN treatment in the hospitalized patient with cirrhosis. Because the FDA-approved indications for TO have removed cirrhosis as an approved population, it is important that treatment with TO in these patients be performed in a carefully controlled manner.

Data Availability

This was an observational study, not a randomized, controlled clinical trial, supported by Otsuka America Pharmaceutical, Inc. All statistical analyses were performed by an outside organization, Mapi Group, using predefined definitions and independent of Otsuka. In the event there were issues, they were adjudicated by 2 members of the Hyponatrema Registry Steering Committee. We would be happy to answer any additional questions. As indicated in the Methods section, all statistical analyses were performed by MAPI using predefined criteria. MAPI is an independent biostatistical organization that is routinely employed for independent analysis of data sets. All analyses were performed independently of investigator in-put. The data for each patient is present in an elaborate Excel spreadsheet that requires detailed explanation in its use.

Ethical Approval

The study was reviewed and approved by each site's respective Institutional Review Board.

Consent

Depending on the local site requirements, study participants, or their legal guardian, provided informed written consent prior to study enrollment. Because the study was observational only and treatment was provided based on routine clinical practice of the patient's treating physician, selected sites permitted enrollment with a waiver of consent.

Conflicts of Interest

Samuel H. Sigal is a consultant, has received travel support and fees for data review, and has served on a speakers' bureau for Otsuka. His institution received research support for serving as an investigational site for the Hyponatremia Registry and for an investigator-initiated trial from Otsuka. Alpesh Amin is a consultant for the Hyponatremia Registry,

has received travel support and fees for data review activities, and has served on a speakers' bureau for Otsuka. Joseph A. Chiodo III was employed by Otsuka America Pharmaceutical, Inc. at the time the manuscript was developed. Arun Sanyal is President of Sanyal Biotechnology and has stock options in Genfit, Akarna, Tiziana, Indalo, Durect. He has served as a consultant to Hemoshear, Echosens, AbbVie, Astra Zeneca, Nitto Denko, Ardelyx, Conatus, Nimbus, Amarin, Salix, Tobira, Takeda, Novo Nordisk, Fibrogen, Jannsen, Gilead, Boehringer, Lilly, Zafgen, Novartis, Pfizer, Immuron, Exhalenz, and Genfit. He has been an unpaid consultant to Intercept, Immuron, Galectin, Fractyl, Syntlogic, Affimune, Chemomab, Nordic Bioscience, and Bristol Myers Squibb. His institution has received grant support from Gilead, Salix, Tobira, Bristol Myers, Shire, Echosens, Intercept, Merck, Astra Zeneca, Malinckrodt, Cumberland, and Novartis. He receives royalties from Elsevier and UptoDate.

Authors' Contributions

Samuel H. Sigal participated in data acquisition and analysis and wrote the paper. Alpesh Amin and Arun Sanyal participated in data acquisition and analysis and manuscript preparation. Joseph A. Chiodo III participated in data analysis and manuscript preparation.

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Supplementary Materials

Supplemental Table 1: this table provides the clinical characteristics, LOS, and mortality of cirrhotic patients in the Hyponatremia Registry. It includes a breakdown between those who presented with HN at hospital admission versus those who developed HN during the hospital stay. Supplemental Table 2: this table shows the clinical characteristics including HN severity of the cirrhotic patients with HN at admission based on initial therapy selected by the treating physician. Supplemental Table 3: this table presents the outcomes of the initial therapies of cirrhotic patients admitted with HN, including the percentages switched to a second therapy, discharged with persistent HN, mortality, and length of stay. Supplemental Table 4: this table provides the outcomes of the secondary therapies of the patients who presented with HN and did not respond to the initial therapy. The initial treatment is indicated in the far-left column and the secondary therapy is indicated in the top row. Supplemental Table 5: this table presents the outcomes of the secondary therapies of cirrhotic patients who were admitted with HN and did not respond to the initial therapy. *(Supplementary Materials)*

References

[1] Y. Iwakiri and R. J. Groszmann, "The hyperdynamic circulation of chronic liver diseases: From the patient to the molecule," *Hepatology*, vol. 43, no. 2, pp. S121–S131, 2006.

[2] P. Ginès and M. Guevara, "Hyponatremia in cirrhosis: Pathogenesis, clinical significance, and management," *Hepatology*, vol. 48, no. 3, pp. 1002–1010, 2008.

[3] P. Angeli, F. Wong, H. Watson et al., "Hyponatremia in cirrhosis: Results of a patient population survey," *Hepatology*, vol. 44, no. 6, pp. 1535–1542, 2006.

[4] S. Deitelzweig, A. Amin, R. Christian, K. Friend, J. Lin, and T. J. Lowe, "Hyponatremia-associated healthcare burden among us patients hospitalized for cirrhosis," *Advances in Therapy*, vol. 30, no. 1, pp. 71–80, 2013.

[5] Vaprisol, "Nashville, TN: Cumberland Pharmaceuticals, Inc," 2014.

[6] Samsca, "Tokyo, Japan: Otsuka Pharmaceutical Co, Ltd," 2012.

[7] P. B. Watkins, J. H. Lewis, N. Kaplowitz et al., "Clinical pattern of tolvaptan-associated liver injury in subjects with autosomal dominant polycystic kidney disease: analysis of clinical trials database," *Drug Safety*, vol. 38, no. 11, pp. 1103–1113, 2015.

[8] A. Greenberg, J. G. Verbalis, A. N. Amin et al., "Current treatment practice and outcomes. Report of the hyponatremia registry," *Kidney International*, vol. 88, no. 1, pp. 167–177, 2015.

[9] P. S. Kamath and W. R. Kim, "The model for end-stage liver disease (MELD)," *Hepatology*, vol. 45, no. 3, pp. 797–805, 2007.

[10] J. G. Verbalis, S. R. Goldsmith, A. Greenberg et al., "Diagnosis, evaluation, and treatment of hyponatremia: expert panel recommendations," *American Journal of Medicine*, vol. 126, no. 10, supplement 1, pp. S1–S42, 2013.

[11] F. Wong, A. T. Blei, L. M. Blendis, and P. J. Thuluvath, "A vasopressin receptor antagonist (VPA-985) improves serum sodium concentration in patients with hyponatremia: A multicenter, randomized, placebo-controlled trial," *Hepatology*, vol. 37, no. 1, pp. 182–191, 2003.

[12] A. L. Gerbes, V. Gülberg, P. Ginès et al., "Therapy of hyponatremia in cirrhosis with a vasopressin receptor antagonist: A randomized double-blind multicenter trial," *Gastroenterology*, vol. 124, no. 4, pp. 933–939, 2003.

[13] European Association for the Study of the Liver, "EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis," *Journal of Hepatology*, vol. 53, no. 3, pp. 397–417, 2010.

[14] A. Steele, M. Gowrishankar, S. Abrahamson, C. D. Mazer, R. D. Feldman, and M. L. Halperin, "Postoperative hyponatremia despite near-isotonic saline infusion: a phenomenon of desalination," *Annals of Internal Medicine*, vol. 126, pp. 20–25, 1997.

[15] S. John and P. J. Thuluvath, "Hyponatremia in cirrhosis: pathophysiology and management," *World Journal of Gastroenterology*, vol. 21, no. 11, pp. 3197–3205, 2015.

[16] G. Liamis, H. Milionis, and M. Elisaf, "A review of drug-induced hyponatremia," *American Journal of Kidney Diseases*, vol. 52, no. 1, pp. 144–153, 2008.

[17] A. Amin, S. Deitelzweig, R. Christian et al., "Evaluation of incremental healthcare resource burden and readmission rates associated with hospitalized hyponatremic patients in the US," *Journal of Hospital Medicine*, vol. 7, no. 8, pp. 634–639, 2012.

[18] A. Amin, S. Deitelzweig, R. Christian, K. Friend, J. Lin, and T. J. Lowe, "Healthcare resource burden associated with hyponatremia among patients hospitalized for heart failure in the US," *Journal of Medical Economics*, vol. 16, no. 3, pp. 415–420, 2013.

[19] R. Berardi, M. Caramanti, M. Castagnani et al., "Hyponatremia is a predictor of hospital length and cost of stay and outcome in cancer patients," *Supportive Care in Cancer*, vol. 23, no. 10, pp. 3095–3101, 2015.

[20] A. Garioud, J. Cadranel, A. Pauwels et al., "Albumin use in patients with cirrhosis in France: results of the 'ALBU-LIVE' survey: a case for better EASL guidelines diffusions and/or revision," *Journal of Clinical Gastroenterology*, vol. 51, pp. 831–838, 2017.

[21] S. Patel, D. Nguyen, A. Rastogi, M. Nguyen, and M. K. Nguyen, "Treatment of cirrhosis-associated hyponatremia with midodrine and octreotide," *Frontiers in Medicine*, vol. 4, 2017.

[22] V. Ahluwalia, D. M. Heuman, G. Feldman et al., "Correction of hyponatraemia improves cognition, quality of life, and brain oedema in cirrhosis," *Journal of Hepatology*, vol. 62, no. 1, pp. 75–82, 2016.

[23] J. R. Chiong, S. Kim, J. Lin, R. Christian, and J. F. Dasta, "Evaluation of costs associated with tolvaptan-mediated length-of-stay reduction among heart failure patients with hyponatremia in the US, based on the EVEREST trial," *Journal of Medical Economics*, vol. 15, no. 2, pp. 276–284, 2012.

[24] A. K. Salahudeen, N. Ali, M. George, A. Lahoti, and S. Palla, "Tolvaptan in hospitalized cancer patients with hyponatremia: A double-blind, randomized, placebo-controlled clinical trial on efficacy and safety," *Cancer*, vol. 120, no. 5, pp. 744–751, 2014.

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Review Article

Systemic Inflammation and Acute-on-Chronic Liver Failure: Too Much, Not Enough

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ACLF is a specific, but complex and multifactorial form of acute decompensation of cirrhosis and is characterized by an extraordinary dynamic natural course, rapidly evolving organ failure, and high short-term mortality. Dysbalanced immune function is central to its pathogenesis and outcome with an initial excessive systemic inflammatory response that drives organ failure and mortality. Later in its course, immuno-exhaustion/immunoparalysis prevails predisposing the patient to secondary infectious events and reescalation in end-organ dysfunction and mortality. The management of patients with ACLF is still poorly defined. However, as its pathophysiology is gradually being unravelled, potential therapeutic targets emerge that warrant further study such as restoring or substituting albumin via plasma exchange or via albumin dialysis and evaluating usefulness of TLR4 antagonists, modulators of gut dysbiosis (pre- or probiotics), and FXR-agonists.

1. Acute-on-Chronic Liver Failure and Systemic Inflammation

Acute clinical deterioration of a patient with cirrhosis remains a decisive time point in terms of medical management, since it is frequently associated with rapidly evolving multiorgan dysfunction, significant morbidity, and high short-term mortality. In the latter clinical constellation, this syndrome has been referred to as acute-on-chronic liver failure (ACLF) [1, 2]. The CANONIC study, the largest prospective multicenter study on ACLF so far with inclusion of 1343 patients admitted with acute decompensation of cirrhosis, has substantiated its relevance and clinical impact by documenting a prevalence of ACLF in this cohort of 30.9% accompanied by a high short-term mortality of 33 and 51% at 28 and 90 days, respectively (Table 1) [2]. In addition, the CANONIC study [2] and subsequent analyses [3] have exposed several premises with regard to the pathophysiology of ACLF and in particular a pivotal role for *dysregulated inflammation*. More specifically, the degree of inflammatory response, as estimated by the leukocyte count and C-reactive protein, was found to be an independent predictor of post-enrolment development of ACLF and paralleled the severity and outcome of ACLF (Figure 1). All patients with ACLF showed a high leukocyte count and C-reactive protein which in 60% of patients could be attributed to an inflammatory

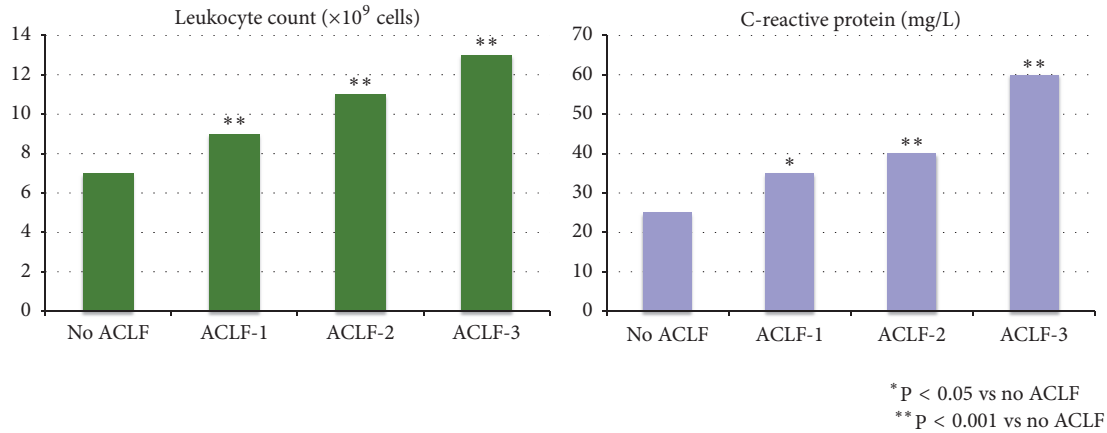

Figure 1: Proof of dysbalanced inflammatory response: relationship between the degree of inflammatory reaction, as estimated by the leukocyte count and C-reactive protein, and the severity of ACLF.

Table 1: Definition and prognosis of ACLF.

Grade of ACLF	28-day mortality	90-day mortality
ACLF grade 1		
(i) Single kidney failure		
(ii) Single "non-kidney" organ failure with serum creatinine ranging from 1.5 mg/dl to 1.9 mg/dl and/or grade I or II hepatic encephalopathy	**22.1%**	**40.7%**
ACLF grade 2: Presence of 2 organ failures	**32%**	**52.3%**
ACLF grade 3: Presence ≥3 organ failures	**76.7%**	**79.1%**

Minimal organ failures defined by the modified Sequential Organ Failure Assessment (SOFA) score for patients with cirrhosis:
(i) Liver: bilirubin ≥ 12mg%
(ii) Kidney: creatinine ≥ 2.0mg%
(iii) Cerebral: hepatic encephalopathy ≥ grade 3
(iv) Coagulation: INR ≥ 2.5 or platelets < 20.000 per mm^3
(v) Circulation: need of vasopressors
(vi) Lungs: PaO/FiO2 > 100

trigger such as bacterial infection or acute alcoholic liver injury whereas in the remaining 40% this remains undetermined at present. Together these findings suggest an altered host response to injury, both infectious and noninfectious, and immune dysfunction leading to an inappropriate inflammatory response. On the other hand, the CANONIC-trial also taught us that ACLF patients, who had earlier episodes of acute hepatic decompensation, developed a less dramatic course of ACLF with lower levels of inflammatory mediators and lower mortality rates compared to patients presenting with a first episode. This suggests that ACLF is not only associated with exaggerated inflammatory response but also with tolerance, a host defence strategy that reduces the negative impact of inflicted injury on host fitness [4].

In the next paragraphs, we will focus first on the basic mechanisms of inflammation (resistance and tolerance), secondly on the different elements contributing to this dysfunction and predominantly in the context of ACLF in patients with underlying cirrhosis (cirrhosis-associated immune dysfunction), and thirdly on additional reinforcing accomplices.

2. Systemic Inflammation: Resistance and Tolerance

If we focus on the healthy liver, it is to be considered as a frontline immunological organ as it balances between "resistance" and "tolerance" [5]. It acts as a gatekeeper via its unique double blood supply, the arterial blood of the hepatic artery, and the portal-venous blood delivering the products resorbed in the intestine. On the one hand, the liver maintains immune surveillance. To accomplish this role, the liver contains numerous resident antigen presenting cells strategically located to allow maximal "border control". These involve the reticuloendothelial system (endothelial cells, Kupffer cells) and dendritic cells, which following detection participate in coordinated immune responses leading to pathogen clearance, leukocyte recruitment, and antigen presentation to lymphocytes within the unique hepatic vasculature. In addition to this local surveillance role, the liver is responsible for the bulk production of proteins involved in innate and adaptive immune responses following stimulation by proinflammatory cytokines (such as interleukin- [IL-] 6, tumor necrosis factor- [TNF-] alpha), including acute phase proteins such as C-reactive protein and lipopolysaccharide binding protein, and complement factors. Conversely, its defensive reactive role is being tightly regulated by, amongst others, high IL-10 production by Kupffer cells and Kupffer cell mediated T-cell suppression to ensure that inappropriate immune responses are not raised against nonpathogenic exogenous blood-borne molecules, such as those derived from food and conventional gut microbial antigens [5].

When a threat arises to our physical integrity, it is primarily dealt by ***"resistance"*** mechanisms. This refers to the attempts of the host immune system to "search and destroy". Crucial in the initial resistance phase of an *infectious threat*

Table 2: Examples of well-characterized DAMPs (danger signals or alarmins).

DAMPs	Receptors	Outcome of receptor ligation
Extracellular nucleotides (ATP, ADP, adenosine)	PI, P2X, and P2Y receptors (ATP, ADP); Al, A2A, A2B, and A3 receptors (adenosine)	Dendritic cell (DC) maturation, chemotaxis, secretion of cytokines (IL-1β, IL-18), inflammation
Extracellular heat shock proteins	CD14, CD91, scavenger receptors, TLR4, TLR2, CD40	DC maturation, cytokine induction, DC, migration to lymph nodes
Extracellular HMGB1	RAGE, TLR2, TLR4	Chemotaxis, cytokine induction, DC activation, neutrophil recruitment, inflammation, activation of immune cells
Uric acid crystals	CD14, TLR2, TLR4	DC activation, cytokine induction, neutrophil recruitment, gout induction
Laminin	Integrins	Neutrophil recruitment, chemotaxis
S100 proteins or calgranulins	RAGE	Neutrophil recruitment, chemotaxis, cytokine secretion, apoptosis
Hyaluronan	TLR2, TLR4, CD44	DC maturation, cytokine production, adjuvant activity
IL-1 family		
IL-1α	IL1R1 and IL1RAP	Inflammatory; promotes activation, costimulation, and secretion of cytokines and other acute-phase proteins; pyrogenic
IL-33	IL1RL1 and IL1RAP	Inducer of type 2 immune responses, activating T helper 2 (TH2) cells and mast cells; stimulates group 2 innate lymphoid cells (ILC2s), regulatory T (Treg) cells, TH1 cells, CD8+ T cells and natural killer (NK) cells.
Mitochondrial DAMPs		
mtDNA	TLR9	Proinflammatory cytokines, neutrophil chemoattraction and matrix metalloproteinase secretion, type I IFN responses 91
N-Formylated peptides	FPR	Neutrophil chemoattraction

are toll-like receptors (TLRs), which recognize distinct conserved structures in pathogens (pathogen-associated molecular patterns, PAMPs) and lead to sensing pathogen invasion, triggering innate immune responses, and priming antigen-specific adaptive immunity [6, 7]. The intracellular cascades triggered by TLR-activation lead to transcription factor activation (e.g., nuclear factor NF-$\kappa\beta$, AP-1) and subsequent transcriptional activation of hundreds of inflammatory mediator genes coding, for instance, for cytokines (i.e., TNF-α, IL-6, IL-1β, or type 1 interferons), which further shape the immune response and the elimination of bacteria and infected cells. Safeguarding the host from invading pathogens is an intricate task that requires cooperation between different pattern recognition receptors (PRRs). While responses to extracellular PAMPs are mainly mediated by membrane bound receptors such as TLRs, other cytosolic receptors (the nucleotide-binding oligomerization domain- (NOD-) like receptor, NLRs) are specialized for detection of PAMPs that reach the cytosol or intracellular organelles [7]. Several members of the NLR gene family are involved in the assembly of macromolecular protein complexes termed "inflammasomes" that lead to the activation of the inflammatory cysteine protease, caspase-1 (also known as interleukin-1 converting enzyme or ICE). Caspase-1 in turn cleaves pro-IL-1β or pro-IL-18, resulting in secretion of the mature and active forms of these cytokines [8].

In *noninfectious threats* due to acute tissue necrosis or immune-inflicted damage (such as fulminant hepatitis or acute alcoholic hepatitis), necrotic cells release damage/danger-associated molecular patterns (DAMPs), consisting of denaturated nuclear or cytosolic proteins, nucleic acids, and so forth, which also interact with TLRs and other specific receptors [9] (Table 2). Therefore, the host response to infectious and noninfectious ("sterile") injury is not substantially different. DAMPs also activate inflammasomes that process the release of IL-1β, which initiates the activation of cytokines, as mentioned earlier [8, 9]. However, differences in host (genetic variants in genes coding for cytokines and other regulatory factors of the innate and adaptive immune systems) and pathogen (virulence, load) factors may lead to variable intensity of immune responses and susceptibility to certain pathogens. Either way, the trade-off for an exaggerated "search and destroy" strategy is collateral damage leading to "immunopathology", defined as the negative impact of immune defence on host fitness.

To deal with this endogenous endangered physical integrity, a 2nd mechanism is activated called "**disease tolerance**" [4]. This refers to a distinct defence strategy that

decreases host susceptibility to tissue damage caused by a pathogen or local factor or by the immune response directed against them (immunopathology). Whereas direct damage caused by pathogens relates to their burden and virulence and an incompetent immune response, immunopathology correlates positively with the magnitude and duration of the immune response. Therefore an optimal immune response balances between an efficient pathogen clearance and acceptable level of immunopathology.

Although much of the knowledge regarding the mechanisms involved in tolerance remains to be elucidated, logically these would be expected to prevent, reduce, or counter inflicted damage and thus involve engagement of basal and inducible homeostatic systems (amongst others by induction of stress-response genes to tone down hypersensitivity) restoring/reducing fitness costs following infectious aggression. An example of such a counterbalancing reaction is the activation of compensatory anti-inflammatory mechanisms in order to restrain a potential overzealous proinflammatory process in patients with infectious or noninfectious systemic inflammatory response. These mechanisms concert with an adapted compartmentalized response with the aim of silencing some acute proinflammatory genes and to maintain the possible expression of certain genes involved in the anti-infectious process (and by combination thus to reduce the burden of immunopathology) [10, 11]. Enhanced release of anti-inflammatory mediators such as IL-10, IL-1 receptor antagonist (IL-1RN), and soluble TNF-α receptor, as well as decreased HLA-DR expression (altering antigen presentation capacity) on different antigen presenting cells amongst others, dampens the inflammatory component. In contrast to what was initially postulated, the anti-inflammatory response is no longer considered a generalized damping phenomenon sequentially following a systemic inflammatory response but rather a concomitant compartmentalized reprogramming of leukocytes leading to an oscillating balance (immune dissonance) between the two opposed forces driving outcome [11, 12]. This particular premise is substantiated by the finding that repeated exposure of in vitro murine macrophages to bacterial endotoxin/ lipopolysaccharides (LPS) led to transient silencing of proinflammatory genes (e.g., TNF-α, IL-6, IL-1ß, IL-12, and type 1 IFN), priming of anti-inflammatory (e.g., IL-10, transforming growth factor- (TGF-) β, and IL-1RN) and antimicrobial effector genes, and impairing antigen presenting capacity (via decreased expression of HLA-DR), leading to a phenomenon called "endotoxin tolerance" [12–14]. These adaptive changes are also commonly associated phenotype switch (M1 $\longrightarrow$M2) and altered substrate utilization. These findings illustrate an adaptive response in macrophages and reveal component-specific regulation of inflammation adding up to the complexity of the balancing act between resistance and tolerance.

3. Resistance and Tolerance in ACLF: Cirrhosis-Associated Immune Dysfunction (Figure 2)

In a cirrhotic patient, immune function becomes an even more complex and often confusing matter as it can take a rapidly interchangeable and highly fluctuating course of either "too much, or not enough" resistance/inflammation [1–3, 10, 15, 16]. Recently, this immune dysfunction syndrome in the context of cirrhosis has been referred to as cirrhosis-associated immune dysfunction [17]. It consists of two concomitant, interlinked, and seemingly opposed forces: systemic inflammation and acquired immunodeficiency. These reciprocally and dynamically drive immune-(in)competence during the course of cirrhosis. This fragile balancing act is already activated in the early stages of cirrhosis (compensated state) as the cause that drives the cirrhogenesis process primes a proinflammatory phenotype by the activation of DAMPs from injury-inflicted tissue damage which is proportional to the etiological force driving chronic liver disease (such as alcohol, HBV, and HCV). As cirrhosis progresses and thus hepatocellular injury and intrahepatic shunting via completely vascularized fibrotic septae increase, the "gate-keeper function" of the liver is hollowed out. More specifically, hepatocellular insufficiency leads to the decreased production of PRRs, acute phase proteins, albumin, complement, and so forth and therefore progressive loss of opsonization, bacterial phagocytosis, and killing, while increased shunting leads to evasion of portal and systemic bacteria to the action of the reticuloendothelial system. Both features explain why bacterial products such as endotoxins and cytokines are insufficiently cleared and further prime systemic inflammation. As the severity of cirrhosis increases, bacterial translocation from the gut (see below) amplifies. This is paralleled by increased levels of PAMPs (and subsequent TLR/NLRs activation), which instigate the activation of the hepatic innate immune system, reinforcing further hepatic injury directly and indirectly through immunopathology. Initially, the spill-over of PAMPs furthers amplifies, and sometimes infuriates, the already primed proinflammatory systemic response as witnessed in LPS-stimulated cirrhotic peripheral blood mononuclear cells (PBMCs) which showed a massive induction of proinflammatory cytokines and chemokines [13, 14, 17–23].

Later on, as circulating and intestinal populations of immune cells are more and more compromised with evolving liver damage and mechanisms like "endotoxin tolerance" (with priming of anti-inflammatory (e.g., IL-10, TGF-β, and IL-1RA) and impairing antigen presenting capacity (via decreased expression of HLA-DR), see earlier) become generalized, the dynamic balance switches over to a predominant immunodeficient phenotype [17–19]. One of the recently elucidated pathways in this latter context is the finding of Bernsmeier et al. [24] who showed that patients with ACLF in comparison to patients with compensated and mere acute decompensation of cirrhosis had increased numbers of MER receptor tyrosine kinase (MERTK) expressing monocytes and macrophages. MERTK negatively controls innate immune response. In ACLF, MERTK expression correlated with the severity of hepatic and extrahepatic disease and systemic inflammatory response. Moreover, in vitro MERTK-inhibitors were able to restore the production of inflammatory cytokines in response to lipopolysaccharide stimulation. Additional work in this context further highlighted the important role of immunosuppressive

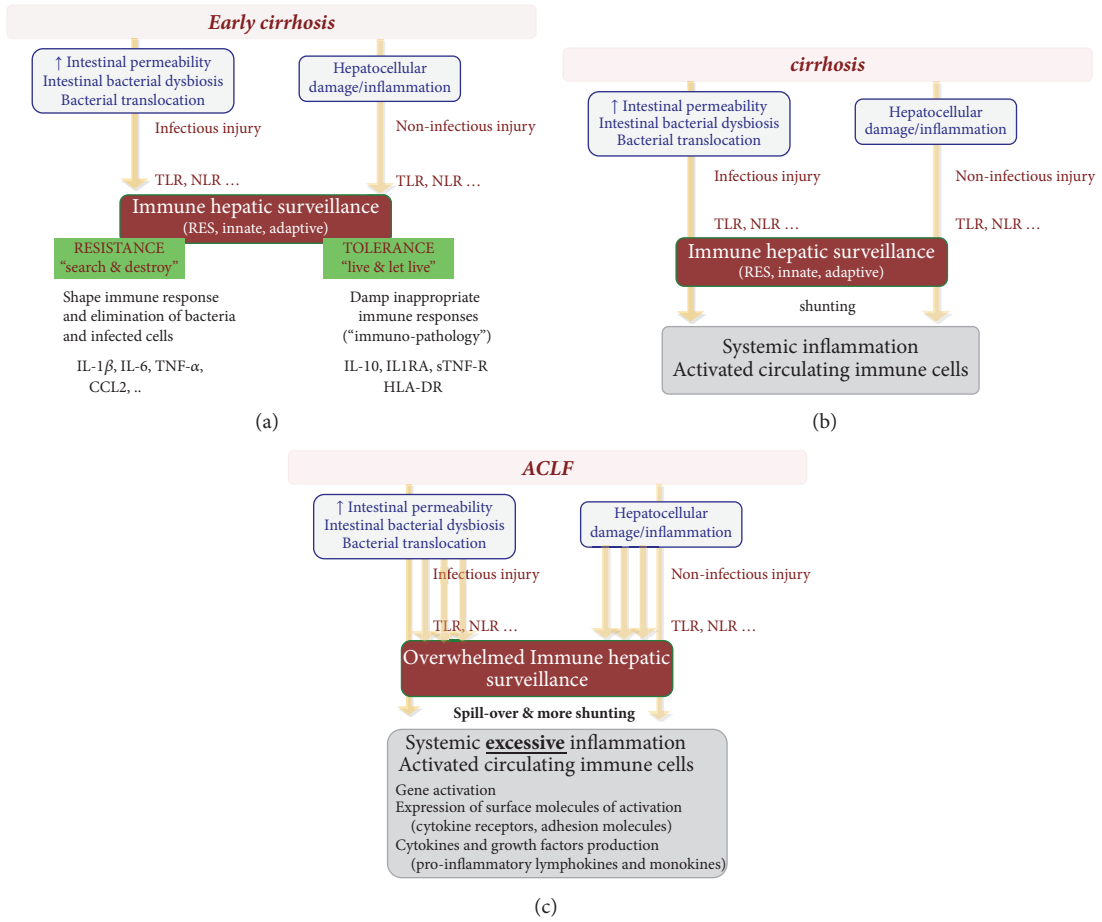

Figure 2: The dynamic course of immune function in evolving cirrhosis: cirrhosis-associated immune: (a) early cirrhosis sets the stage for ACLF; (b) evolving cirrhosis primes the systemic immune system and finally culminates in ACLF (c).

mononuclear $CD14^{+}HLA\text{-}DR^{-}$ myeloid-derived suppressor cells (M-MDSCs) who equally quell on antimicrobial defences in ACLF [25].

However, immune activation and deficiency can coexist. Intestinal macrophages in cirrhosis are activated due to bacterial translocation (compartmentalized immune activation while at the same time immune responses may fail systemically) [20].

While endotoxin tolerance, and an anti-inflammatory response in general, is conceived as a primarily protective mechanism, its protracted duration and outbalanced intensity have been associated with high risks of secondary infections and death [11, 12, 18, 19].

The clinical implications of this skewed homeostatic balance between resistance and tolerance in cirrhosis translate in essence in the end-organ failure that determines ACLF. Although tolerance capacity differs depending on organ (given the difference in intrinsic damage susceptibility, repair capacity, functional autonomy, and damage or malfunction sequelae), dysbalanced inflammation may eventually cause organ failure through different mechanisms. First, through the action of circulating proinflammatory mediators and membrane-shed microparticles, it causes an escalation of the portal hypertensive syndrome leading to an aggravated systemic circulatory dysfunction characterized by arterial vasodilation, impairment in cardiac function, organ hypoperfusion, and end-organ ischemia [26–30]. Second, the direct extension of systemic inflammation to organs impairs cell function and may cause necrosis and/or apoptosis. In lung and kidney, detection of TLR4 forms the direct link between increasing circulating microbial products and the subsequent proinflammatory cascade in this end-organ injury [31, 32]. A recent paper by Clària et al. [3] has documented high circulating levels of pro- and anti-inflammatory cytokines in ACLF, whose levels significantly correlated with the number of organ failures. Moreover, different profiles of cytokine response were identified depending on the type of precipitating event (like, for example, alcoholic steatohepatitis or bacterial infection).

This same study revealed that not only inflammatory markers, but also markers of oxidative stress (e.g., oxidized albumin, see below) known to drive systemic inflammation, might help to identify patients with ACLF and predict their outcome. Other clinical observations underscoring the impact of this dysbalance are, for example, the observation that in patients with increasing grade of ACLF and HRS the response to terlipressin and albumin is blunted [33]. Additionally, the exaggerated anti-inflammatory

response/immune-tolerance may facilitate the appearance of bacterial infections. Indeed, in the CANONIC study cohort, 46% of patients with ACLF without bacterial infections at diagnosis of ACLF developed a bacterial infection within 4 weeks, with devastating impact on short term mortality [34].

Finally, inflammation increases the release of local procoagulant factors (including tissue factor and membrane microparticles) from the endothelial cells, inducing microthrombosis in the microcirculation of different organs [35].

In conclusion, in addition to impaired circulatory function, systemic inflammation may lead to organ failure by a direct effect of the inflammatory mediators on microvascular integrity, cell function, and death mechanisms. As such the peripheral vasodilation theory no longer exclusively explains the mechanism of organ failure but embraces the systemic inflammation hypothesis in evolving cirrhosis [36].

4. Other Partners in Crime

In addition to inflammation, the following factors are thought to contribute as "accomplices" in the hold-up ACLF imposes on a patient.

4.1. Inflammaging and Immunosenescence. Patients with ACLF in the CANONIC study were of younger age underlining that the younger patients have the stronger response and thereby are more susceptible to develop ACLF [2]. In subsequent publications using different independent cohorts for the elaboration of the CLIF-C-AD score, a measure to calculate the risk of ACLF and death, younger age seems to be associated with ACLF development [37]. Previously, such trends did not reach statistical significance, but were also described [38, 39]. However, looking on the other side, TLR expression and function declines with age, thereby leading to an inadequate response to infections [40]. But on the other side, rate and severity of infections are higher and the outcome is poorer in older patients [41].

The concepts of immunosenescence and inflammaging might render these thoughts even more complex [42, 43]. Immunosenescence, characterized by impaired adaptive and innate immune systems (from decrease in naïve T-cells, increase in memory cells, skewing of myeloids, impaired chemotaxis, and effector functions in neutrophils to defects in NK-cells and monocyte dysregulation [44]), leads also to unsustained memory response to new antigens and might increase the rate of autoimmune responses, as well as inflammaging [45]. Inflammaging is a lingering, low-grade chronic inflammation. This proinflammatory environment is mainly due to the senescence-associated secretory phenotype (SASP) of the senescent immune cells [42, 43], resembling on the one side the processes during chronic liver injury and fibrogenesis [45] and on the other side the processes in mitochondria and autophagy-inflammation-cell death axis, which are quite similar to those described for alcoholic hepatitis and NASH [46–49].

Interestingly, chronic latent viral infections such as CMV and HCV might promote immunosenescence [50, 51] and thereby predispose even younger patients to ACLF. Most importantly the immunological ageing is additionally shaped by infections, and those might tailor the inflammatory response to specific insults [45, 52–54]. Chronic latent viral infections, especially CMV, but also HCV and HIV, might promote chronic systemic inflammation and increased levels of proinflammatory cytokines (IL-6, TNF-α), associated with premature death [51, 52, 55–57]. The low-grade inflammation after latent viral infections also induces premature ageing, predisposing cirrhotic patients to ACLF. This is supported by the fact that reactivation of HBV is a major precipitating factor for the development of ACLF, especially in Asia [39, 58, 59]. Hepatitis E might also be an important trigger for the development of ACLF, whose role is still in discussion [39].

Moreover, inflammaging is associated with impaired production of estrogen and androgen, an impairment that is also present in cirrhosis [60, 61]. Therefore, besides age, the latent infections, mitochondria damage, and decreased sexual hormones might lead to premature immunosenescence and inflammaging in chronic liver disease and predisposing for ACLF development.

4.2. Albumin and Prostaglandin E_2 (PGE_2). Albumin, the most abundant extracellular protein in our system and synthetized exclusively by the liver, is pivotal in maintaining colloid osmotic pressure (for about 70%) but is also endowed with other vital non-oncotic properties, such as antioxidant and scavenging activity (via its sulfhydryl-groups), binding of highly toxic reactive metal species (Cu, Ni, Co, and Fe), and transport of endogenous (such as bilirubin, endotoxin, long-chain fatty acids) and exogenous toxins via the amino-terminal NH2 [62]. Albumin has been found to be a predictor of survival both in compensated and decompensated cirrhosis and ACLF [63, 64]. Studies have shown that in patients with cirrhosis albumin is subjected to posttranscriptional modifications leading to oxidized forms of albumin with impairment of its non-oncotic biological properties and thus leading to decreased "effective" albumin concentration [60].

Emerging recent evidence links this decreased "effective" (no longer native and reduced) albumin in decompensated cirrhosis to increased circulating PGE_2-bioavailability [65]. PGE_2, a cyclooxygenase-derived lipid mediator, is known to play a dual role in immunity since it is a major mediator of inflammation and fever, but a potent inducer of immune suppression by depressing the effector functions of macrophages and neutrophils [66]. Increased free PGE_2 levels, due to decreased effective binding capacity of albumin, might therefore explain the profound immunodeficiency and associated bacterial infections typical of acutely decompensated cirrhosis. Turning this paradigm around, the authors showed that treatment of five patients with acutely decompensated cirrhosis with 200 ml of 20% HSA increased serum albumin concentrations from 23 g/l to 30 g/l and reversed immunosuppression [65]. In an extended and larger sample size feasibility study of 20% HSA infusions, the same group has meanwhile confirmed that infusions to raise serum albumin above 30 g/L reversed plasma-mediated immune dysfunction [67]. However, in this study the reversal of immune dysfunction following HAS therapy appeared to be mediated by changes in the circulating levels of a novel series of anti-inflammatory and proresolving lipid mediators

generated from long-chain omega-3 polyunsaturated fatty acids rather than by binding of PGE_2 [67].

Attempts to substitute or cleanse albumin (via albumin dialysis or plasma exchange) might therefore prove interest and warrant further investigation. In favour of this premise is the recently presented ANSWER study [68]. This randomised, controlled trial of 440 patients with cirrhosis and uncomplicated ascites compared standard diuretic therapy with standard diuretic therapy plus human albumin (40 g intravenously twice weekly in the first two weeks and then once weekly). Treatment with human albumin reduced the risk of death by 38%. In addition, albumin infusions also rendered significant benefits with regard to management of ascites, complications of cirrhosis (spontaneous bacterial peritonitis (SBP), non-SBP bacterial infection, renal dysfunction, hepatorenal syndrome, and hepatic encephalopathy), quality of life, and hospital admissions.

4.3. Farnesoid X-Receptor (FXR). FXR is a ligand-activated transcription factor belonging to the nuclear receptor superfamily and acts as sensor for a broad range of natural ligands with bile acids as the most potent ones, in particular chenodeoxycholic acid. Therefore, FXR is highly expressed in bile acid-handling tissues such as liver, intestine, and kidney. Upon binding of bile acids to FXR, the receptor translocates to the nucleus where it forms a heterodimer with its binding partner retinoid-X receptor (RXR) and through its DNA-binding domain directly influences the transcription of a large variety of target genes [65, 66]. Since FXR is at the crossroad of metabolic regulation, inflammation, and regeneration in normal tissue, it is driving key regulator functions.

Recent translational research has suggested a central role for defective farnesoid-X-receptor signaling in hepatic inflammation, portal hypertension, and intestinal bacterial translocation, factors which are known to promote and shape ACLF and are potentially targetable through pharmacological agonists [69–74].

4.4. Gut Microbiota. Intestinal dysbiosis is characterized by imbalanced quantitative and qualitative changes in the composition of the gut microbiota and is associated with alterations of metabolic activity as well as an altered distribution of its microbial members. In recent years, accumulating evidence has indicated that microbial products trigger and instigate liver inflammation and that progressive qualitative changes in the gut microbiome (autochthonous to non-autochthonous taxa abundance) accompany cirrhosis and become more severe in the setting of decompensation [75]. In a recent case-control study in patients with ACLF of diverse etiology, the severity of gut dysbiosis was found worse in ACLF than in cirrhosis (considered as "a press disturbance" implying long-term impact on an ecosystem) with only moderate impact of antibiotics on its composition [76]. Additionally, the authors found that the specific gut dysbiosis in ACLF was associated with outcome, with abundance of Pasteurellaceae as independent predictor of mortality. More specifically, network-analysis comparison showed robust correlations between specific bacterial families (Ruminococcaceae and Lachnospiraceae) and inflammatory cytokines (IL-6, TNF-α, IL-2) in ACLF patients, indicating that gut microbiota constitutes a major backbone in ACLF pathogenesis and perpetuation [48].

5. Conclusions

ACLF is a specific, but complex and multifactorial form of acute decompensation of cirrhosis and is characterized by an extraordinary dynamic natural course, rapidly evolving organ failure, and high short-term mortality. Dysbalanced inflammation is central to its pathogenesis and outcome with an initial excessive systemic inflammatory response associated that drives organ failure and mortality. Later in its course, immuno-exhaustion/immunoparalysis prevails predisposing the patient to secondary infectious events and reescalation in end-organ dysfunction and mortality.

Further studies are needed to evaluate and characterize the evolving course of systemic inflammation starting from compensated cirrhosis over mere acute decompensation to ACLF. In addition, specific systemic inflammation signatures and organ dysfunction/failure are to be assessed as are specific inflammatory prophiles per triggering event. These studies hopefully will be able to guide future management, in terms of both prevention and treatment and/or organ specific approaches, for patients with AD and ACLF. For now, as its pathophysiology is gradually being unravelled, potential therapeutic targets emerge that warrant further study such as restoring or substituting albumin via plasma exchange or via albumin dialysis and evaluating usefulness of TLR4 antagonists, modulators of gut dysbiosis (pre- or probiotics), and FXR-agonists.

Abbreviations

ACLF:	Acute-on-chronic liver failure
PRR:	Pattern recognition receptor
IL:	Interleukin
TNF:	Tumor necrosis factor
NLR:	Nucleotide-binding oligomerization domain-(NOD-) like receptor
TLR:	Toll-like receptor
PAMPs:	Pathogen-associated molecular patterns
DAMPs:	Damage/danger-associated molecular patterns
SASP:	Senescence-associated secretory phenotype
FXR:	Farnesoid-X-receptor
EASL-CLIF:	European Association for the Study of the Liver-Consortium on Chronic Liver Insufficiency.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] W. Laleman, L. Verbeke, P. Meersseman et al., "Acute-on-chronic liver failure: Current concepts on definition, pathogenesis, clinical manifestations and potential therapeutic interventions,"

Expert Review of Gastroenterology & Hepatology, vol. 5, no. 4, pp. 523–537, 2011.

[2] R. Moreau, R. Jalan, P. Gines et al., "Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis," *Gastroenterology*, vol. 144, no. 7, pp. 1426.e9–1437.e9, 2013.

[3] J. Clària, R. E. Stauber, M. J. Coenraad et al., "Systemic inflammation in decompensated cirrhosis: Characterization and role in acute-on-chronic liver failure," *Hepatology*, vol. 64, no. 4, pp. 1249–1264, 2016.

[4] R. Medzhitov, D. S. Schneider, and M. P. Soares, "Disease tolerance as a defense strategy," *Science*, vol. 335, no. 6071, pp. 936–941, 2012.

[5] C. N. Jenne and P. Kubes, "Immune surveillance by the liver," *Nature Immunology*, vol. 14, no. 10, pp. 996–1006, 2013.

[6] T. Kawai and S. Akira, "The role of pattern-recognition receptors in innate immunity: update on toll-like receptors," *Nature Immunology*, vol. 11, no. 5, pp. 373–384, 2010.

[7] S. Pandey, T. Kawai, and S. Akira, "Microbial sensing by toll-like receptors and intracellular nucleic acid sensors," *Cold Spring Harbor Perspectives in Biology*, vol. 7, no. 1, Article ID a016246, 2015.

[8] N. Saïd-Sadier and D. Ojcius, "Alarmins, inflammasomes and immunity," *Biomedical Journal*, vol. 35, no. 6, pp. 437–449, 2012.

[9] H. Kono and K. L. Rock, "How dying cells alert the immune system to danger," *Nature Reviews Immunology*, vol. 8, no. 4, pp. 279–289, 2008.

[10] L. Verbeke, F. Nevens, and W. Laleman, "Bench-to-beside review: acute-on-chronic liver failure - linking the gut, liver and systemic circulation," *Critical Care*, vol. 15, no. 5, article 233, 2011.

[11] M. Adib-Conquy and J.-M. Cavaillon, "Compensatory anti-inflammatory response syndrome," *Thrombosis and Haemostasis*, vol. 101, no. 1, pp. 36–47, 2009.

[12] S. L. Foster, D. C. Hargreaves, and R. Medzhitov, "Gene-specific control of inflammation by TLR-induced chromatin modifications," *Nature*, vol. 447, no. 7147, pp. 972–978, 2007.

[13] S. K. Biswas and E. Lopez-Collazo, "Endotoxin tolerance: new mechanisms, molecules and clinical significance," *Trends in Immunology*, vol. 30, no. 10, pp. 475–487, 2009.

[14] E. Weiss, P. Rautou, M. Fasseu et al., "Type I interferon signaling in systemic immune cells from patients with alcoholic cirrhosis and its association with outcome," *Journal of Hepatology*, vol. 66, no. 5, pp. 930–941, 2017.

[15] S. K. Sarin, A. Kumar, J. A. Almeida et al., "Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL)," *Hepatology International*, vol. 3, no. 1, pp. 269–282, 2009.

[16] R. Jalan, C. Yurdaydin, J. S. Bajaj et al., "Toward an improved definition of acute-on-chronic liver failure," *Gastroenterology*, vol. 147, no. 1, pp. 4–10, 2014.

[17] A. Albillos, M. Lario, and M. Álvarez-Mon, "Cirrhosis-associated immune dysfunction: Distinctive features and clinical relevance," *Journal of Hepatology*, vol. 61, no. 6, pp. 1385–1396, 2014.

[18] C.-Y. Lin, I.-F. Tsai, Y.-P. Ho et al., "Endotoxemia contributes to the immune paralysis in patients with cirrhosis," *Journal of Hepatology*, vol. 46, no. 5, pp. 816–826, 2007.

[19] H. E. Wasmuth, D. Kunz, E. Yagmur et al., "Patients with acute on chronic liver failure display 'sepsis-like' immune paralysis," *Journal of Hepatology*, vol. 42, no. 2, pp. 195–201, 2005.

[20] J. Du Plessis, H. Vanheel, C. E. I. Janssen et al., "Activated intestinal macrophages in patients with cirrhosis release NO and IL-6 that may disrupt intestinal barrier function," *Journal of Hepatology*, vol. 58, no. 6, pp. 1125–1132, 2013.

[21] M. Cazzaniga, E. Dionigi, G. Gobbo, A. Fioretti, V. Monti, and F. Salerno, "The systemic inflammatory response syndrome in cirrhotic patients: Relationship with their in-hospital outcome," *Journal of Hepatology*, vol. 51, no. 3, pp. 475–482, 2009.

[22] S. Gandoura, E. Weiss, P.-E. Rautou et al., "Gene- and exon-expression profiling reveals an extensive LPS-induced response in immune cells in patients with cirrhosis," *Journal of Hepatology*, vol. 58, no. 5, pp. 936–948, 2013.

[23] A. Albillos, A. De La Hera, E. Reyes et al., "Tumour necrosis factor-alpha expression by activated monocytes and altered T-cell homeostasis in ascitic alcoholic cirrhosis: amelioration with norfloxacin," *Journal of Hepatology*, vol. 40, no. 4, pp. 624–631, 2004.

[24] C. Bernsmeier, O. T. Pop, A. Singanayagam et al., "Patients with acute-on-chronic liver failure have increased numbers of regulatory immune cells expressing the receptor tyrosine kinase MERTK," *Gastroenterology*, vol. 148, no. 3, pp. 603–615.e14, 2015.

[25] C. Bernsmeier, E. Triantafyllou, R. Brenig et al., "$CD14^+$ $CD15^-$ $HLA\text{-}DR^-$ myeloid-derived suppressor cells impair antimicrobial responses in patients with acute-on-chronic liver failure," *Gut*, vol. 67, no. 6, pp. 1155–1167, 2018.

[26] P.-E. Rautou, J. Bresson, Y. Sainte-Marie et al., "Abnormal plasma microparticles impair vasoconstrictor responses in patients with cirrhosis," *Gastroenterology*, vol. 143, no. 1, pp. 166–176.e6, 2012.

[27] P. Bellot, J. C. García-Pagán, R. Francés et al., "Bacterial DNA translocation is associated with systemic circulatory abnormalities and intrahepatic endothelial dysfunction in patients with cirrhosis," *Hepatology*, vol. 52, no. 6, pp. 2044–2052, 2010.

[28] G. Mehta, R. P. Mookerjee, V. Sharma, and R. Jalan, "Systemic inflammation is associated with increased intrahepatic resistance and mortality in alcohol-related acute-on-chronic liver failure," *Liver International*, vol. 35, no. 3, pp. 724–734, 2015.

[29] A. Albillos, A. D. la Hera, M. González et al., "Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement," *Hepatology*, vol. 37, no. 1, pp. 208–217, 2003.

[30] W. Laleman, A. Wilmer, P. Evenepoel et al., "Effect of the molecular adsorbent recirculating system and Prometheus devices on systemic haemodynamics and vasoactive agents in patients with acute-on-chronic liver failure," *Critical Care*, vol. 10, no. 4, article R108, 2006.

[31] J. Villar, N. Cabrera, and M. Casula, "Mechanical ventilation modulates Toll-like receptor signaling pathway in a sepsis-induced lung injury model," *Intensive Care Medicine*, vol. 36, no. 6, pp. 1049–1057, 2010.

[32] N. Shah, F. E. Mohamed, M. Jover-Cobos et al., "Increased renal expression and urinary excretion of TLR4 in acute kidney injury associated with cirrhosis," *Liver International*, vol. 33, no. 3, pp. 398–409, 2013.

[33] S. Piano, H. H. Schmidt, X. Ariza et al., "Association between grade of acute on chronic liver failure and response to terlipressin and albumin in patients with hepatorenal syndrome," *Clinical Gastroenterology and Hepatology*, 2018, In press.

[34] J. Fernández, J. Acevedo, R. Wiest et al., "Bacterial and fungal infections in acute-on-chronic liver failure: prevalence, characteristics and impact on prognosis," *Gut*, 2017.

[35] T. Lisman, S. H. Caldwell, A. K. Burroughs et al., "Hemostasis and thrombosis in patients with liver disease: the ups and downs," *Journal of Hepatology*, vol. 53, no. 2, pp. 362–371, 2010.

[36] M. Bernardi, R. Moreau, P. Angeli, B. Schnabl, and V. Arroyo, "Mechanisms of decompensation and organ failure in cirrhosis: From peripheral arterial vasodilation to systemic inflammation hypothesis," *Journal of Hepatology*, vol. 63, no. 5, pp. 1272–1284, 2015.

[37] R. Jalan, M. Pavesi, F. Saliba et al., "The CLIF Consortium Acute Decompensation score (CLIF-C ADs) for prognosis of hospitalised cirrhotic patients without acute-on-chronic liver failure," *Journal of Hepatology*, vol. 62, pp. 831–840, 2015.

[38] R. Jalan, V. Stadlbauer, S. Sen, L. Cheshire, Y.-M. Chang, and R. P. Mookerjee, "Role of predisposition, injury, response and organ failure in the prognosis of patients with acute-on-chronic liver failure: A prospective cohort study," *Critical Care*, vol. 16, no. 6, article R227, 2012.

[39] H. Garg, A. Kumar, V. Garg, P. Sharma, B. C. Sharma, and S. K. Sarin, "Clinical profile and predictors of mortality in patients of acute-on-chronic liver failure," *Digestive and Liver Disease*, vol. 44, no. 2, pp. 166–171, 2012.

[40] R. Aspinall, G. Del Giudice, R. B. Effros, B. Grubeck-Loebenstein, and S. Sambhara, "Challenges for vaccination in the elderly," *Immunity & Ageing*, vol. 4, article 9, 2007.

[41] G. Gavazzi and K.-H. Krause, "Ageing and infection," *The Lancet Infectious Diseases*, vol. 2, no. 11, pp. 659–666, 2002.

[42] C. Franceschi, M. Bonafè, S. Valensin et al., "Inflamm-aging. An evolutionary perspective on immunosenescence," *Annals of the New York Academy of Sciences*, vol. 908, pp. 244–254, 2000.

[43] C. Franceschi, M. Capri, D. Monti et al., "Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans," *Mechanisms of Ageing and Development*, vol. 128, no. 1, pp. 92–105, 2007.

[44] M. Deleidi, M. Jäggle, and G. Rubino, "Immune ageing, dysmetabolism and inflammation in neurological diseases," *Frontiers in Neuroscience*, vol. 9, article 172, 2015.

[45] J. J. Goronzy and C. M. Weyand, "Understanding immunosenescence to improve responses to vaccines," *Nature Immunology*, vol. 14, no. 5, pp. 428–436, 2013.

[46] S. L. Friedman, "Mechanisms of hepatic fibrogenesis," *Gastroenterology*, vol. 134, no. 6, pp. 1655–1669, 2008.

[47] D. R. Green, L. Galluzzi, and G. Kroemer, "Mitochondria and the autophagy-inflammation-cell death axis in organismal aging," *Science*, vol. 333, no. 6046, pp. 1109–1112, 2011.

[48] L. Dubuquoy, A. Louvet, G. Lassailly et al., "Progenitor cell expansion and impaired hepatocyte regeneration in explanted livers from alcoholic hepatitis," *Gut*, vol. 64, no. 12, pp. 1949–1960, 2015.

[49] C. Koliaki, J. Szendroedi, K. Kaul et al., "Adaptation of Hepatic Mitochondrial Function in Humans with Non-Alcoholic Fatty Liver Is Lost in Steatohepatitis," *Cell Metabolism*, vol. 21, no. 5, pp. 739–746, 2015.

[50] J. Trebicka and R. Schierwagen, "Hepatic mitochondrial dysfunction in nonalcoholic steatohepatitis: Read-out or reason?" *Hepatology*, vol. 63, no. 5, pp. 1729–1732, 2016.

[51] S. Koch, A. Larbi, D. Özcelik et al., "Cytomegalovirus infection: A driving force in human T cell immunosenescence," *Annals of the New York Academy of Sciences*, vol. 1114, pp. 23–35, 2007.

[52] N. H. Gruener, F. Lechner, M.-C. Jung et al., "Sustained dysfunction of antiviral CD8+ T lymphocytes after infection with hepatitis C virus," *Journal of Virology*, vol. 75, no. 12, pp. 5550–5558, 2001.

[53] J. Nikolich-Žugich, "Ageing and life-long maintenance of T-cell subsets in the face of latent persistent infections," *Nature Reviews Immunology*, vol. 8, no. 7, pp. 512–522, 2008.

[54] L. F. Su, B. A. Kidd, A. Han, J. J. Kotzin, and M. M. Davis, "Virus-specific $CD4^+$ memory-phenotype T cells are abundant in unexposed adults," *Immunity*, vol. 38, no. 2, pp. 373–383, 2013.

[55] E. Derhovanessian, A. B. Maier, K. Hähnel et al., "Infection with cytomegalovirus but not herpes simplex virus induces the accumulation of latedifferentiated CD4 + and CD8 + T-cells in humans," *Journal of General Virology*, vol. 92, no. 12, pp. 2746–2756, 2011.

[56] L. H. Mortensen, A. B. Maier, P. E. Slagbom et al., "Early-life environment influencing susceptibility to cytomegalovirus infection: Evidence from the Leiden Longevity Study and the Longitudinal Study of Aging Danish Twins," *Epidemiology and Infection*, vol. 140, no. 5, pp. 835–841, 2012.

[57] M. Beyer, Z. Abdullah, J. M. Chemnitz, and etaL, "TNF impairs CD4+ T-cell mediated immune control in chronic viral infection," *Nature Immunology*, 2016, In press.

[58] L. J. Sun, J. W. Yu, Y. H. Zhao, P. Kang, and S. C. Li, "Influential factors of prognosis in lamivudine treatment for patients with acute-on-chronic hepatitis B liver failure," *Journal of Gastroenterology and Hepatology*, vol. 25, no. 3, pp. 583–590, 2010.

[59] K. A. Wlodzimirow, S. Eslami, A. Abu-Hanna, M. Nieuwoudt, and R. A. F. M. Chamuleau, "A systematic review on prognostic indicators of acute on chronic liver failure and their predictive value for mortality," *Liver International*, vol. 33, no. 1, pp. 40–52, 2013.

[60] M. Maggio, S. Basaria, A. Ble et al., "Correlation between testosterone and the inflammatory marker soluble interleukin-6 receptor in older men," *The Journal of Clinical Endocrinology & Metabolism*, vol. 91, no. 1, pp. 345–347, 2006.

[61] M. Abu-Taha, C. Rius, C. Hermenegildo et al., "Menopause and ovariectomy cause a low grade of systemic inflammation that may be prevented by chronic treatment with low doses of estrogen or losartan," *The Journal of Immunology*, vol. 183, no. 2, pp. 1393–1402, 2009.

[62] M. Bernardi, C. S. Ricci, and G. Zaccherini, "Role of human albumin in the management of complications of liver cirrhosis," *Journal of Clinical and Experimental Hepatology*, vol. 4, no. 4, pp. 302–311, 2014.

[63] C. Ripoll, K. Bari, and G. Garcia-Tsao, "Serum albumin can identificy patients with compensated cirrhosis," *Journal of Clinical Gastroenterology*, vol. 49, no. 7, pp. 613–619, 2015.

[64] R. Jalan, K. Schnurr, R. P. Mookerjee et al., "Alterations in the functional capacity of albumin in patients with decompensated cirrhosis is associated with increased mortality," *Hepatology*, vol. 50, no. 2, pp. 555–564, 2009.

[65] A. J. O'Brien, J. N. Fullerton, K. A. Massey et al., "Immunosuppression in acutely decompensated cirrhosis is mediated by prostaglandin E2," *Nature Medicine*, vol. 20, no. 5, pp. 518–523, 2014.

[66] P. Kalinski, "Regulation of immune responses by prostaglandin E_2," *The Journal of Immunology*, vol. 188, no. 1, pp. 21–28, 2012.

[67] L. China, A. Maini, S. S. Skene et al., "Albumin counteracts immune-suppressive effects of lipid mediators in patients with advanced liver disease," *Clinical Gastroenterology and Hepatology*, vol. 16, no. 5, pp. 738–747.e7, 2018.

[68] P. Caraceni, O. Riggio, P. Angeli et al., "Long-term albumin administration improves survival in patients with decompensated cirrhosis: final results of the "ANSWER" study," *Journal of Hepatology*, vol. 66, no. 1, p. S93, 2017.

[69] L. Verbeke, R. Farre, J. Trebicka et al., "Obeticholic acid, a farnesoid X receptor agonist, improves portal hypertension by two distinct pathways in cirrhotic rats," *Hepatology*, vol. 59, no. 6, pp. 2286–2298, 2014.

[70] L. Verbeke, R. Farre, B. Verbinnen et al., "The FXR agonist obeticholic acid prevents gut barrier dysfunction and bacterial translocation in cholestatic rats," *The American Journal of Pathology*, vol. 185, no. 2, pp. 409–419, 2014.

[71] M. Ubeda, M. Borrero, M. Lario et al., "O153 the farnesoid X receptor agonist, obeticholic acid, improves intestinal antibacterial defense and reduces gut bacterial translocation and hepatic fibrogenesis in CCl4-cirrhotic rats with ascites," *Journal of Hepatology*, vol. 60, no. 1, p. S63, 2014.

[72] Y.-D. Wang, W.-D. Chen, M. Wang, D. Yu, B. M. Forman, and W. Huang, "Farnesoid X receptor antagonizes nuclear factor κB in hepatic inflammatory response," *Hepatology*, vol. 48, no. 5, pp. 1632–1643, 2008.

[73] P. Lutz, C. Berger, B. Langhans et al., "A farnesoid X receptor polymorphism predisposes to spontaneous bacterial peritonitis," *Digestive and Liver Disease*, vol. 46, no. 11, pp. 1047–1050, 2014.

[74] V. Massafra, A. Milona, H. R. Vos et al., "Farnesoid X Receptor Activation Promotes Hepatic Amino Acid Catabolism and Ammonium Clearance in Mice," *Gastroenterology*, vol. 152, no. 6, pp. 1462–1476.e10, 2017.

[75] J. S. Bajaj, D. M. Heuman, P. B. Hylemon et al., "Altered profile of human gut microbiome is associated with cirrhosis and its complications," *Journal of Hepatology*, vol. 60, no. 5, pp. 940–947, 2014.

[76] Y. Chen, J. Guo, G. Qian et al., "Gut dysbiosis in acute-on-chronic liver failure and its predictive value for mortality," *Journal of Gastroenterology and Hepatology*, vol. 30, no. 9, pp. 1429–1437, 2015.

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Research Article

The Correlation between miR-122 and Lipoprotein Lipase Expression in Chronic Hepatitis C Patients

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Chronic HCV infection is strictly associated with host lipid/lipoprotein metabolism disorders. The study aimed to analyze the relationship between viral load, lipid profile, IFNγ, and the expression of miR-122 and LPL in the liver and PBMCs. Sera, PBMCs, and matching liver biopsies from 17 chronic hepatitis C patients were enrolled in this study. Collected data shows that liver (not PBMCs) miR-122 expression is positively correlated with HCV RNA load and IFNγ and reversely with LPL expression in CHC patients. Presented, for the first time, in this study, the reverse correlation of miR-122 and LPL expression in liver; miR-122 and LPL seem to be important factors of CHC infection.

1. Introduction

Hepatitis C virus (HCV), with an estimated over 185 million people infected worldwide [1], is the major etiological cause of chronic hepatitis. Chronic hepatitis C (CHC) is directly associated with development of liver cirrhosis and hepatoma. HCV RNA triggers production of interferon gamma (IFNγ) but is often abrogated in CHC patients [2]. Reduced IFNγ level implicates an immunological impairment that in turn consolidates the state of chronicity.

HCV infection affects host lipids/lipoproteins homeostasis. Alteration of metabolism seems to be essential for the HCV entire "life cycle": entry, replication, and the secretory pathway [3]. Circulating HCV virions are associated with VLDL and LDL, designated as lipo-viro-particles. Lipoprotein lipase (LPL) that catalyzes triglyceride (TG) hydrolysis of chylomicrons and VLDL has been identified as a promising host factor against HCV infection [4]. Lipo-viro HCV particles constitute effective substrate for LPL; thus reduction of HCV infectivity can be achieved by modifying the virion-associated lipid composition [5]. Additionally, LPL increases the binding capacity of HCV particles and reduces infectivity of the virus, possibly by blocking virions on the surface of cells [6]. Also, LPL-induced peroxisomal proliferators-activated receptor-α (PPAR-α) signaling pathway activation was suggested as a factor that suppresses HCV infection [7].

Previous reports underscore the importance of miR-122 in liver biology and in the control of HCV infection (for review [8]). miR-122 exerts in vitro a positive effect on HCV replication through a direct interaction with 5'UTR region of viral genome. Inhibition of miR-122 reduces serum cholesterol level [9]. HCV RNA sequences were detected in vivo, not only in hepatocytes but also in fresh peripheral blood mononuclear cell (PBMC) preparations from HCV-infected patients as well as cultured PBMCs [10]. In previous studies we showed not only changes in serum lipid profile in CHC patients but also in cholesterol expression in PBMCs [11]. Neither this lipid's alteration nor HCV RNA presence in cells was related to miR-122 expression in PBMCs.

This study compares miR-122 and lipoprotein lipase expression in the liver and PBMCs and investigates how these parameters are associated with lipid profile, with HCV load and IFN-gamma, revealing the reverse correlation between lipoprotein lipase and miR-122 expression in the liver of CHC patients. To the best of our knowledge this study represents the first attempt to evaluate the relationship between miR-122 and LPL expression.

2. Materials and Methods

2.1. Participant Enrollment. This study recruited 17 patients with CHC (genotype 1) and 26 healthy donors (HD). Blood

samples obtained from both groups, CHC patients and healthy donors, were used for PBMCs and sera isolation. Serum HCV RNA was detected by the Amplicor HCV test, version 2.0 (Roche Diagnostics). In case of CHC patients, matching human liver specimens from fine needle biopsies were collected. The study was approved by the Bioethical Committee of the Medical University of Lodz (RNN/93/07/kB); signed informed consent from all participants was obtained.

2.2. Determination of Biochemical Parameters in Sera and PBMCs. We followed the methods of Sidorkiewicz et al. 2017 [12]. Serum total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and triglycerides (TG) were measured enzymatically in Olympus AU 640 and the kits of Olympus. The IFNγ concentration in sera was measured using Human IFNγ ELISA kit (BioVendor).

Intracellular cholesterol level in PBMC (IC) was evaluated using the cholesterol assay kit, Cholesterol CHOD-PAP (BIOLABO S.A., France) as per manufacturer's recommendation, and the results were normalized to the protein concentration in cell lysates and presented as relative expression of (r.e. IC).

2.3. miR-122 Analysis. For miR-122 analysis, RNA fraction <200 nt was extracted from PBMCs, using mirVana miRNA Isolation Kit (Ambion), and total RNA from biopsy samples using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. The reverse transcription (RT) was done, on 10 ng of RNA using the TaqMan MicroRNA Assay specific for miRNA-122 (Applied Biosystems). RT product (3.3 μL) was used in Real-Time PCR with miRNA-122-specific primers/probe mix and TaqMan Universal PCR Master Mix (Applied Biosystems). The reaction was performed on the ABI Prism 7900 Fast Real-Time PCR System (Applied Biosystems). Relative expression (r.e.) of miR-122 presents the mean Ct value for miRNA-122 Ct, calculated from triplicate reactions and normalized to average Ct values, obtained for snRNA U6.

2.4. Determination of LPL Expression. LPL expression was determined by Western Blotting. A total of 50 μg of protein from PBMCs lysates was subjected to SDS-PAGE, transferred into nitrocellulose, and incubated with rabbit anti-LPL primary antibodies (sc-32885, Santa Cruz Biotechnology, dilution 1:300) and secondary goat anti-rabbit antibodies (sc-2004, Santa Cruz Biotechnology, dilution 1:4000). As a control, goat antibodies against β-actin (sc-1615, Santa Cruz Biotechnology, dilution 1:300) were used with secondary rabbit anti-goat (1:10000) from Sigma. The positive bands were detected using enhanced chemiluminescence system (ECL) and were quantitatively analyzed using the Bio-Rad Quantity One System. Relative expression (r.e.) of LPL was calculated by normalization to the actin expression for each sample.

2.5. Statistical Analysis. Statistical analysis was performed with STATISTICA 8,0 PL software (StatSoft). Groups were compared using the Mann–Whitney *U* test. Spearman's correlation analysis was applied to measure the strength of relationship between parameters. Differences were considered statistically significant at $p<0,05$.

All origin data supporting the results reported in this paper are collected and available in the Department of Medical Biochemistry.

Table 1: Comparison of analyzed parameters in the group of CHC patients and healthy donors.

Analyzed parameters (mean values)	CHC patients	Healthy donors	p value
Number (M/F)	17 (13/4)	26 (15/11)	n.a.
Age (ys)	22,7	23,2	n.a.
ALT (IU/ml)	69,18	20,6	0,003
IFN gamma (pg/ml)	4,41	5,52	0,015
TC (mmol/L)	2,55	3,61	<0,0001
LDL-C (mmol/L)	1,4	2,03	0,003
HDL-C (mmol/L)	0,8	1,09	0,002
TG (mmol/L)	0,77	1,1	0,16 (ns)
r.e. IC (PBMCs)	1,12	1,76	<0,0001
HCV RNA load (IU/ml)	$3,79 \times 10^5$	n.a.	n.a.
r.e. LPL (PBMCs)	2,37	1,32	0,008
r.e. LPL (Liver)	3,12	n.a.	n.a.
r.e. miR-122 (PBMCs)	0,88	0,76	0,001
r.e. miR-122 (Liver)	1,15	n.a.	n.a.

ALT: alanine aminotransferase; F: female; M: male; n.a.: not analyzed; ns: not statistically confirmed result; r.e.: relative expression; ys: years.

3. Results

This study recruited 17 patients with CHC (genotype 1) and 26 healthy donors (HD). General characteristics of studied subjects are included in Table 1. As presented, the level of total cholesterol, LDL, and HDL fractions in sera as well as intracellular cholesterol level in PBMCs were significantly lower in CHC patients compared to healthy donors. Similarly, serum IFNγ was significantly lower in CHC patients than in HD. Observed decreased level of triglycerides in CHC patients was not statistically significant. Comparison of LPL and miR-122 expression in PBMCs showed, for both parameters, a higher expression in cells from CHC patients than from HD.

Spearman rank correlation analysis performed on data collected from CHC patients (Table 2) indicated strong reverse correlation for miR-122 expression between liver and PBMCs as well as for LPL expression between these two origins. miR-122 expression in liver was positively correlated with serum HCV RNA load and IFNγ and reversely correlated with hepatic LPL expression. We found also the tendency for hepatic LPL expression to be reversely correlated with viral load. Neither for miR-122 nor for LPL expression in PBMCs of CHC patients, significant correlation with HCV

Table 2: The list of correlations (Spearman rank correlation test) found for data obtained by the analysis of sera (S), liver biopsies (L), and PBMCs (P) samples collected from CHC patients.

Compared parameters	Correlation coefficient value	P value
miR-122 (L) vs HCV RNA (S)	+0,63	0,006
miR-122 (L) vs IFN γ (S)	+0,54	0,024
miR-122 (P) vs HCV RNA (S)	-0,81	0,026
miR-122 (P) vs miR-122 (B)	-0,93	0,001
LPL (L) vs LPL (P)	-0,56	0,028
LPL (L) vs miR-122 (B)	-0,47	0,014
LPL (L) vs HCV RNA (S)	-0,31	0,06$*$
LPL (P) vs HCV RNA (S)	+0,42	0,036

$*$ - indicates a tendency.

RNA load was revealed. Additionally, in the group of healthy donors, strong reverse correlation was found between serum IFNγ and both TC (r= -0,52, p=0,008) and LDL (r= -0,42, p=0,039), not detected in the group of CHC patients.

4. Discussion

The strategic role of hepatitis C virus in manipulating of host lipids/lipoproteins metabolism was described [3] as essential for the entry, secretion, and replication of virus. Our results showed that TC, LDL, and HDL fractions, as well as intracellular cholesterol level in PBMCs, were significantly decreased in CHC patients compared to healthy donors. These data remain in line with previous observations that associates CHC infections not only with liver steatosis but also with decreased cholesterol expression [13, 14]. HCV may modulate lipid metabolism in host cells in order to create a more hospitable environment for its own "life cycle".

Concerning IFN, we observed that serum IFNγ level was significantly reduced in CHC patients compared to healthy donors, which remains in agreement with earlier reports [15, 16]. Additionally, we found that serum IFNγ was reversely correlated with total cholesterol and LDL level, but only in the group of healthy donors. Lack of such a relationship in the group of CHC patients may suggest perturbation between cholesterol metabolism and IFNγ signaling. It was shown by others [17] that the alteration of cholesterol pathway may abrogate the IFN-gamma-dependent immune response.

Lipoprotein lipase, which plays a main role in lipoprotein/lipid homeostasis, emerged recently as a host-protecting factor against HCV infection. Indeed, a strong tendency to opposite correlation between HCV RNA load and hepatic expression of lipoprotein lipase seems to confirm these observations in our study. Lack of such a relationship for LPL in PBMCs can be explained by opposite correlation between LPL expression in liver and PBMCs. Interestingly, while LPL may regulate TG metabolism, no correlation was found between LPL expression and TG level.

According to the suggested role of miR-122 in HCV replication [8], our results demonstrated the positive correlation between the HCV RNA load and miR-122 expression in liver (not in PBMCs). Like in case of LPL, miR-122 showed also opposite expression in the liver and in PBMCs. Hepatic miR-122 was reversely correlated with the expression of lipoprotein lipase. It is especially interesting observation as liver-specific miR-122 is known to promote hepatitis C virus replication and lipoprotein lipase to protect the host from HCV infection.

As was mentioned earlier, LPL may suppress HCV infectivity by the breakdown of viral lipid coat and bridging between HCV and hepatocytes that blocks the viral entry to the cell [4, 6]. The third mechanism of anti-HCV action of LPL is based on activation of peroxisomal proliferators-activated receptor-α (PPAR-α) signaling pathway [7]. After LPL hydrolysis free fatty acids create ligands for this pathway in target cells. That in turn may cause an antiviral action in infected patients via reduction of viral assembly and secretion. Independently of LPL activity, the same pathway may be triggered directly by alteration of miR-122 expression. The study of Gatfield [18] demonstrated that the knockdown of miR-122 expression resulted in the activation of PPAR-α signaling pathway.

Additionally, we found that miR-122 expression in liver (not in PBMCs) is positively correlated with IFNγ concentration. Interestingly, it was found earlier that IFNγ is involved in lipoprotein lipase inhibition, at the level of LPL gene transcription [19]. Thus, we cannot exclude that, in CHC patients, IFN-gamma may participate in reducing of LPL expression along with miR-122.

In conclusion, this study provides evidence for opposite expression of both miR-122 and lipoprotein lipase in liver and PBMCs. Hepatic miR-122 expression in CHC patients seems to be of high importance for HCV infection which was evidenced by strong positive relationship between miR-122 and HCV RNA load. The reverse relationship between miR-122 and lipoprotein lipase in liver suggests that the modulation of lipoprotein lipase may be another manifestation of the proinfective action of miR-122. Therefore, a future study attempting to explain the mechanism of cross-reactivity between miR-122 and LPL is worth considering.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] A. P. Thrift, H. B. El-Serag, and F. Kanwal, "Global epidemiology and burden of HCV infection and HCV-related disease," *Nature Reviews Gastroenterology & Hepatology*, vol. 14, no. 2, pp. 122–132, 2017.

[2] L. M. Pfeffer, M. A. Madey, C. A. Riely, and J. F. Fleckenstein, "The induction of type i interferon production in hepatitis C-infected patients," *Journal of Interferon & Cytokine Research*, vol. 29, no. 5, pp. 299–306, 2009.

[3] C.-I. Popescu, L. Riva, O. Vlaicu, R. Farhat, Y. Rouillé, and J. Dubuisson, "Hepatitis C virus life cycle and lipid metabolism," *Biology*, vol. 3, no. 4, pp. 892–921, 2014.

[4] Y. Shimizu, T. Hishiki, K. Sugiyama et al., "Lipoprotein lipase and hepatic triglyceride lipase reduce the infectivity of hepatitis C virus (HCV) through their catalytic activities on HCV-associated lipoproteins," *Virology*, vol. 407, no. 1, pp. 152–159, 2010.

[5] Y. Shimizu, T. Hishiki, S. Ujino, K. Sugiyama, K. Funami, and K. Shimotohno, "Lipoprotein component associated with hepatitis C virus is essential for virus infectivity," *Current Opinion in Virology*, vol. 1, no. 1, pp. 19–26, 2011.

[6] P. Maillard, M. Walic, P. Meuleman et al., "Lipoprotein lipase inhibits hepatitis C virus (HCV) infection by blocking virus cell entry," *PLoS ONE*, vol. 6, no. 10, Article ID e26637, 2011.

[7] H.-Y. Sun, C.-C. Lin, P.-J. Tsai et al., "Lipoprotein lipase liberates free fatty acids to inhibit HCV infection and prevent hepatic lipid accumulation," *Cellular Microbiology*, vol. 19, no. 4, Article ID e12673, 2017.

[8] S. Bandiera, S. Pfeffer, T. Baumert, and M. Zeisel, "miR-122 a key factor and therapeutic target in liver disease," *Journal of Hepatology*, vol. 62, pp. 448–457, 2015.

[9] C. Esau, S. Davis, S. F. Murray et al., "miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting," *Cell Metabolism*, vol. 3, no. 2, pp. 87–98, 2006.

[10] I. Castillo, E. Rodríguez-Iñigo, J. Bartolomé et al., "Hepatitis C virus replicates in peripheral blood mononuclear cells of patients with occult hepatitis C virus infection," *Gut*, vol. 54, no. 5, pp. 682–685, 2005.

[11] M. Sidorkiewicz, M. Grek, B. Jozwiak, and A. Piekarska, "Decreased level of intracellular cholesterol in peripheral blood mononuclear cells is associated with chronic hepatitis C virus infection," *Virus Research*, vol. 178, no. 2, pp. 539–542, 2013.

[12] M. Sidorkiewicz, M. Grek, B. Jozwiak, A. Krol, and A. Piekarska, "The impact of chronic hepatitis C infection on cholesterol metabolism in PBMCs is associated with microRNA-146a expression," *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 36, no. 4, pp. 697–702, 2017.

[13] D. Siagris, M. Christofidou, G. J. Theocharis et al., "Serum lipid pattern in chronic hepatitis C: Histological and virological correlations," *Journal of Viral Hepatitis*, vol. 13, no. 1, pp. 56–61, 2006.

[14] D. J. Felmlee, M. L. Hafirassou, M. Lefevre, T. F. Baumert, and C. Schuster, "Hepatitis C virus, cholesterol and lipoproteins—impact for the viral life cycle and pathogenesis of liver disease," *Viruses*, vol. 5, no. 5, pp. 1292–1324, 2013.

[15] Y. Kondo, V. M. Sung, K. Machida, M. Liu, and M. M. Lai, "Hepatitis C virus infects T cells and affects interferon-γ signaling in T cell lines," *Virology*, vol. 361, no. 1, pp. 161–173, 2007.

[16] M. Malaguarnera, I. Di Fazio, A. Laurino, G. Pistone, S. Restuccia, and B. A. Trovato, "Decrease of interferon gamma serum levels in patients with chronic hepatitis C," *Biomedicine & Pharmacotherapy*, vol. 51, no. 9, pp. 391–396, 1997.

[17] J. M. Jameson, J. Cruz, A. Costanzo, M. Terajima, and F. A. Ennis, "A role for the mevalonate pathway in the induction of subtype cross-reactive immunity to influenza A virus by human $\gamma\delta$ T lymphocytes," *Cellular Immunology*, vol. 264, no. 1, pp. 71–77, 2010.

[18] D. Gatfield, G. Le Martelot, C. E. Vejnar et al., "Integration of microRNA miR-122 in hepatic circadian gene expression," *Genes & Development*, vol. 23, no. 11, pp. 1313–1326, 2009.

[19] S. M. Harris, E. J. Harvey, T. R. Hughes, and D. P. Ramji, "The interferon-γ-mediated inhibition of lipoprotein lipase gene transcription in macrophages involves casein kinase 2- and phosphoinositide-3-kinase-mediated regulation of transcription factors Sp1 and Sp3," *Cellular Signalling*, vol. 20, no. 12, pp. 2296–2301, 2008.

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Research Article

Serum Metabonomics Analysis of Liver Failure Treated by Nonbioartificial Liver Support Systems

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Objective. To analyze the small molecular metabolic compounds of nonbioartificial liver for treatment of hepatic failure and make further efforts to study the clinical efficacy, mechanism of action, and pathogenesis of hepatic failure. *Methods*. 52 patients who met the standard of artificial liver treatment for liver failure were enrolled; these patients included 6 cases of acute liver failure (11.54%), 3 cases of subacute liver failure (5.77%), acute-on-chronic liver failure in 10 cases (19.23%), and 33 cases of chronic liver failure (63.46%). Treatment modes included plasma exchange in 34 patients (65.38%), bilirubin adsorption in 9 patients (17.31%), and hemofiltration in 9 patients (17.31%). The clinical efficacy of artificial liver was assessed by monitoring the effects in the near future. Significant changes in metabolic compounds of liver failure in the treatment before and after artificial liver were screened by using Ultra-Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS). Related metabolic pathways were analyzed by MetaboAnalyst. *Results*. After artificial liver treatment, the liver function and coagulation function of liver failure patients were significantly improved ($P < 0.01$), the Meld score was lower than that before treatment, and the difference was statistically significant ($P < 0.05$). Serum metabolomics identified 29 small metabolic compounds and 12 metabolic pathways with variable projection importance (VIP) greater than 1 before and after artificial liver treatment. There were 11 metabolic compounds of VIP over 1 and 7 metabolic pathways in the different modes of artificial liver treatment for chronic liver failure. Among them, bile acid metabolism, fatty acid metabolism, and amino acid metabolism are the main sources. *Conclusion*. Artificial liver treatment can effectively improve liver function and blood coagulation function and Meld score, clinical symptoms and signs in patients with liver failure; the curative effect of artificial liver was verified, which reflected the clinical value of artificial liver in the treatment of liver failure. Artificial liver treatment of liver failure on fatty acids and primary bile acid synthesis pathway was the most significant. The difference of fatty acid, primary bile acid synthesis pathway, and phenylalanine metabolic pathway in different artificial liver patterns of chronic liver failure was the most significant. This provides a new basis for understanding the mechanism of hepatic failure and the mechanism of liver failure by artificial liver treatment.

1. Introduction

Hepatic failure is a serious hepatic damage that can be caused by many factors. In China, the main cause of liver failure is hepatitis B virus (HBV), followed by drugs and hepatotoxicity [1, 2]. Treatment of liver failure includes comprehensive internal medical treatment, artificial liver support therapy, liver transplantation, and other methods. In recent years, the nonbioartificial liver technology has become one of the most effective methods for treating liver failure. It includes a variety of managements, and plasma exchange, hemofiltration, and bilirubin adsorption are the most common ones [3].

Metabolomics is a technique for studying the metabolic network of biological systems. The object which has been researched is mainly the endogenous small molecules with relative molecular mass below one thousand [4]. The most widely used and effective metabolomics techniques are gas chromatography-mass spectrometry [5] and liquid chromatography-mass spectrometry (GC-MS) [6]; the latter is used more often. At present, the use of metabonomics to analyze the changes of serum metabolites in patients with liver failure by nonbioartificial liver treatment is relatively rare. Therefore, changes of serum metabolites before and after nonbioartificial liver treatment were detected by the platform

of UPLC-MS, to further analyze the pathogenesis of liver failure and investigate the effect of nonbioartificial liver in patients with liver failure metabolites. This provides a basis for further study of artificial liver technology in the treatment of liver failure. It was expected to provide a new basis for further clinical diagnosis and treatment.

2. Materials and Methods

2.1. Inclusion and Exclusion Criteria. All the patients in the group met the criteria for the diagnosis of "guideline for diagnosis and treatment of liver failure" (2012 update) and "guideline for nonbioartificial liver support systems in treatment of liver failure" (2016 update). Liver failure is a serious liver damage caused by a variety of factors and a group of clinical symptoms; the main manifestations include coagulation dysfunction, jaundice, hepatic encephalopathy, and ascites. Acute liver failure is a clinical manifestation of acute onset within 2 weeks. The clinical manifestations of subacute hepatic failure were 2-26 weeks. Acute-on-chronic liver failure is a clinical syndrome with acute or subacute liver decompensation occurring on the basis of chronic liver disease in a short time. Chronic liver failure is a progressive decline and decompensation of liver function on the basis of liver cirrhosis. Exclusion criteria included patients with active bleeding or disseminated intravascular coagulation (DIC); hemodynamic instability in patients; allergic to blood products or medications during treatment. According to the above criteria, 68 patients were evaluated, and 6 cases of active bleeding, 4 cases of allergy to blood products, 6 patients with DIC, and 52 patients with liver failure treated with nonbioartificial liver were selected from December 2015 to November 2016 at the First Affiliated Hospital of Dalian Medical University finally (Figure 1). The aetiology of individual causes of liver failure was different; there were 6 cases of drug hepatitis, 5 cases of alcoholic hepatitis, 3 cases of chronic viral hepatitis B, 2 cases of chronic viral hepatitis E, 3 cases of autoimmune hepatitis, and 33 cases of cirrhosis. According to the diagnosis and classification of hepatic failure, 52 cases were divided into acute hepatic failure in 6 cases (11.54%), subacute hepatic failure in 3 cases (5.77%), acute-on-chronic liver failure in 10 cases (19.23%), and chronic liver failure in 33 cases (63.46%).

2.2. Methods of Each Artificial Liver Support. Plasma exchange (PE) is to filter out the plasma containing toxins in the blood out of the membrane and back into the body with the same amount of fresh plasma or fresh frozen plasma with the blood type components that are withheld in the membrane. It can clear liver failure toxins and some pathogenic factors, supplement the essential substances such as coagulation factors that are lacking in liver failure, and correct metabolic disorders caused by liver failure. The disadvantage of PE is that it cannot effectively remove water-soluble substances from small molecules. PE has a certain risk of adverse reactions in the treatment of liver failure, mainly allergic reactions, but symptomatic treatment relieved symptoms and did not affect the treatment effect. Hemofiltration applies a membrane with larger aperture.

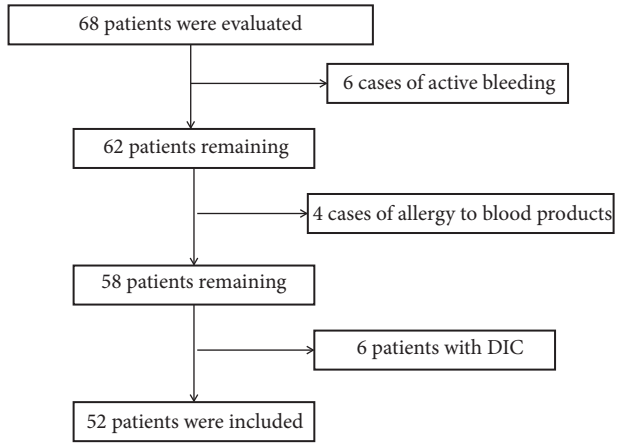

Figure 1: Flowchart of included patients.

The pressure difference between the liquid on both sides of the membrane is used as a transmembrane pressure. In the form of convection, the toxins in the blood are removed with water. Hemofiltration is more close to the function of glomerular filtration in the human kidney. If the replacement fluid is contaminated, complications such as fever and septicemia can occur, and this can be avoided by prevention and treatment. The main mechanism of bilirubin adsorption is to separate the plasma from the patient and send the plasma to the bilirubin adsorption column of BS series, so as to completely adsorb the bile acid and bilirubin in the serum and then send the purified plasma back to the human body, so as to effectively remove the blood poisoning metabolites and inflammatory cytokines. Bilirubin adsorption treatment increased the toxin clearance ability, and the safety was high. Studies have shown that PE, hemofiltration, and bilirubin adsorption are safe and effective in the treatment of liver failure and can prolong the survival time of patients.

2.3. Defining Criteria to Patients with Different Modalities of Artificial Liver Support. Plasma exchange is the most commonly used method, which can retain the relatively large molecular weight of coagulation factors, hepatocyte growth factor. Hemofiltration is suitable for all kinds of liver failure with acute kidney injury, including hepatorenal syndrome, hepatic encephalopathy, electrolyte imbalance, and acid-base imbalance. Bilirubin adsorption is applied to severe hyperbilirubinemia caused by various reasons and patients with severe cholestasis of liver disease treated by poorly internal medicine. According to the above pattern of artificial liver, 52 patients were divided into plasma exchange in 34 cases (65.38%), 9 cases were bilirubin absorption (17.31%), and 9 cases were hemofiltration (17.31%). Because chronic liver failure accounts for 63.46% of liver failure and plasma replacement is 65.38% of the artificial liver pattern, this article focuses on chronic liver failure with different artificial liver treatment and plasma exchange for liver failure.

2.4. Clinical Efficacy Observation Index. According to the guidelines, the clinical efficacy of artificial liver and the

Table 1: Diagnosis and classification of liver failure.

Diagnosis	acute hepatic failure	subacute hepatic failure	acute-on-chronic liver failure	chronic liver failure	Total
Cases	6	3	10	33	52
Proportion	11.54%	5.77%	19.23%	63.46%	100%

Table 2: Diagnosis and classification of plasma exchange for liver failure.

Diagnosis	acute hepatic failure	subacute hepatic failure	acute-on-chronic liver failure	Chronic liver failure	Total
Cases	6	3	10	15	34
Proportion	17.65%	8.82%	29.41%	44.12%	100%

changes of the patient's condition were evaluated by monitoring the effects in the near future, including serum bilirubin lowering, PTA or international standardization ratio improvement, MELD score decline, other laboratory indexes improvement, and digestive tract symptom improvement. The metabolic compounds of liver failure were observed before and after artificial liver by using UPLC-MS, and related metabolic pathways were analyzed by MetaboAnalyst.

2.5. Instruments and Reagents. The following reagents and instruments were used for this study: 7600 automatic biochemical analyzer from Japan's Hitachi company, blood coagulation analyzer from German BE company, Wasters UPLC-Q Exactive HF MS (Thermo Fisher Scientific, Rockford, IL, USA), centrifuge (Microfuge 22, Beckman Coulter company), vortex mixer (Vortex Genius 3, German IKA group), Acetonitrile and formic acid (from the Fisher company in the United States), ammonium acetate, and analytically pure (from the Sigma-Aldrich company in the United States), and ultrapure water was prepared by the MILLI-Q ultra purified water preparation system (US Millipore Corporation).

2.6. Samples Collection and Processing. Fasting venous blood and venous blood after treatment were taken immediately, and the serum was collected after 3500 rpm centrifugation for 8 minutes. Using a constant temperature container with dry ice for transportation, the serum finally were placed in -80 centigrade refrigerator to storage. Serum samples were thawed at room temperature, taking 50 uL serum add into 200 uL acetonitrile containing the internal standard and centrifuging at 10,000 g for 10 min. 180 μl lyophilized supernatant was taken and dissolved in 80 uL volume ratio of acetonitrile/water of 1/4, and, after whirling 30s, centrifugal supernatant was analyzed by LC-MS.

2.7. Analysis Condition. Chromatographic condition was as follows: chromatographic column: ACQUITY UPLC HSS T3 (standard: 100 mm × 2.1 mm, 1.8 μm), column temperature: 50°C, and current speed: 0.35 ml/min. Mass spectrometry condition was as follows: MS full scan range of positive ion: 80-1200 m/z; spraying voltage: 3.50 kV; anion: 70-1100; spraying voltage: 3.00 kV. Capillary temperature 300°C, auxiliary gas temperature 350°C. The velocities of sheath gas and auxiliary gas are, respectively, 45 and 10 (arbitrary units), resolution set to $12e^4$.

2.8. Data Processing and Statistical Analysis. The raw data collected by mass spectrometry was processed by SIEVE software and 80% principles were used to deal with missing values [7], and data were imported into SIMCA-P 11.5 software, pattern recognition using PLS-DA, and the VIP (Variable Importance in Projection) value of each variable in PLS-DA model was obtained, screening out potential biomarkers. Statistical software was using SPSS 17.0, and $P < 0.05$ was statistically significant.

3. Results

3.1. Classification of Liver Failure and Nonbioartificial Liver Patterns

3.1.1. Diagnosis and Classification of Liver Failure. According to the diagnosis and classification of hepatic failure, 52 cases were divided into acute hepatic failure in 6 cases (11.54%), subacute hepatic failure in 3 cases (5.77%), acute-on-chronic liver failure in 10 cases (19.23%), and chronic liver failure in 33 cases (63.46%), Table 1.

3.1.2. Diagnosis and Classification of Plasma Exchange for Liver Failure. Out of 52 patients, 34 cases (65.38%) underwent plasma exchange in the treatment of liver failure diagnosis, according to the classification, divided into 6 cases of acute liver failure (17.65%), 3 cases of subacute liver failure (8.82%), acute-on-chronic liver failure in 10 cases (29.41%), and 15 cases of chronic liver failure (44.12%), Table 2.

3.1.3. Classification of Chronic Hepatic Failure with Different Patterns of Nonbioartificial Liver Treatment. Out of 52 patients, there was chronic liver failure in 33 cases (63.46%); they are in the majority, so the following further on chronic liver failure of artificial liver pattern was analyzed, including 15 cases of plasma exchange (45.454%), 9 cases of bilirubin adsorption (27.273%), and 9 cases of hemofiltration (27.273%), Table 3.

3.1.4. Classification of Nonbioartificial Liver Treatment Patterns. According to the classification of nonbioartificial liver treatment patterns, 52 patients were divided into 34 cases of plasma exchange (65.38%), 17.31% cases were bilirubin absorption, 9 cases were hemofiltration (17.31%), Table 4.

Table 3: Classification of chronic hepatic failure with different patterns of nonbioartificial liver treatment.

therapeutic methods	plasma exchange	bilirubin adsorption	hemofiltration	Total
Cases	15	9	9	33
Proportion	45.454%	27.273%	27.273%	100%

Table 4: Classification of nonbioartificial liver models.

therapeutic method	plasma exchange	bilirubin adsorption	hemofiltration	Total
Cases	34	9	9	52
Proportion	65.38%	17.31%	17.31%	100%

Table 5: Meld score analysis of liver failure treated with nonbioartificial liver treatment.

	Before treatment	After treatment	The value of P
Meld score	23.38 ± 5.32	21.38 ± 4.81	0.001*

Note. The Meld score before and after nonbioartificial liver treatment was P ⩽ 0.001, which was statistically significant.

3.2. Clinical Efficacy Analysis

3.2.1. Meld Score Analysis of Liver Failure Treated with Nonbioartificial Liver Treatment. The Meld score of all patients before and after nonbioartificial liver treatment was statistically analyzed, and the results showed that the Meld score after nonbioartificial liver treatment was lower than before; the difference was statistically significant (P = 0.001), Table 5.

3.2.2. Clinical Analysis of Liver Failure Treated by Nonbioartificial Liver Treatment. The liver function and coagulation function of the 52 patients treated with nonbioartificial liver were compared and analyzed, and the levels of AST, ALB, ALP, γ-GT, TBIL, and PT in nonbioartificial liver were significantly lower than those before nonbioartificial liver treatment (P < 0.01), with statistical significance. The level of ALT after treatment decreased but had no statistically significance (P > 0.05), Table 6.

3.2.3. Clinical Analysis of Plasma Exchange in the Treatment of Liver Failure. When the plasma exchange was used in the treatment of acute liver failure, acute-on-chronic liver failure and chronic liver failure, liver function, and blood coagulation function indicators were compared, and the levels of AST, γ-GT, TBIL, PT, and ALB after treatment were significantly lower than before (P < 0.05); the difference was statistically significant, Table 7.

3.2.4. Clinical Analysis of Different Nonbioartificial Liver Treatment Patterns in Patients with Chronic Hepatic Failure. Liver function and blood coagulation function indicators were compared in different patterns of nonbioartificial liver treatment of chronic liver failure, and the levels of AST, γ-GT, TBIL, and PT were significantly lower than that before plasma exchange treatment (P < 0.05); there was statistical significance; only TBIL was significantly decreased after bilirubin adsorption treatment (P < 0.05), and there was statistical significant differences; only PT significantly decreased after hemofiltration treatment (P < 0.05), and the difference was statistically significant, Table 8.

3.3. Serum Metabonomics Analysis

3.3.1. Serum Metabonomics Analysis of Liver Failure Treated by Nonbioartificial Liver. The LC-MS data before and after liver failure treated by nonbioartificial liver were processed, and the ion information in negative ion mode was obtained by SIEVE software. All data were processed by PLS-DA analysis and got Figures 2–4. In the scoring chart (Figure 2), the samples were distinguished before and after the nonbioartificial liver treatment, which validated the effectiveness of the nonbioartificial liver treatment; the load graph (Figure 3) showed a number of variables away from the center, indicating that these variables contributed significantly to the model, and there was a significant change before and after the nonbioartificial liver treatment; the validation model showed that R2 = (0, 0.222) and Q2 = (0, -0.228), indicating that the PLS-DA model did not have overfitting, that the prediction between groups was reliable, and that the model was successfully established. We analyzed metabolite with significant difference before and after nonbioartificial liver treatment and VIP greater than 1, which used the MetaboAnalyst analysis. Combined with Figures 2-3, we identified 29 differential metabolites and, as shown in Table 9, the fatty acids and bile acids were taken as the principal thing. Metabolic pathways were analyzed through the MetaboAnalyst analysis, and 12 metabolic pathways were identified, which relates to the proportion of fatty acid metabolites metabolism and bile acid metabolism in the highest (Figure 4, Table 10). These results indicated that nonbioartificial liver treatment had significant effects on fatty acid metabolism and bile acid metabolism.

3.3.2. Serum Metabonomics Analysis of Chronic Liver Failure Treated by Nonbioartificial Liver. The 52 patients were included, 33 cases of chronic liver failure (63.46%), among them there were 15 cases of plasma exchange (45.454%), 9 cases of bilirubin adsorption (27.273%), 9 cases of hemofiltration(27.273%). Serum LC-MS data of chronic liver failure after treatment with different nonbioartificial liver patterns were analyzed by PLS-DA, and Figures 5–7 was obtained. In the score plot (Figure 5) in different nonbioartificial liver treatment pattern distinguishing, which showed that there were obvious differences between the treatment pattern, load graphs (Figure 6) showed multiple variables

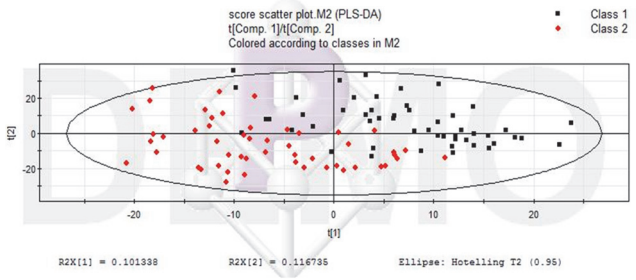

Figure 2: Scoring chart of PLS-DA pattern in treatment of liver failure with nonbioartificial liver. *Note.* Class1, Before nonbioartificial liver treatment; Class2, After nonbioartificial liver treatment.

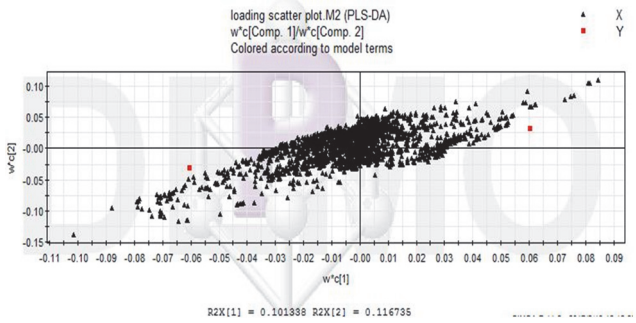

Figure 3: Load chart of PLS-DA pattern in treatment of liver failure with nonbioartificial liver.

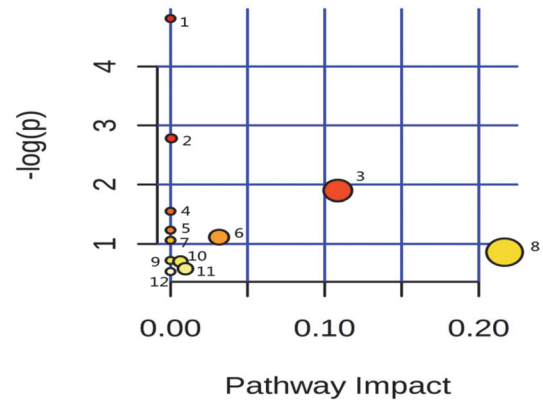

Figure 4: Differential metabolite related pathway. Note that numbers 1-12 represent different metabolic pathways, the name of metabolic pathways, and distribution parameters as shown in Table 10.

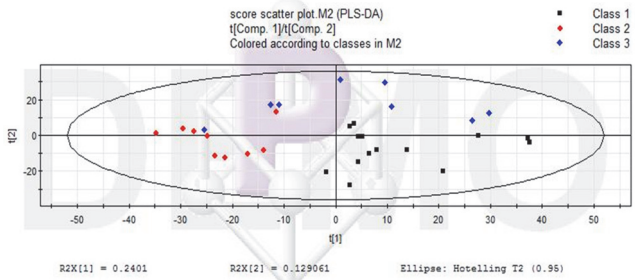

Figure 5: Scoring chart of PLS-DA pattern in treatment of chronic liver failure with nonbioartificial liver. Note the following: Class1: plasma exchange for chronic liver failure; Class2: treatment of chronic liver failure with bilirubin adsorption; Class3: hemofiltration in the treatment of chronic liver failure.

away from the center, indicating that these variables are important in distinguishing different treatment modalities; verification model showed that R2 = (0, 0.537) and Q2 = (0, -0.441), which indicated the PLS-DA model without overfitting; internal group prediction model was established successfully reliable. According to the variable importance projection (VIP > 1), combined with Figures 5-6, we identified 11 different metabolites, shown in Table 11, with fatty acids, bile acids, and amino acids; 7 metabolic pathways were identified through the MetaboAnalyst analysis, as shown in Figure 7 and Table 12. The proportion of metabolites involved bile acid metabolism, fatty acid metabolism, and amino acid metabolism in the metabolism was the highest. It indicated that the different treatment patterns had significant effects on bile acid metabolism, fatty acid metabolism, and amino acid metabolism.

4. Discussion

Liver failure is one of the most common serious liver disease syndromes, with high fatality rate [1]. There are three main treatment methods for liver failure: medical treatment, nonbioartificial liver support therapy, and liver transplantation treatment [7]. Because of comprehensive internal medical treatment is limited, liver transplantation improved survival rate but affected by the shortage of donor, high cost, and other factors; nonbioartificial liver support therapy can improve liver function, until you find the suitable donor liver or liver regeneration, which is particularly important in the clinical application of liver failure [8–11]. Nonbioartificial liver treatment can significantly improve liver function and coagulation indexes in the early and middle stages of liver failure. It can improve the symptoms in the late stage of liver failure and the survival time of patients waiting for liver transplantation and the survival value of patients [12–14]. In this study, we used metabonomics to analyze the changes of the organism before and after the nonbioartificial liver treatment.

Hepatic uptake of free fatty acids (FFA) plays an important role in the synthesis, storage, and transport of lipids [15]. With necrosis and apoptosis of hepatocytes, the absorbance of free fatty acids decreased and free fatty acids in serum increased liver failure patients. Studies have shown that different forms of fatty acids have different effects [16]. A protective role for endogenously generated unsaturated fatty acids was also indicated by in vivo experiments using genetically modified mice bearing an inactivating mutation in the gene encoding the enzyme stearoyl-CoA desaturase [17]. Exposure of a variety of cell types, including hepatocytes, to long-chain saturated fatty acids led to increased expression of proinflammatory cytokines, inhibition of insulin signaling, induction of endoplasmic reticulum (ER) stress, and

Table 6: Clinical analysis of hepatic failure treated by nonbioartificial liver treatment.

Index	Mean (before and after)	Standard deviation (front and back)	The value of P
ALT(U/L)	117.06, 69.60	236.50, 76.28	0.051
AST(U/L)	185.15, 141.23	250.61, 138.12	0.003∗
ALB(g/L)	30.59, 28.33	4.61, 2.81	0.001∗∗
ALP(U/L)	95.54, 86.33	33.33, 31.24	0.01∗
r-GT(U/L)	60.77, 49.06	36.66, 21.87	0.003∗
TBil(umol/L)	372.53, 263.53	229.01, 170.05	0.001∗∗
PT(s)	24.66, 19.74	8.36, 5.82	0.001∗∗

Note. ∗Compared with the preclinical indexes in the nonbioartificial liver treatment $P < 0.01$. ∗∗Compared with the preclinical indexes in the nonbioartificial liver treatment $P \leqslant 0.001$. There was significant statistical significance.

Table 7: Clinical analysis of plasma exchange in the treatment of liver failure.

Liver failure type	Index	Mean (before and after)	Standard deviation (front and back)	The value of P
acute hepatic failure	ALT(U/L)	130.00, 76.33	147.77, 51.69	0.08
	AST(U/L)	180.50, 100.17	117.18, 52.11	0.028∗
	ALB(g/L)	31.82, 25.98	3.26, 1.06	0.042∗
	ALP(U/L)	124.33, 93.00	24.19, 21.93	0.027∗
	r-GT(U/L)	139.83, 79.83	30.65, 18.14	0.028∗
	TBil(umol/L)	383.88, 206.82	127.49, 71.11	0.028∗
	PT(s)	19.12, 16.87	2.50, 1.03	0.046∗
subacute hepatic failure	ALT(U/L)	427.67, 313.67	20.79, 104.18	0.285
	AST(U/L)	343.67, 367.33	24.66, 267.99	1.00
	ALB(g/L)	34.83, 28.00	2.60, 7.43	0.109
	ALP(U/L)	117.00, 67.67	22.61, 21.50	0.109
	r-GT(U/L)	56.33, 31.67	12.50, 13.58	0.109
	TBil(umol/L)	633.90, 384.87	67.76, 80.85	0.109
	PT(s)	37.77, 20.17	4.80, 1.80	0.109
acute-on-chronic liver failure	ALT(U/L)	272.90, 88.00	469.06, 56.50	0.008∗∗
	AST(U/L)	371.20, 187.80	506.46, 193.74	0.005∗∗
	ALB(g/L)	33.24, 29.85	1.92, 1.74	0.007∗∗
	ALP(U/L)	74.00, 66.00	36.24, 25.79	0.541
	r-GT(U/L)	70.50, 54.80	20.70, 13.10	0.047∗
	TBil(umol/L)	559.37, 408.53	380.74, 321.44	0.005∗∗
	PT(s)	20.24, 15.37	6.26, 1.53	0.028∗
Chronic liver failure	ALT(U/L)	37.8, 37.53	27.83, 18.69	0.955
	AST(U/L)	97.20, 78.20	90.85, 76.31	0.019∗
	ALB(g/L)	28.60, 27.86	6.21, 1.96	0.394
	ALP(U/L)	89.00, 80.13	28.71, 23.39	0.053
	r-GT(U/L)	41.40, 36.40	19.73, 18.53	0.033∗
	TBil(umol/L)	319.73, 223.11	145.33, 79.61	0.001∗∗∗
	PT(s)	24.06, 17.86	5.78, 2.60	0.001∗∗∗

Note. ∗Compared with the preclinical indexes in the treatment of plasma exchange $P < 0.05$. ∗∗Compared with the preclinical indexes in the treatment of plasma exchange $P < 0.01$. ∗∗∗Compared with the preclinical indexes in the treatment of plasma exchange $P \leqslant 0.001$. There was significant statistical significance.

promotion of cell death, mainly by apoptosis [18]. After nonbioartificial liver treatment, the levels of various saturated fatty acids decreased and the levels of unsaturated fatty acids increased; this showed that fatty acids were involved in the process of inflammation and repair of hepatocytes. And nonbioartificial liver treatment can effectively affect the distribution of fatty acids in the environment and create conditions for the regeneration of liver cells.

Bile acid is composed of cholesterol in the cytoplasm and microsome of liver cells. Bile acids can be divided into hydrophobic and hydrophilic bile acids according to water solubility [19, 20]. Bile acid enters the cell and can inhibit the oxidative phosphorylation of mitochondria, and the ATP synthesis decreased calcium pump inactivation, extracellular Ca2+ influx, and activation of various proteolytic enzymes, which caused the decomposition of DNA, RNA and protein,

Table 8: Clinical analysis of different nonbioartificial liver treatment patterns in patients with chronic liver failure.

Non-bioartificial liver treatment method	Index	Mean (before and after)	Standard deviation (front and back)	The value of P
Plasma exchange	ALT(U/L)	37.80, 37.53	27.83, 18.69	0.955
	AST(U/L)	97.20, 78.20	90.85, 76.31	0.019∗
	ALB(g/L)	28.60, 27.86	6.21, 1.96	0.394
	ALP(U/L)	89.00, 80.13	28.71, 23.39	0.053
	r-GT(U/L)	41.40, 36.40	19.73, 18.53	0.033∗
	TBil(umol/L)	319.73, 223.11	145.33, 79.61	0.001∗∗
	PT(s)	24.06, 17.86	5.78, 2.60	0.001∗∗
Bilirubin adsorption	ALT(U/L)	55.33, 60.22	40.57, 38.87	0.05
	AST(U/L)	162.56, 178.33	70.30, 79.48	0.123
	ALB(g/L)	29.14, 28.82	4.32, 3.76	0.859
	ALP(U/L)	106.00, 104.78	20.71, 23.65	0.398
	r-GT(U/L)	54.00, 58.67	12.51, 13.56	0.095
	TBil(umol/L)	301.80, 210.37	90.99, 33.98	0.011∗
	PT(s)	20.89, 21.38	5.76, 2.66	0.213
Hemofiltration	ALT(U/L)	25.56, 26.11	12.33, 6.95	0.767
	AST(U/L)	97.89, 109.44	85.78, 99.71	0.260
	ALB(g/L)	30.21, 28.61	3.22, 1.90	0.066
	ALP(U/L)	93.56, 102.56	40.73, 45.90	0.097
	r-GT(U/L)	37.78, 39.44	21.38, 21.15	0.233
	TBil(umol/L)	229.00, 220.31	106.43, 92.20	0.441
	PT(s)	33.64, 27.87	9.01, 8.88	0.011∗

Note: ∗Compared with the preclinical indexes in the treatment of nonbioartificial liver in Chronic liver failure $P < 0.05$, ∗∗Compared with the preclinical indexes in the treatment of nonbioartificial liver in Chronic liver failure $P < 0.001$. There was significant statistical significance.

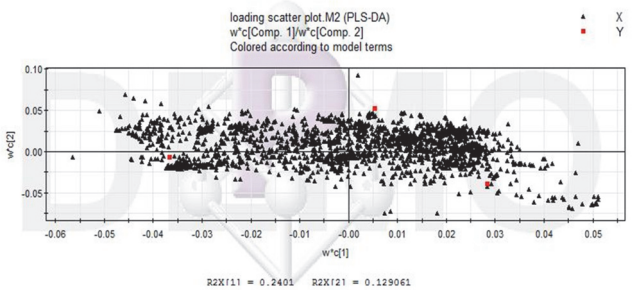

Figure 6: Load chart of PLS-DA pattern in treatment of chronic liver failure with nonbioartificial liver.

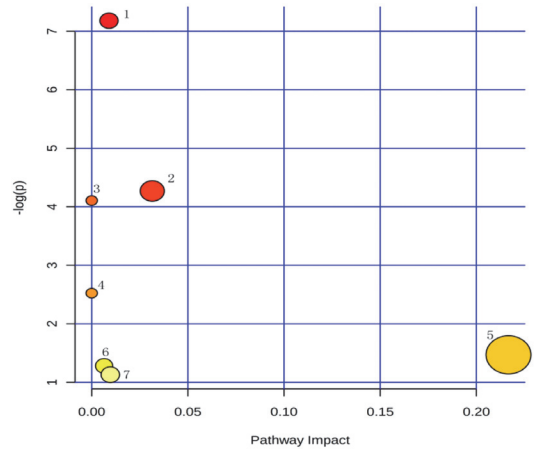

Figure 7: Differential metabolite related pathway in treatment of chronic liver failure with nonbioartificial liver. Note that numbers 1-7 represent different metabolic pathways, the name of metabolic pathways, and distribution parameters as shown in Table 12.

cell dysfunction, and ultimately apoptosis [21, 22]. During liver failure, a large number of liver cells were mortified and bile acid was released into the blood, resulting in elevated levels of serum bile acids, and elevated bile acids reaction on liver cells can further lead to necrosis and apoptosis of liver cells. In this study, the hydrophobic bile acid decreased and the hydrophilic bile acid increased after nonbioartificial liver treatment. The positive correlation between hydrophobic bile acids and liver injury was confirmed, and the antagonism between hydrophilic bile acids and hydrophobic bile acids was also confirmed.

The liver plays a key role in amino acid metabolism [23], liver injury can cause imbalance of amino acid metabolism and change of amino acid levels in human body [24, 25]. Phenylalanine is an essential amino acid, it can synthesize proteins and be converted into nonessential amino acid tyrosine. Under the influence of hepatitis virus invasion or other physical and chemical factors, the parenchymal cells

Table 9: Identification of characteristic metabolites.

Number	Detection of mass/charge ratio	The theory of mass/charge ratio	metabolite	VIP value
1	112.1	113.1	creatinine	2.96
2	391.3	392.6	Ursodeoxycholic acid	2.74
3	367.2	288.4	Dehydroepiandrosterone	2.70
4	203.1	204.2	L- tryptophan	2.12
5	217.0	200.1	1,3 hydroxy uric acid	2.07
6	178.1	178.2	benzoyl-glycine	2.05
7	281.3	282.5	FFA 18_1	1.84
8	204.1	205.2	indolelactic acid	1.81
9	303.2	304.5	FFA 20_4	1.60
10	448.3	467.6	Glycerol deoxycholate	1.52
11	309.3	310.0	FFA 20_1	1.46
12	185.2	186.3	FFA 11_0	1.39
13	407.3	408.6	cholalic acid	1.37
14	167.0	168.1	uric acid	1.37
15	391.3	392.6	deoxycholic acid	1.35
16	157.1	158.3	FFA 9_0	1.33
17	339.3	338.6	FFA 22_0	1.32
18	286.3	284.5	FFA 18_0-d3	1.32
19	305.3	306.3	FFA 20_3	1.30
20	199.2	200.3	FFA 12_0	1.29
21	241.2	242.4	FFA 15_0	1.28
22	391.3	391.6	Ursodeoxycholic acid	1.28
23	171.1	172.3	FFA 10_0	1.25
24	295.3	296.3	FFA 19_1	1.24
25	337.3	338.4	FFA 22_1	1.23
26	301.2	302.5	FFA 20_5	1.20
27	369.2	269.4	DHES-3	1.20
28	528.3	449.4	glycoursodeoxycholic acid	1.19
29	498.3	499.7	Tauroursodeoxycholic Acid	1.18

Note. FFA: free fatty acid; DHES-3: 3-dehydro estrone; VIP: variable importance in projection.

Table 10: Metabolic pathway name and distribution parameter.

Number	Metabolic pathway name	Ordinate value (-log(p))	Abscissa value (pathway impact)
1	Fatty acid synthesis	4.8098	0.0
2	Primary bile acid synthesis	2.7816	5.4E-4
3	Tryptophan metabolism	1.8992	0.10853
4	Phenylalanine, tyrosine and tryptophan biosynthesis	1.5525	0.0
5	Nitrogen metabolism	1.233	0.0
6	Phenylalanine metabolism	1.1137	0.0315
7	Glycine, serine and threonine metabolism	1.061	0.0
8	arachidonic acid metabolism	0.85981	0.21669
9	Aminoacyl-tRNA biosynthesis	0.71939	0.0
10	Arginine and proline metabolism	0.70068	0.00645
11	Purine metabolism	0.57911	0.00969
12	Steroid hormone biosynthesis	0.53171	0.0

Note. The number of 1-12 corresponding to the 1-12 metabolic pathway in Figure 4.

Table 11: Identification of characteristic metabolites about chronic liver failure with nonbioartificial liver.

Comparison of treatment methods	Detection of mass/charge ratio	The theory of mass/charge ratio	metabolite	VIP value
Plasma exchange and Bilirubin adsorption	167.0	168.1	uric acid	2.03
	407.3	408.6	Cholic acid	1.61
	112.1	113.1	creatinine	1.58
	391.3	392.6	anthropodesoxycholic acid	1.16
	303.2	304.5	FFA 20_4	1.09
	167.0	515.7	cholaic acid	1.11
	286.3	284.5	FFA 18_0-d3	1.08
Plasma exchange and Hemofiltration	167.0	168.1	uric acid	2.03
	407.3	408.6	cholalic acid	1.61
	112.1	113.1	creatinine	1.58
	391.3	392.6	anthropodesoxycholic acid	1.16
	303.2	304.5	FFA 20_4	1.09
	281.3	282.5	FFA 18_1	1.40
	178.1	178.2	hippuric acid	1.33
	203.1	204.2	L- tryptophan	1.16
Hemofiltration and Bilirubin adsorption	281.3	282.5	FFA 18_1	1.40
	498.3	281.2	phenylacetylglutamine	1.28

Note. VIP: variable importance in projection.

Table 12: Metabolic pathway name and distribution parameter.

Number	Metabolic pathway name	Ordinate value (-log(p))	Abscissa value (pathway impact)
1	Primary bile acid synthesis	7.1808	0.009
2	Phenylalanine metabolism	4.2699	0.0315
3	Fatty acid synthesis	4.1066	0.0
4	Taurine and hypotaurine metabolism	2.5233	0.0
5	arachidonic acid metabolism	1.4694	0.21669
6	Arginine and proline metabolism	1.2801	0.00645
7	Purine metabolism	1.1293	0.00969

Note. The number of 1-7 corresponding to the 1-7 metabolic pathway in Figure 7.

of the liver are seriously damaged and even destroyed. Any of various enzymes responsible for biochemical metabolism in the liver cells that are reduced or released into the body fluid for inactivation. Thus, the catabolic pathway of aromatic amino acids slows down, and its content in the blood increases [26, 27]. After the nonbioartificial liver treatment of liver failure, the aromatic amino acids, especially phenylalanine, decreased significantly, indicating the gradual recovery of hepatocytes, which prove that the nonbioartificial liver treatment is helpful for the regeneration of liver cells and the recovery of liver function. In this study, phenylalanine metabolism in patients with chronic liver failure treated with different nonbioartificial liver models were significantly different. It was further proved that the levels of aromatic amino acids changed significantly in the treatment of chronic liver failure by nonbioartificial liver treatment.

In summary, nonbioartificial liver treatment of liver failure mainly through the impact of fatty acid synthesis pathway, primary bile acid synthesis pathway, and phenylalanine metabolic pathway affects the clinical efficacy of patients with liver failure. Through the study of metabolic changes and metabolic pathway of the organism, this study will establish the clinical basis for further study on the pathogenesis of liver failure and the mechanism of nonbioartificial liver treatment.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

References

[1] "Chinese Medicine Association Infectious Diseases Branch Liver Failure and Artificial Liver," *Chinese Journal of Hepatology*, vol. 5, no. 6, pp. 321–327, 2012.

[2] M. J. W. McPhail, S. Kriese, and M. A. Heneghan, "Current management of acute liver failure," *Current Opinion in Gastroenterology*, vol. 31, no. 3, pp. 209–214, 2015.

[3] "Chinese Medical Association of Infectious Diseases Branch of liver failure and artificial liver group, Treatment of Hepatic Failure with Abiotic Artificial Liver," *Chinese Journal of Clinical Infectious Diseases*, vol. 9, no. 2, pp. 97–103, 2016.

[4] T. L. Hai, L. Lian et al., "The successful practice of metabolomics in medical research," *Progress in Modern Biomedicine*, vol. 10, pp. 1582–1584, 2007.

[5] N. Krone, B. A. Hughes, G. G. Lavery, P. M. Stewart, W. Arlt, and C. H. L. Shackleton, "Gas chromatography/mass spectrometry (GC/MS) remains a pre-eminent discovery tool in clinical steroid investigations even in the era of fast liquid chromatography tandem mass spectrometry (LC/MS/MS)," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 121, no. 3–5, pp. 496–504, 2010.

[6] D. M. Drexler, M. D. Reily, and P. A. Shipkova, "Advances in mass spectrometry applied to pharmaceutical metabolomics," *Analytical and Bioanalytical Chemistry*, vol. 399, no. 8, pp. 2645–2653, 2011.

[7] M. Zhen and W. Yun, "Treatment status of liver failureif possible," *Journal of Clinical Hepatology*, vol. 32, 2016.

[8] S. K. Sarin, C. K. Kedarisetty, Z. Abbas et al., "Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) 2014," *Hepatology International*, vol. 8, no. 4, pp. 453–471, 2014.

[9] J. Lee, D. Lee, S. Lee et al., "Functional Evaluation of a Bioartificial Liver Support System Using Immobilized Hepatocyte Spheroids in a Porcine Model of Acute Liver Failure," *Scientific Reports*, vol. 7, no. 1, 2017.

[10] J. Chen, J. Huang, Q. Yang 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 Diseases International*, vol. 15, no. 3, pp. 275–281, 2016.

[11] M. L. Schilsky, "Acute liver failure and liver assist devices," *Transplantation Proceedings*, vol. 43, no. 3, pp. 879–883, 2011.

[12] M. Li, J. Sun, J. Li et al., "Clinical observation on the treatment of acute liver failure by combined non-biological artificial liver," *Experimental and Therapeutic Medicine*, vol. 12, no. 6, pp. 3873–3876, 2016.

[13] S. L. Nyberg, "Bridging the gap: Advances in artificial liver support," *Liver Transplantation*, vol. 18, no. S2, pp. S10–S14, 2012.

[14] X. Xu, X. Liu, Q. Ling et al., "Artificial Liver Support System Combined with Liver Transplantation in the Treatment of Patients with Acute-on-Chronic Liver Failure," *PLoS ONE*, vol. 8, no. 3, p. e58738, 2013.

[15] G. Musso, R. Gambino, and M. Cassader, "Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD)," *Progress in Lipid Research*, vol. 48, no. 1, pp. 1–26, 2009.

[16] M. D. Mantzaris, E. V. Tsianos, and D. Galaris, "Interruption of triacylglycerol synthesis in the endoplasmic reticulum is the initiating event for saturated fatty acid-induced lipotoxicity in liver cells," *FEBS Journal*, vol. 278, no. 3, pp. 519–530, 2011.

[17] Z. Z. Li, M. Berk, T. M. McIntyre, and A. E. Feldstein, "Hepatic lipid partitioning and liver damage in nonalco-holic fatty liver disease: role of stearoyl-CoA desaturase," *Biological Chemistry*, vol. 284, pp. 5637–5644, 2009.

[18] Y. Akazawa, S. Cazanave, J. L. Mott et al., "Palmitoleate attenuates palmitate-induced Bim and PUMA up-regulation and hepatocyte lipoapoptosis," *Journal of Hepatology*, vol. 52, no. 4, pp. 586–593, 2010.

[19] R. E. Poupon, Y. Chrétien, R. Poupon, and G. Paumgartner, "Serum bile acids in primary biliary cirrhosis: Effect of ursodeoxycholic acid therapy," *Hepatology*, vol. 17, no. 4, pp. 599–604, 1993.

[20] H. Sagawa et al., "Protection against hydrophobic bile salt-induced cell membrance damage by liposomes and hydrophilic bile salts," *American Journal of Physiology*, vol. 264, pp. 835–839, 2001.

[21] A. P. Rolo, C. M. Palmeira, and K. B. Wallace, "Mitochondrially mediated synergistic cell killing by bile acids," *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, vol. 1637, no. 1, pp. 127–132, 2003.

[22] T. Nakajima, Y. Okuda, K. Chisaki et al., " Bile acids increase intracellular Ca ," *British Journal of Pharmacology*, vol. 130, no. 7, pp. 1457–1467, 2000.

[23] B. Kinny-Köster, M. Bartels, S. Becker et al., "Plasma amino acid concentrations predict mortality in patients with end-stage liver disease," *PLoS ONE*, vol. 11, no. 7, Article ID e0159205, 2016.

[24] M. Holecek, "Ammonia and amino acid profiles in liver cirrhosis: Effects of variables leading to hepatic encephalopathy," *Nutrition Journal* , vol. 31, no. 1, pp. 14–20, 2015.

[25] M. Plauth and T. Schütz, "Branched-chain amino acids in liver disease: New aspects of long known phenomena," *Current Opinion in Clinical Nutrition & Metabolic Care*, vol. 14, no. 1, pp. 61–66, 2011.

[26] W. Jiangping, *Determination of Aromatic Amino Acids in Serum by High Performance Liquid Chromatography and Its Clinical Application*, Central South University, 2003.

[27] M. Kong, S. Hongbo, L. Bai, J. Yao, and Y. Chen, "Changes and Characteristics of Plasma Amino Acid Spectra in Patients with Hepatitis B Virus Related Chronic Hepatitis, Clinical meta," *Cirrhosis and Chronic Hepatic Failure*, vol. 7, pp. 732–735, 2016.

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Research Article

Alpha-Fetoprotein as a Predictive Marker for Patients with Hepatitis B-Related Acute-on-Chronic Liver Failure

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Background and Aims. The value of alpha-fetoprotein (AFP) in hepatitis B-related acute-on-chronic liver failure (HBACLF) is not fully understood. The present study aimed to evaluate the prognostic effect of AFP on the prediction of HBACLF outcomes. *Methods*. We investigated a cohort of patients with HBACLF admitted from January 2013 to May 2017. The endpoint of followup was 180 days, death, or liver transplantation. AFP concentrations were estimated on admission. To make statistical comparisons, we used chi-squared test, receiver operating characteristic (ROC) curve analysis, survivorship curve analysis, and Cox proportional-hazards model. *Results*. A total of 92 patients (81.5% male, median age of 46 years) were included. Overall survival rate within 180 days was 43.48%, and the value of $\log_{10}{}^{\mathrm{AFP}} \geq 2.04$ indicated a better prognosis with 76.9% specificity and 62.5% sensitivity for patients with HBACLF. Age (HR 1.041), total bilirubin (HR 1.004), $\log_{10}{}^{\mathrm{AFP}}$ (HR 2.155), and INR (HR 1.446) were found to be risk factors of survival. *Conclusion*. AFP could be a useful marker to predict outcomes of acute-on-chronic liver failure.

1. Introduction

Acute-on-chronic liver failure (ACLF) is defined by a rapid progression in hepatic dysfunction induced by certain precipitating events due to previous liver diseases, resulting in multisystem organ failure and high short-term mortality [1]. There is no specific treatment for ACLF, and the most effective therapy is liver transplantation. However, the shortage of liver donations has largely hindered its wide implementation. Supporting the regeneration of hepatocytes and preventing the complications tend to decrease the mortality of ACLF, and artificial liver support is a useful method to manage ACLF [2]. The aetiologies of ACLF vary between territories. Viral infections are more common in Asia, while there is a wide prevalence of alcoholic cirrhosis and nonalcoholic fatty liver in American and European countries [3–6]. With a carrier rate of hepatitis B virus (HBV) surface antigen at approximately 8% in adults, China exhibits a high morbidity of hepatitis B, which is the most common aetiological factor of ACLF [7].

Some scoring systems efficiently assess the severity and mortality of ACLF, such as SOFA score [8], MELD [9], and King's College Criteria [10]. Other parameters including bilirubin and international normalized ratio (INR) are treated as prognostic markers in clinical practice as well. Those methods primarily focus on the impaired hepatic function, while seldom did researchers concentrate on parameters of hepatocyte regeneration and evaluate their prognostic value for ACLF. Typically, AFP is the most abundant plasma protein in foetuses, and serum AFP remains elevated in infant livers until several weeks after birth. High serum AFP expression in adults generally indicates a high possibility of hepatocellular carcinoma in patients with chronic hepatitis or cirrhosis [11]. Meanwhile, AFP is considered a biomarker of proliferating liver stem cells in liver injury conditions as well, and the recruitment of liver progenitor cells is associated with a better outcome for liver failure [12, 13].

The discovery of AFP dates back to 1960s. Since then, AFP has been investigated in the field of liver diseases. Previous

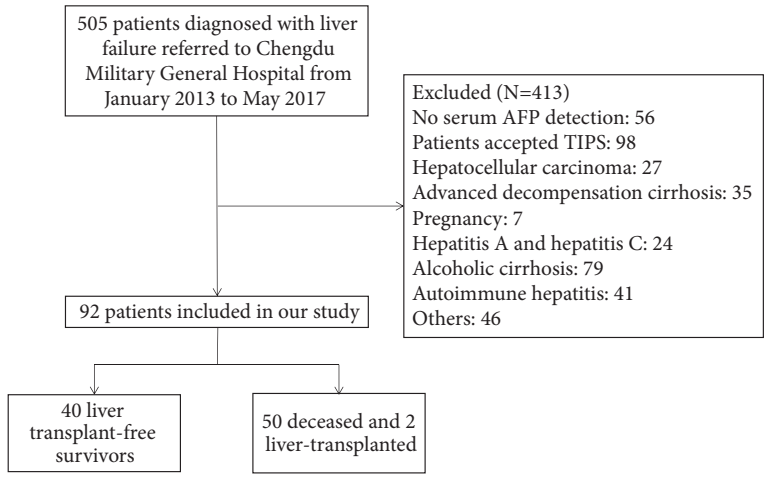

Figure 1: Inclusion and exclusion criteria for the study.

studies demonstrated that AFP was a vital prognostic marker for outcomes in patients with acute liver failure [14–16]. However, few studies evaluated the outcomes of ACLF from the perspective of hepatocyte regeneration. As we know, ACLF exhibits a small window during which liver dysfunction may be reversed, and the repair of liver tissues is tightly correlated with hepatocyte regeneration, which presents as increased AFP levels. Therefore, we performed a retrospective study to identify whether AFP was a valid predictive indicator of outcomes in ACLF patients.

2. Materials and Methods

2.1. Study Population. We concentrated on a cohort of patients with hepatitis B-related acute-on-chronic liver failure (HBACLF). A total of 505 patients with suspected ACLF were enrolled in our study from January 2013 to May 2017 at Chengdu Military General Hospital, Sichuan, China. Researchers who undertook the work of selecting cases as the main subjects were blind to the serum AFP concentrations of those patients. ACLF is diagnosed according to the criteria of Asian Pacific Association for the Study of the Liver (APASL): serum bilirubin $\geq 85\,\mu$mol/L, INR ≥ 1.5 or prothrombin activity $\leq 40\%$, any degree of encephalopathy and/or clinical ascites within 4 weeks, and an evidence of ongoing chronic liver diseases [3]. Patients who were diagnosed with ACLF and aged 18 to 75 years were included. A total of 413 patients in our database were excluded for the following reasons: (1) lack of serum AFP concentrations; (2) manifestation of decompensated liver cirrhosis prior to ACLF diagnosis, such as ascites and variceal haemorrhage; (3) patients with portal hypertension who received a transjugular intrahepatic portosystemic shunt (TIPS); (4) patients pathologically diagnosed with or clinically suspected for hepatocellular carcinoma; (5) other malignancies such as gastric cancer; (6) pregnancy; (7) HIV or hepatotropic virus infection other than HBV; and (8) other preexisting chronic liver diseases, such as fatty liver, autoimmune hepatitis, and alcoholic cirrhosis (Figure 1). The final cohort contained 92 patients with a median age of 46 years (range, 18–75 years), and all patients received antiviral therapy by orally taking tenofovir or entecavir. Besides, reduced glutathione was given to protect the liver from subsequent damage.

2.2. Clinical and Biological Parameters. The clinical parameters included ascites and hepatic encephalopathy (HE). The biological parameters included AFP, INR, and total bilirubin. The serum AFP levels were measured on admission.

2.3. Followup. The end point of observation was 180 days, death, or liver transplantation. Forty of the 92 patients survived spontaneously, 50 patients died, and 2 patients received liver transplantation.

2.4. Statistical Analysis. Results are presented as means and standard deviations (SDs) and median and range appropriately. The chi-squared test was used to compare rates between groups. Receiver operating characteristic (ROC) curve analysis was performed. Survival was estimated by Kaplan-Meier method, and differences were evaluated with log-rank test. Cox proportional-hazards model was adopted to estimate the risk factors of survival. Data were analyzed using SPSS version 16.0 software (IBM Corporation, Somers, NY, USA). Differences were considered to be of statistical significance when the P value ≤ 0.05.

3. Results

3.1. Baseline Characteristics. Ninety-two patients were incorporated in our study, including 17 women (18.5%). The population was divided into two groups based on the prognosis of ACLF. In total, there were 40 liver transplant-free survivors, 50 deceased patients, and 2 liver-transplanted patients. Table 1 depicts the demographic and biochemical characters of the two groups. Age, total bilirubin, AFP, and INR differed significantly between transplant-free survivors and those who deceased or got liver-transplanted.

3.2. AFP as a Predictor for Prognosis of HBACLF. The recruitment of functional hepatocytes is the key to the recovery

Table 1: Characteristic comparisons between subgroups.

Variables	Transplant-free survival group ($n = 40$)	Deceased and transplanted group ($n = 52$)	P value
Age*, y	41.83 (12.37)	50.31 (13.10)	0.002
Male n, %	33 (82.50%)	42 (80.77%)	0.832
Total bilirubin#, μmol/L	261.89 (86.98–496.59)	386.55 (137.45–723.22)	<0.001
AFP#, ng/ml	148.80 (8.50–2375.30)	43.21 (1.10–1495.80)	<0.001
INR#	1.83 (1.50–4.31)	2.21 (1.51–5.70)	0.001

AFP, alpha-fetoprotein; INR, international normalized ratio; *normally distributed continuous variable; #not normally distributed continuous variable.

Table 2: Univariate and multivariate Cox regression analysis for survival.

Parameter	Univariate Cox regression ($n = 92$)			Multivariate Cox regression ($n = 92$)		
	P	HR	CI	P	HR	CI
Age*	0.003	1.033	1.011 1.056	<0.001	1.041	1.019 1.063
Total bilirubin*	<0.001	1.004	1.002 1.006	<0.001	1.004	1.002 1.006
INR*	0.001	1.564	1.200 2.039	0.015	1.446	1.074 1.948
$\log_{10}^{\text{AFP}\#}$	<0.001	2.908	1.603 5.274	0.018	2.155	1.139 4.076
Ascites#	0.407					
HE#	0.163					

AFP, alpha-fetoprotein; INR, international normalized ratio; HE, hepatic encephalopathy; HR, hazard ratio; and CI, confidence interval; *continuous variables and #categorical variables ($\log_{10}^{\text{AFP}}$ was sorted into subgroups: $\log_{10}^{\text{AFP}} \geq 2$ and $\log_{10}^{\text{AFP}} < 2$. No statistical significance was attained between patients with or without ascites and HE).

of the impaired liver function. To estimate the outcome of ACLF from the perspective of hepatocyte regeneration, we evaluated the predictive value of AFP by creating an equation, namely, $\log_{10}^{\text{AFP}}$, to assess the prognosis of HBACLF. A receiver operating characteristic curve was created for this parameter to predict the outcome of ACLF patients. The area under the curve was 0.725. A cut-off point of $\log_{10}^{\text{AFP}} \geq 2.04$ was suggested to indicate a better outcome with 76.9% specificity and 62.5% sensitivity (Figure 2).

3.3. $\log_{10}^{\text{AFP}}$ *Is a Risk Factor of Survival for HBACLF.* Patients with chronic hepatitis B-related ACLF exhibited an incredibly high mortality within 180 days in our observation. The transplant-free survival rate at 30 days was 72.83%, and it gradually declined to 43.48% at 180 days of followup (Figure 3(a)). Given that patients would probably have a better outcome when $\log_{10}^{\text{AFP}}$ was approximately higher than 2, we then chose $\log_{10}^{\text{AFP}}$ as a categorical variable to assess the survival of HBACLF. In addition, we also adopt demographic parameters and serum biochemical parameters which were considered as representatives of the hepatic function. By using Cox proportional-hazards model, $\log_{10}^{\text{AFP}}$ was found to be an independent factor of survival as a categorical variable, as with continuous variables including age, INR, and levels of total bilirubin (Table 2). There were 41 patients whose $\log_{10}^{\text{AFP}} \geq 2$, and this group of patients was more likely to exhibit a longer survival time. The transplant-free survival rates at 30, 90, and 180 days were 58.82% versus 90.24% ($P = 0.001$), 39.22% versus 83.93% ($P < 0.001$), and 29.41% versus 58.54% ($P = 0.005$), respectively, in groups of patients with $\log_{10}^{\text{AFP}} < 2$ and ≥ 2 (Figure 3(b)).

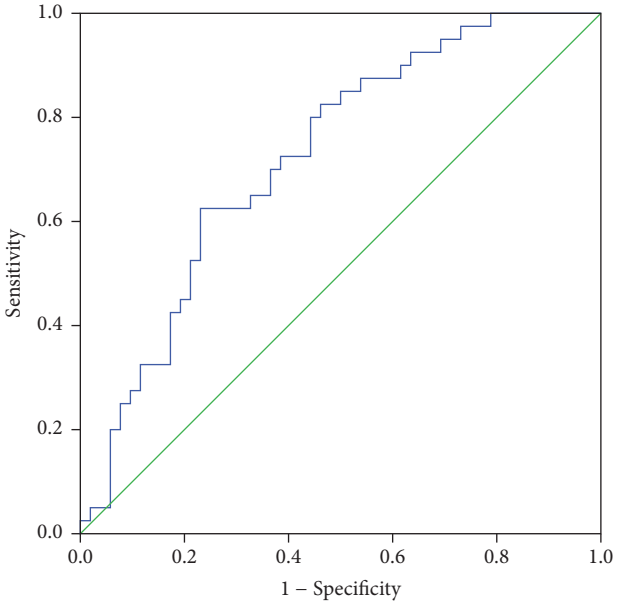

Figure 2: ROC curve for $\log_{10}^{\text{AFP}}$ in predicting the outcome of HBACLF ($n = 92$).

4. Discussion

The idea of ACLF was first proposed to describe the acute liver damage of an ongoing chronic liver disease in 1995 [17]. There

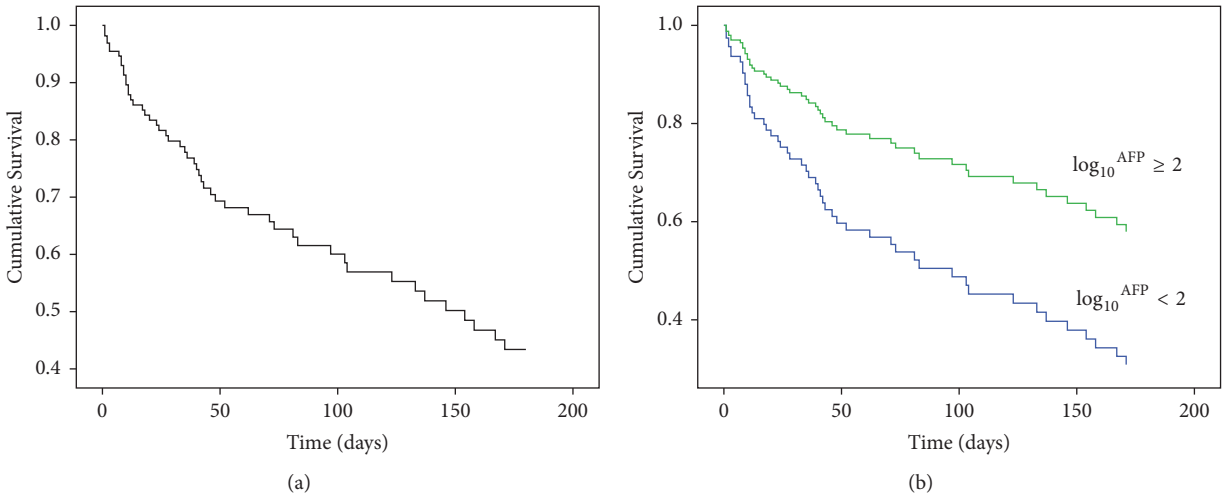

FIGURE 3: (a) Graph of multivariate Cox regression survival curve of the total population ($n = 92$). (b) Graph of multivariate Cox regression survival curve for patients with $\log_{10}{}^{AFP} \geq 2$ ($n = 41$) and $\log_{10}{}^{AFP} < 2$ ($n = 51$).

are more than 13 different definitions of ACLF worldwide, which vary from the West to the East. An acute insult may lead to rapid and progressive liver failure in patients with chronic liver diseases and result in high short-term mortality of approximately 50–90% because of the limited functional reserve of the liver [1, 18].

China exhibits a high morbidity of hepatitis B and HBA-CLF. There are studies estimating the severity and outcomes of HBACLF by establishing prognostic scoring models. Previous studies illustrated that the liver volume [19], lymphocyte-monocyte ratio [20], albumin-bilirubin score [21], logistic regression model [22], macrophage inflammatory protein 3α [23], and other methods [24–26] could be simple and sensitive models to evaluate the severity and prognosis of ACLF, which would be practically useful in clinic. Those methods mainly concentrated on the severity of liver injury, and the parameters in those models were generally to reflect the condition of liver function. It is known to us that the prognosis of ACLF depends on the extent of liver injury, the capability of hepatocyte regeneration, and the prevention of multiple organ failure. There are a number of studies evaluating the prognosis of ACLF from the perspective of liver function; however, limited researches have assessed the outcome of ACLF by adopting parameters reflecting hepatocyte regeneration. AFP is a biomarker of liver renovation; thus, we investigated the prognostic value of AFP in ACLF for that the magnitude of in the increased AFP levels is closely related to hepatocyte regeneration after acute or superimposed hepatic injury. Previous studies demonstrated that AFP was a prognostic marker in patients with acute liver failure [27, 28]. However, limited studies demonstrated a correlation between AFP levels and the outcomes of patients with ACLF, especially patients with acute hepatic failure based on chronic HBV infection.

AFP is not detected in normal adult serum. In 1963, Abelev et al. [29] found that a type of murine embryonal α-globulin, which was originally called AFP, could be detected in normal or malignant hepatocyte proliferation in adult rats. Then, AFP was considered a marker of hepatocyte regeneration, and the capacity of hepatocyte regeneration is key to the reversal of liver injury. The present study assessed the predictive value of AFP in the prognosis of hepatitis B-related acute-on-chronic liver failure because these patients always exhibited bad progress, and the short-term mortality is dramatically high. Valid predictive models and intensive care are necessary in the management of ACLF because some individuals regain their health following liver injury recovery and hepatocyte regeneration. Our study demonstrated that the parameter $\log_{10}{}^{AFP}$ was a helpful marker to predict the outcomes of ACLF. A higher AFP concentration could predict a better outcome of HBACLF, and $\log_{10}{}^{AFP} \geq 2$ could indicate a longer short-term survival time for patients with ACLF. Therefore, we speculated that the concentration of AFP may be positively related to the capacity for liver regeneration in the condition of acute liver injury on the basis of chronic liver diseases, apart from malignancies such as hepatocellular carcinoma and gastric cancer.

Consistent with Katoonizadeh's research [18], our study indicated that the difference of age was of statistical significance between survivors and those who deceased or got liver transplanted. Elderly patients exhibit a weakened condition of body function, which may result in multiple organ failure when there was a hypohepatia already. And we suspected that the number of functional hepatocytes may be lower in the elderly than young adults. In addition, total bilirubin and INR were found to be risk factors of survival as well. Previous studies demonstrated that a higher level of bilirubin was associated with a poor outcome in patients with liver failure because the dysfunctional liver exhibited deficient bilirubin metabolism, which demonstrates that the extent of damage to hepatocytes was extremely serious [8, 30]. Impeded hepatic synthetic function reduces the production of prothrombin and other proteins in the liver, which accounts for the

higher level of INR, and a higher degree of INR indicates an unfavourable prognosis [31].

One limitation of our study was the lack of dynamic observations of AFP levels. A persistently low AFP may be interpreted as regenerative failure in protracted cases but ultimately leads to death. Some patients with low AFP concentrations on admission ultimately survived because liver tissue repair occurred followed with a serially intensive care. Other parameters, such as HBeAg, creatinine, albumin, and some scoring systems, could also help assess the outcome of ACLF. Therefore, further studies of multifactor-correlated prognostic models are needed.

5. Conclusion

In summary, AFP is an indicator of the prognosis of hepatitis B-related acute-on-chronic liver failure. Higher levels of AFP concentrations could predict a better outcome of HBACLF, and $\log_{10}^{\mathrm{AFP}} \geq 2$ would indicate a longer survival time.

Data Availability

All data arising from this study are contained within the manuscript.

Conflicts of Interest

The authors declare that they have no competing financial interest.

Authors' Contributions

Shanhong Tang designed the study and carried it out. Sen Qin and Haijun Zeng joined in data collection. Xianjun Yang and Jianjiang Yang conducted data analysis. Xiaoping Wang and Caifei Shen drafted the manuscript. Xiaoling Wu and Weizheng Zeng helped to finalize the manuscript. All of the authors read and approved the manuscript. Xiaoping Wang and Caifei Shen equally contributed to this work.

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References

[1] S. K. Sarin and A. Choudhury, "Acute-on-chronic liver failure: Terminology, mechanisms and management," *Nature Reviews Gastroenterology & Hepatology*, vol. 13, no. 3, pp. 131–149, 2016.

[2] L. L. Kjaergard, J. Liu, B. Als-Nielsen, and C. Gluud, "Artificial and bioartificial support systems for acute and acute-on-chronic liver failure: A systematic review," *Journal of the American Medical Association*, vol. 289, no. 2, pp. 217–222, 2003.

[3] S. K. Sarin, C. K. Kedarisetty, Z. Abbas et al., "Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) 2014," *Hepatology International*, vol. 8, no. 4, pp. 453–471, 2014.

[4] J. Wendon, J. Cordoba, A. Dhawan et al., "EASL clinical practical guidelines on the management of acute (fulminant) liver failure," *Journal of Hepatology*, vol. 66, no. 5, pp. 1047–1081, 2017.

[5] J. Polson and W. M. Lee, "AAFOL Disease, AASLD position paper: the management of acute liver failure: update 2011," *National Guideline Clearinghouse*, vol. 65, no. 1, pp. 1433–1441, 2011.

[6] J. Cordoba, M. Ventura-Cots, M. Simón-Talero et al., "Characteristics, risk factors, and mortality of cirrhotic patients hospitalized for hepatic encephalopathy with and without acute-on-chronic liver failure (ACLF)," *Journal of Hepatology*, vol. 60, no. 2, pp. 275–281, 2014.

[7] I. Merican, R. Guan, D. Amarapuka et al., "Chronic hepatitis B virus infection in Asian countries," *Journal of Gastroenterology and Hepatology*, vol. 15, no. 12, pp. 1356–1361, 2000.

[8] S. Sen, R. Williams, and R. Jalan, "The pathophysiological basis of acute-on-chronic liver failure," *Journal of Liver*, vol. 22, no. 2, pp. 5–13, 2002.

[9] P. S. Kamath and W. R. Kim, "The model for end-stage liver disease (MELD)," *Hepatology*, vol. 45, no. 3, pp. 797–805, 2007.

[10] J. Aguirre-Valadez, A. Torre, M. Vilatobã et al., "Indications for liver transplant," *Revista De Investigacion Clinica*, vol. 66, no. 6, pp. 534–546, 2014.

[11] Y.-M. Zhou, J.-M. Yang, B. Li et al., "Clinicopathologic characteristics of intrahepatic cholangiocarcinoma in patients with positive serum a-fetoprotein," *World Journal of Gastroenterology*, vol. 14, no. 14, pp. 2251–2254, 2008.

[12] K. Kakisaka, K. Kataoka, M. Onodera et al., "Alpha-fetoprotein: A biomarker for the recruitment of progenitor cells in the liver in patients with acute liver injury or failure," *Hepatology Research*, vol. 45, no. 10, pp. E12–E20, 2015.

[13] G. G. Karvountzis and A. G. Redeker, "Relation of alpha fetoprotein in acute hepatitis to severity and prognosis," *Annals of Internal Medicine*, vol. 80, no. 2, pp. 156–160, 1974.

[14] S. K. Jain, A. Rohatgi, K. K. Raman, and V. K. Sharma, "Study of serum prealbumin and serum alpha fetoprotein in cases of fulminant hepatic failure.," *Journal of the Association of Physicians of India*, vol. 43, no. 7, pp. 462-463, 1995.

[15] I. M. Murray-Lyon, A. H. Orr, B. Gazzard, J. Kohn, and R. Williams, "Prognostic value of serum alpha fetoprotein in fulminant hepatic failure including patients treated by charcoal haemoperfusion," *Gut*, vol. 17, no. 8, pp. 576–580, 1976.

[16] A. Varshney, R. Gupta, S. K. Verma, and S. Ahmad, "Alpha-fetoprotein as a prognostic marker in acute liver failure: a pilot study," *Tropical Doctor*, vol. 47, no. 3, pp. 202–205, 2017.

[17] H. Ohnishi, J. Sugihara, H. Moriwaki et al., "Acute-on-chronic liver failure," *Journal of Hepatology*, vol. 13, no. 12, pp. 2128–2139, 1995.

[18] A. Katoonizadeh, W. Laleman, C. Verslype et al., "Early features of acute-on-chronic alcoholic liver failure: A prospective cohort study," *Gut*, vol. 59, no. 11, pp. 1561–1569, 2010.

[19] S. Lin, J. Chen, M. Wang et al., "Prognostic nomogram for acute-on-chronic hepatitis B liver failure," *Oncotarget* , vol. 8, no. 65, pp. 109772–109782, 2017.

[20] S. M. Zhu, Y. Waili, X. T. Qi, Y. M. Chen, and Y. F. Lou, "Lymphocyte-monocyte ratio at admission predicts possible outcomes in patients with acute-on-chronic liver failure," *European Journal of Gastroenterology & Hepatology*, vol. 29, no. 1, pp. 31–35, 2017.

[21] B. Chen and S. Lin, "Albumin-bilirubin (ALBI) score at admission predicts possible outcomes in patients with acute-on-chronic liver failure," *Medicine (United States)*, vol. 96, no. 24, Article ID e7142, 2017.

[22] M.-H. Zheng, K.-Q. Shi, Y.-C. Fan et al., "A Model to Determine 3-Month Mortality Risk in Patients With Acute-on-Chronic Hepatitis B Liver Failure," *Clinical Gastroenterology and Hepatology*, vol. 9, no. 4, pp. 351–e3, 2011.

[23] J. Xin, W. Ding, S. Hao et al., "Serum macrophage inflammatory protein 3α levels predict the severity of HBV-related acute-on-chronic liver failure," *Gut*, vol. 65, no. 2, pp. 355–357, 2016.

[24] Y. Yan, L. Mai, Y.-B. Zheng et al., "What MELD score mandates use of entecavir for ACLF-HBV HBeAg-negative patients?" *World Journal of Gastroenterology*, vol. 18, no. 33, pp. 4604–4609, 2012.

[25] T. Wu, J. Li, L. Shao et al., "Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure," *Gut*, p. gutjnl-2017-314641.

[26] L. Chen, Y. Lou, Y. Chen, and J. Yang, "Prognostic value of the neutrophil-to-lymphocyte ratio in patients with acute-on-chronic liver failure," *International Journal of Clinical Practice*, vol. 68, no. 8, pp. 1034–1040, 2014.

[27] L. E. Schmidt and K. Dalhoff, "Alpha-fetoprotein is a predictor of outcome in acetaminophen-induced liver injury," *Hepatology*, vol. 41, no. 1, pp. 26–31, 2005.

[28] F. V. Schiodt, G. Ostapowicz, N. Murray et al., "Alpha-fetoprotein and prognosis in acute liver failure," *Liver Transplantation*, vol. 12, no. 12, pp. 1776–1781, 2006.

[29] G. I. Abelev, S. D. Perova, N. I. Khramkova, Z. A. Postnikova, and I. S. Irlin, "Production of embryonal α-globulin by transplantable mouse hepatomas," *Transplantation*, vol. 1, no. 2, pp. 174–180, 1963.

[30] Y. R. Krishna, V. A. Saraswat, K. Das et al., "Clinical features and predictors of outcome in acute hepatitis A and hepatitis E virus hepatitis on cirrhosis," *Liver International*, vol. 29, no. 3, pp. 392–398, 2009.

[31] H. Garg, A. Kumar, V. Garg, P. Sharma, B. C. Sharma, and S. K. Sarin, "Clinical profile and predictors of mortality in patients of acute-on-chronic liver failure," *Digestive and Liver Disease*, vol. 44, no. 2, pp. 166–171, 2012.

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Clinical Study

Good Tolerance of Citrate Accumulation due to Plasma Exchange among Patients with Acute-on-Chronic Liver Failure: A Prospective, Observational Study

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Aim. To assess the tolerance of citrate accumulation due to plasma exchange (PE) among patients with acute-on-chronic liver failure (ACLF). *Methods*. A prospective, observational study was conducted among patients with ACLF who received heparin anticoagulation during PE-centered therapy without filtration and dialysis. Citrate accumulation was defined as the value of total calcium (Ca_{tot}) to ionized calcium (Ca_{ion}) ratio (Ca_{tot}/Ca_{ion}) greater than or equal to 2.5 ($Ca_{tot}/Ca_{ion} \geq 2.5$). *Results*. Fifty-four patients were enrolled. The mean age and MELD score were 50.0 ± 11.3 years old and 25 ± 7, respectively. Thirty-three patients had liver cirrhosis. The total 3-month survival rate was 57.4% (31/54). The mean Ca_{tot}/Ca_{ion} at the time before PE was 2.05 ± 0.14. $Ca_{tot}/Ca_{ion} \geq 2.5$ occurred in 100.0% (54/54) and 29.6% (16/54) of patients with mean Ca_{tot}/Ca_{ion} of 4.34 ± 1.52 and 2.36 ± 0.32 immediately after PE and 1 hour after PE, respectively, and these levels were much higher than those before PE ($p < 0.01$). However, all values returned to lower than 2.5 by the next morning with no difference from those before PE (2.10 ± 0.14 versus 2.05 ± 0.14, $p > 0.05$). Hypocalcemia (ionized calcium) and mild alkalosis were the main metabolic alterations. No symptoms associated with hypocalcemia occurred. *Conclusions*. Citrate accumulation is well tolerated by patients with ACLF who receive PE-centered therapy without filtration and dialysis. This study is regeristed with ChiCTR-OOC-17013618.

1. Introduction

Acute-on-chronic liver failure (ACLF) remains an important cause of mortality. The accumulation of various toxins and inflammatory cytokines leads to life-threatening complications, including renal failure, altered immune response, hepatic coma, and systemic hemodynamic dysfunction, which eventually culminate in multiorgan failure [1, 2]. Current medical therapy involves the management of the precipitating event and treatment of complications until the liver eventually recovers. Removal of toxins improves the capacity of the liver to regenerate, or artificial liver support system (ALSS) therapy can be a bridge to liver transplantation until a suitable organ is available [2–7].

To adequately maintain extracorporeal circulation, heparin or low-molecular-weight heparin anticoagulation is often used in clinical practice, but its side effects such as bleeding and thrombocytopenia threaten the safety of the patient. In recent years, regional citrate anticoagulation (RCA) has become the preferred anticoagulation method in continuous renal replacement therapy (CRRT) for patients with acute kidney injury (AKI) [8]. Several studies have focused on the safety and efficacy of RCA during blood purification for patients with liver failure and have concluded that RCA seems safe and feasible for these patients [9–16]. However, the blood purification techniques used in these studies, including continuous venovenous hemodialysis (CVVHD) [9, 11, 16, 17], sustained low-efficiency dialysis [12], molecular adsorbent recirculating system (MARS) [10, 14, 15], and fractionated plasma separation and adsorption (FPSA; the Prometheus system) [13], contain a dialysis technique that has the ability to remove citrate. Despite partial removal of the

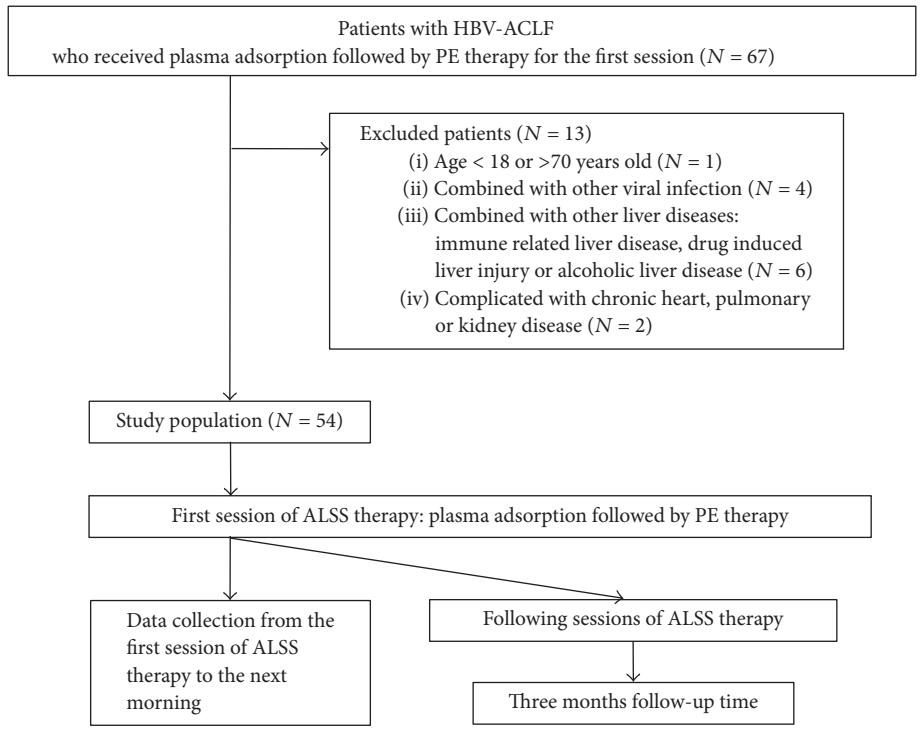

Figure 1: Flow diagram of patient selection and study process. PE, plasma exchange; HBV-ACLF, hepatitis B virus-related acute-on-chronic liver failure; ALSS, artificial liver support system.

citrate by the dialyzer as a complex bound with ionized calcium (Ca_{ion}), a certain amount of citrate enters the systemic circulation, which might lead to citrate accumulation, acid-base imbalance, and hypocalcemia and so on [18].

MARS and FPSA have been widely used for liver failure in Western countries during the past 3 decades, while plasma exchange- (PE-) centered ALSS therapy has been widely used in China for nearly the last 2 decades. With the benefit of having no effect on the blood clotting mechanism in vivo, RCA seems safe and feasible in patients with liver failure who received MARS or FPSA [10, 13–15]. However, without dialysis and filtration techniques, it remains uncertain whether RCA is still effective and safe for patients with liver failure and bleeding tendency or active bleeding who received PE-centered ALSS therapy or not.

Although citrate metabolism is severely impaired and the risk of adverse effects is high in patients with acute liver failure who receive PE therapy with frozen plasma containing citrate [19], it might be not necessarily the same as ACLF for there are big differences between acute liver failure and ACLF [20], and, more importantly, two prospective, controlled studies have found that PE plus standard medical therapy versus standard medical therapy alone could improve the short-term prognosis of patients with acute liver failure [4] and patients with ACLF [3]. Thus, we predict that patients with liver failure still have a certain ability to tolerate and metabolize citrate. However, the tolerance of citrate in patients with ACLF has not been studied in detail. Here, we conducted a prospective, observational study to assess the tolerance of citrate accumulation due to PE among patients with hepatitis B virus (HBV) related ACLF (HBV-ACLF) who received PE-centered ALSS therapy but did not receive filtration or dialysis.

2. Materials and Methods

2.1. Study Design and Patients. To assess the tolerance of citrate accumulation due to PE among patients with HBV-ACLF, a prospective, observational study was conducted in the Center of Infectious Diseases, West China Hospital of Sichuan University. Patients with HBV-ACLF who received PE-centered therapy for the first session were recruited from March 1, 2017, to June 31, 2017.

Patients with HBV-ACLF who received plasma adsorption followed by PE for the first session of ALSS therapy (a kind of nonbioartificial liver support system widely used in China) were enrolled (Figure 1). The diagnosis of HBV-ACLF was mainly based on the following criteria: (i) preexisting chronic hepatitis B virus infection; (ii) progressive hyperbilirubinemia, defined as a >50% increase in bilirubin or up to a level of >171 μmol/L within 4 weeks; (iii) prothrombin time activity (PTA) ≤ 40% or international normalized ratio (INR) ≥ 1.5. Patients whose age was less than 18 years or more than 70 years were excluded.

Patients were excluded if they had the following diseases: drug-induced liver injury, immune-related liver disease, alcoholic liver disease, and hyperthyroidism, and patients who were pregnant were also excluded. In addition, patients with HBV-ACLF were excluded if they were complicated with other viral infections (including hepatitis A, C, D, or E virus, cytomegalovirus, herpes simplex virus, or human immunodeficiency virus) or had evidence of chronic heart, pulmonary,

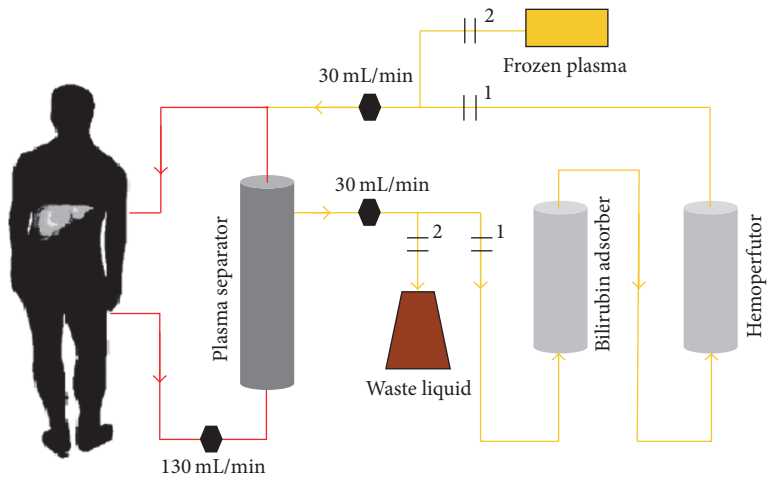

Figure 2: Schematic diagram of the double plasma molecular adsorption system (DPMAS) and plasma exchange (PE). PE (circuit 2) is initiated immediately when DPMAS (circuit 1) is completed in this study.

or kidney diseases. The diagnosis of liver cirrhosis was based on ultrasound and/or computed tomography.

All the patients with HBV-ACLF received standard medication, including antiviral drugs, hepatoprotective agents, and drugs to treat complications. Plasma adsorption followed by PE therapy was performed with heparin anticoagulation. The first session of ALSS therapy was identified as the observational session. Until September 30, 2017, all the patients were followed up for 3 months to identify the status of clinical outcomes.

This study was approved by the Ethics Committee of West China Hospital of Sichuan University. All study components were performed according to the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from each patient or his/her legal guardian.

2.2. ALSS Therapy, Plasma and Citrate. All the study patients received plasma adsorption therapy for 2 hours followed immediately by PE therapy for nearly an hour.

PE-centered ALSS therapy is widely used in China. The dose recommended by the American Society for Apheresis is 1 to 1.5 total plasma volume [21]. In the past, the dose was usually set to one total plasma volume, which was approximately 5% of body weight (approximately 3,000 mL) [22]. Due to the shortage of plasma in recent years and a retrospective study suggesting that plasma adsorption plus PE therapy could reduce the amount of plasma needed for PE therapy without impairing the therapeutic efficiency [23], plasma adsorption plus PE with half the total plasma volume (approximately 1,500 mL) is now widely used for ALSS therapy in China.

Citrate is a regular anticoagulant element in the blood preservation solution. Every 1000 mL of the blood preservation solution consists of sodium citrate 13.2 g, citrate 4.8 g, and glucose 14.7 g. A single blood bag contains 75 mL blood preservation solution. Frozen plasma was provided by the Chengdu Blood Center affiliated to Chengdu Health and Family Planning Commission. Whole blood was collected and stored in a blood bag, and plasma was separated and stored in another blood bag for clinical use. Each patient received 7 to 8 (7.9 ± 0.3) bags of plasma, and the total amount of plasma was 1,500 to 1,550 (1,513 ± 22) mL. Therefore, the mean dose of citrate was approximately 61.3 mmol and the mean concentration of citrate in frozen plasma was approximately 40.5 mmol/L.

Plasma adsorption was combined with a plasma bilirubin adsorption followed by a plasma hemoperfusion (Figure 2), which is referred to as double plasma molecular adsorption system (DPMAS) in China [24]. The extracorporeal blood lines, plasma separator (MICROPLAS MPS05), plasma bilirubin adsorption column (DX350), and plasma hemoperfutor (HA330-II) were manufactured by B.Braun Diapact CRRT, Bellco S.r.l, Biosun Corporation, and Jafron Biomedical Corporation, respectively.

The treatment parameters were set to postdilution fluid replacement for continuous venovenous hemofiltration (CVVH) with blood flow of 130 mL/min, substitution flow of 1800 mL/h, and ultrafiltration flow of 0 mL/h. Therefore, the total time of PE using 1,500 to 1,550 mL plasma for each patient was 50 to 60 min. Patients received an intravenous injection of heparin sodium with an initial dose of 6,250 U at 5 to 10 minutes before plasma adsorption, and those with suspected coagulation would receive an additional heparin sodium injection with a dose of 3,125 U. Patients received 50 mL of 10% calcium gluconate supplement with a speed of 60 mL/h during PE. At the end of ALSS therapy, all patients received an intravenous injection of protamine sulfate with a dose of 50 mg.

2.3. Data Collection and Laboratory Examination. The death or survival information of patients was obtained from electronic medical records in the hospital and/or telephone follow-up. If the survival information could not be obtained from these 2 ways, we made the assumption that the patient had died by the follow-up time. The data of clinical characteristics and laboratory results were obtained from electronic medical records in the hospital. The severity of liver failure

was rated according to the model for end-stage liver disease (MELD) [25].

Venous blood samples from the observational sessions of ALSS therapy were collected at the time before DPMAS (the time before ALSS therapy), before PE (immediately after DPMAS), immediately after PE (the time after ALSS therapy), 1 hour after PE (1 hour after ALSS therapy), and the next morning. All the samples were sent to the Department of Laboratory Medicine, West China Hospital of Sichuan University, for analysis within 30 minutes. Routine blood parameters, biochemical parameters, and coagulation function were measured by automatic analyzers according to standard laboratory procedures at the time before DPMAS, immediately after PE, and the next morning. Blood gas analysis was performed using the COBAS b 123 system (Roche Diagnostics) at the time before PE, immediately after PE, 1 hour after PE, and the next morning.

2.4. Definition. The presence of total calcium (Ca_{tot}) to ionized calcium (Ca_{ion}) ratio (Ca_{tot}/Ca_{ion}) greater than or equal to 2.5 ($Ca_{tot}/Ca_{ion} \geq 2.5$) with or without metabolic acidosis and an enlarged anion gap are the typical manifestations of citrate accumulation. This means that citrate is not being metabolized in a timely manner into bicarbonate, carbon dioxide, and water [9]. Thus, citrate accumulation was defined as $Ca_{tot}/Ca_{ion} \geq 2.5$ in this study [9, 16, 26].

2.5. Statistical Analysis. Quantitative variables were expressed as mean ± standard deviation (SD), and categorical variables as absolute and relative frequencies. One-way ANOVA was performed to calculate differences between quantitative data, while chi-square test or Fisher's exact test was performed to calculate differences between qualitative data. A p value of less than 0.05 was considered to indicate statistical significance. All statistical analyses were performed with SPSS Version 17.0 (SPSS Inc.), and the figures were drawn using GraphPad Prism 6 (GraphPad Software Inc.)

3. Results

3.1. Patients' Characteristics. From March 1, 2017, to June 31, 2017, fifty-four patients with HBV-ACLF who received plasma adsorption followed by PE for the first session of ALSS therapy were enrolled. Of these patients, the mean age was 50.0 ± 11.3 years old, 41 patients were male, 33 patients had a diagnosis of liver cirrhosis, and the mean level of HBV DNA was 3.8 ± 2.2 log IU/mL. The baseline laboratory parameters of patients before the first session of ALSS therapy are summarized in Table 1. The mean levels of total bilirubin, international normalized ratio (INR) of prothrombin time, creatinine, and MELD score were elevated to 408.1 ± 137.1 μmol/L, 2.0 ± 0.6, 101 ± 91 μmol/L, and 25 ± 7, respectively.

3.2. Patient Outcomes. During hospitalization, all the patients with HBV-ACLF received standard medication including antiviral drugs, hepatoprotective agents, and drugs to treat complications. Thirty-seven patients underwent right internal jugular vein catheterization, and the remaining patients right femoral vein catheterization. Patients received a total of 203 sessions of ALSS therapy with a mean number of sessions of 3.8 ± 2.0. The immediate effect of the first session of ALSS therapy is summarized in Table 2.

Table 1: Baseline laboratory parameters of patients before the first session of ALSS therapy.

Hemoglobin, g/dL	11.1 ± 2.1
Platelets, $\times 10^9$/L	126 ± 76
White blood cells, $\times 10^9$/L	8.2 ± 6.5
INR	2.0 ± 0.6
Total bilirubin, μmol/L	408.1 ± 137.1
Direct bilirubin, μmol/L	307.2 ± 107.9
Alanine transaminase, IU/L	200 ± 232
Aspartate transaminase, IU/L	177 ± 178
Alkaline phosphatase, IU/L	223 ± 320
gamma-Glutamyl transferase, IU/L	141 ± 276
Albumin, g/L	32.1 ± 4.4
Globulin, g/L	28.2 ± 8.2
Total bile acid, μmol/L	290.7 ± 78.6
Creatinine, μmol/L	101 ± 91
Ammonia, mmol/L	76 ± 35
Sodium, mmol/L	133.9 ± 5.3
Potassium, mmol/L	3.4 ± 0.6
Chloride, mmol/L	97.1 ± 6.4
MELD scores	25 ± 7

ALSS, artificial liver support system; MELD, model for end-stage liver disease; INR, international normalized ratio; measurement data are represented as mean ± SD.

At the final follow-up on September 30, 2017, all enrolled patients had been discharged. The mean length of hospital stay was 26.0 ± 13.4 days. The survival information was available for all. Four patients received living-donor liver transplantation during hospitalization or the 3-month follow-up period, and then we assumed here that they had died. The occurrences of major complications of liver disease (infection, hemorrhage, hepatorenal syndrome, and hepatic encephalopathy) are summarized in Table 3. The total 3-month survival rate was 57.4% (31/54).

3.3. Alteration in Calcium Status and Safety of Citrate Accumulation. In this study, we used Ca_{tot}/Ca_{ion}, an indicator with the cut-off value of 2.5, to assess whether there was citrate accumulation [9, 16, 26], instead of using citrate concentration to indirectly evaluate the presence of citrate accumulation. Changes of Ca_{tot}, Ca_{ion}, Ca_{tot}/Ca_{ion} and anion gap during and after ALSS therapy are summarized in Table 4 and their trends were shown in Figure 3.

The mean level of Ca_{ion} before PE therapy was 1.05 ± 0.06 mmol/L. Although supplied with 50 mL of 10% calcium gluconate during PE, the mean level of Ca_{ion} decreased noticeably to 0.71 ± 0.16 mmol/L immediately after PE, which was much lower than the level before PE ($p < 0.01$). It is worth noting that the mean level of Ca_{ion} returned to normal levels 1 hour after the ALSS therapy and remained stable until the next morning. Although there was an obviously

Table 2: Immediate effect of the first session of ALSS therapy.

	Rate of reduction between pre- and post-ALSS therapy (%)	Rate of reduction between pre-ALSS therapy and the next morning (%)
Hemoglobin	12.1 ± 6.3	3.3 ± 5.9
Platelets	20.0 ± 18.1	17.4 ± 15.3
White blood cells	−7.3 ± 31.6	7.1 ± 21.5
INR	12.7 ± 17.8	9.4 ± 15.2
Total bilirubin	49.1 ± 6.7	14.1 ± 14.8
Direct bilirubin	47.5 ± 7.2	16.8 ± 9.8
Alanine transaminase	36.9 ± 12.2	27.4 ± 13.5
Aspartate transaminase	30.4 ± 57.8	17.1 ± 11.3
Alkaline phosphatase	24.9 ± 33.6	3.2 ± 61.3
gamma-Glutamyl transferase	31.2 ± 14.1	20.0 ± 18.8
Albumin	5.6 ± 7.6	−2.2 ± 8.9
Globulin	13.4 ± 12.3	12.7 ± 8.6
Total bile acid	19.9 ± 14.1	28.5 ± 14.4
Creatinine	7.7 ± 15.4	3.2 ± 16.6
Ammonia	16.3 ± 36.3	−32.5 ± 68.3
Sodium	−3.1 ± 2.5	−1.9 ± 2.4
Potassium	−6.3 ± 10.0	−10.6 ± 14.0
Chloride	−1.2 ± 4.7	−0.3 ± 4.5
MELD score	21.4 ± 13.4	9.2 ± 10.2

ALSS, artificial liver support system; MELD, model for end-stage liver disease; INR, international normalized ratio; measurement data are represented as mean ± SD.

Table 3: Disease state during hospitalization and short-term prognosis.

Hospital stay, days	26.0 ± 13.4
Spontaneous bacterial peritonitis, yes/no	30/24
Infection, yes/no	16/38
Hemorrhage, yes/no	7/47
Hepatorenal syndrome, yes/no	12/42
Hepatic encephalopathy, yes/no	15/39
Three-month survival, yes/no★	31/23

ALSS, artificial liver support system. ★Four patients received living donor liver transplantation during hospitalization or the 3-month follow-up period, we assume here that they have died. Measurement data are represented as mean ± SD. Enumeration data are represented as frequencies.

lower level of Ca_{ion} during and after ALSS therapy, none of the patients complained about numbness of the mouth and fingers, nor did we observe any twitching of the calf muscles.

Because of calcium supplements, the mean level of Ca_{tot} immediately after PE was much higher than the level before PE (2.90 ± 0.21 mmol/L versus 2.15 ± 0.13 mmol/L, $p < 0.01$), and the mean level of Ca_{tot} 1 hour after PE was much higher than the level before PE (2.52 ± 0.21 mmol/L versus 2.15 ± 0.13 mmol/L, $p < 0.01$). Although the mean level of Ca_{tot} at the next morning was higher than that before PE (2.27 ± 0.14 mmol/L versus 2.15 ± 0.13 mmol/L, $p < 0.01$), it was noticeably decreased from 2.52 ± 0.21 mmol/L at 1 hour after PE ($p < 0.01$), and the level returned to normal next morning.

$Ca_{tot}/Ca_{ion} \geq 2.5$, an indicator of citrate accumulation, did not occur before PE, and the mean value of Ca_{tot}/Ca_{ion} was 2.05 ± 0.14. $Ca_{tot}/Ca_{ion} \geq 2.5$ occurred in 100.0% (54/54) and 29.6% (16/54) of patients immediately after PE and 1 hour after PE, respectively, which were much higher than values before PE ($p < 0.01$). The mean values of Ca_{tot}/Ca_{ion} immediately after PE and 1 hour after PE were 4.34 ± 1.52 and 2.36 ± 0.32, respectively, which were much higher than that before PE ($p < 0.01$). However, the mean value of Ca_{tot}/Ca_{ion} did noticeably decrease 1 hour after PE, and all values returned to lower than 2.5 the next morning. Furthermore, the mean value of Ca_{tot}/Ca_{ion} the next morning was similar to that before PE (2.10 ± 0.14 versus 2.05 ± 0.14, $p > 0.05$).

As shown in Table 4 and Figure 3, the mean level of anion gap had similar trends to the mean value of Ca_{tot}/Ca_{ion}. The mean level of anion gap before PE was 6.2 ± 2.6 mmol/L. The mean level of anion gap was much higher at the time immediately after PE than that before PE (9.8 ± 2.6 mmol/L versus 6.2 ± 2.6 mmol/L, $p < 0.01$). However, the mean level of anion gap returned to the baseline level 1 hour after PE and remained stable the next morning ($p > 0.05$).

3.4. Alterations in Acid-Base Status and Lactate. In the human body, citrate is metabolized into bicarbonate, carbon dioxide, and water. The acid-base status values during and after ALSS therapy are shown in Table 4 and Figure 4.

Although the mean pH immediately after PE was similar to that before PE (7.42 ± 0.04 versus 7.42 ± 0.04, $p > 0.05$), the mean pH noticeably increased 1 hour after PE and the

Table 4: Calcium and acid-base status during and after ALSS therapy.

Time	Before PE	Immediately after PE	1 hour after PE	Next morning★
Ca_{tot}, mmol/L	$2.15 \pm 0.13^{\S}$	$2.90 \pm 0.21^{\#\S}$	$2.52 \pm 0.21^{\#\S}$	$2.27 \pm 0.14^{\#}$
Ca_{ion}, mmol/L	1.05 ± 0.06	$0.71 \pm 0.16^{\#\S}$	1.08 ± 0.10	1.08 ± 0.06
Ca_{tot}/Ca_{ion}	2.05 ± 0.14	$4.34 \pm 1.52^{\#\S}$	$2.36 \pm 0.32^{\#\S}$	2.10 ± 0.14
$Ca_{tot}/Ca_{ion} \geq 2.5$, yes/no	0/54	$54/0^{\#\S}$	$16/38^{\#\S}$	0/54
Anion gap, mmol/L	6.2 ± 2.6	$9.8 \pm 2.6^{\#\S}$	6.7 ± 2.3	6.1 ± 1.8
pH	$7.42 \pm 0.04^{\S}$	$7.42 \pm 0.04^{\S}$	$7.47 \pm 0.05^{\#}$	$7.46 \pm 0.04^{\#}$
HCO_3^-, mmol/L	$25.4 \pm 4.6^{\triangle}$	25.8 ± 4.3	26.6 ± 3.9	$27.4 \pm 3.2^{\divideontimes}$
PCO_2, mmHg	39.5 ± 5.7	40.1 ± 5.6	37.5 ± 7.0	39.4 ± 5.5
Lactate, mmol/L	2.4 ± 0.6	2.5 ± 1.3	2.6 ± 0.8	2.6 ± 0.6

ALSS, artificial liver support system; PE, plasma exchange; Ca_{tot}, total calcium; Ca_{ion}, ionized calcium; Ca_{tot}/Ca_{ion}, total-to-ionized calcium ratio; HCO_3^-, standard bicarbonate; PCO_2, partial pressure of carbon dioxide. ★The interval time between the time after PE therapy and the next morning is 16.5 ± 1.9 hours. Compared with the data at the time before PE, $^{\divideontimes}p < 0.05$, $^{\#}p < 0.01$, the others, $p > 0.05$. Compared with the data at the next morning, $^{\triangle}p < 0.05$, $^{\S}p < 0.01$, the others, $p > 0.05$. Measurement data are represented as mean ± SD. Enumeration data are represented as frequencies.

Table 5: Univariate analysis and multivariate analysis of predictors for citrate accumulation at 1 hour after ALSS therapy.

	Correlation coefficient	p	Regression coefficient	Standard error	p	Odds ratio	95% CI
Gender	0.393	0.003	−2.130	0.938	0.023	0.12	0.02–0.75
Baseline lactate	0.396	0.003	1.710	0.856	0.046	5.53	1.03–29.57
Baseline Ca_{tot}/Ca_{ion}	0.356	0.008	8.632	3.917	0.028	5607.59	2.60–12108390.04
Baseline Ca_{ion}	−0.380	0.005	1.974	8.144	0.809	7.20	0.00–61574425.76
Baseline platelets	−0.368	0.006	0.000	0.006	0.873	1.00	0.99–1.01

ALSS, artificial liver support system; Ca_{tot}, total calcium; Ca_{ion}, ionized calcium; Ca_{tot}/Ca_{ion}, total-to-ionized calcium ratio.

next morning compared with the value before PE (7.47 ± 0.05 versus 7.42 ± 0.04 and 7.46 ± 0.04 versus 7.42 ± 0.04, $p < 0.01$, respectively). However, the mean pH next morning was similar to that 1 hour after PE (7.46 ± 0.04 versus 7.47 ± 0.05, $p > 0.05$).

As shown in Table 4 and Figure 4, the mean level of standard bicarbonate (HCO_3^-) increased during and after ALSS therapy. It was higher the next morning than before and immediately after PE and 1 hour after PE (27.4 ± 3.2 mmol/L versus 25.4 ± 4.6 mmol/L, 27.4 ± 3.2 mmol/L versus 25.8 ± 4.3 mmol/L, and 27.4 ± 3.2 versus 26.6 ± 3.9, $p < 0.05$, resp.). The partial pressure of carbon dioxide (PCO_2) and the lactate level remained stable around the time of ALSS therapy with no difference among the observation time ($p > 0.05$).

3.5. Predictors for Citrate Accumulation. Under normal conditions, citrate's half-life is approximately 5 minutes and citrate is metabolized completely within 30 minutes of discontinuing a citrate infusion [27, 28]. However, $Ca_{tot}/Ca_{ion} \geq 2.5$ occurred in 29.6% (16/54) of our patients 1 hour after PE. Correlation analysis showed that gender ($p = 0.003$), baseline lactate levels ($p = 0.003$), baseline Ca_{tot}/Ca_{ion} ($p = 0.008$), baseline Ca_{ion} ($p = 0.005$), and baseline platelet counts ($p = 0.006$) before the ALSS therapy were all predictors for $Ca_{tot}/Ca_{ion} \geq 2.5$ at 1 hour after PE therapy (Table 5). Multivariate analysis revealed that gender (OR, 0.12; 95% CI, 0.02–0.75; $p = 0.023$), baseline lactate, (OR, 5.53; 95% CI, 1.03–29.57; $p = 0.046$), and baseline Ca_{tot}/Ca_{ion} (OR, 5607.59; 95% CI, 2.60–12108390.04; $p = 0.028$) were the independent predictors for citrate accumulation at 1 hour after PE therapy. The highest AUC value regarding citrate accumulation was observed for serum lactate (AUC, 0.750; 95% CI, 0.601–0.899). An increase in $Ca_{tot}/Ca_{ion} \geq 2.5$ at 1 hour after PE was predicted by the presence of baseline levels of plasma lactate greater than or equal to 2.65 mmol/L (sensitivity 62.5%; specificity 84.2%). The AUC values for gender and baseline Ca_{tot}/Ca_{ion} were 0.684 and 0.725, respectively.

4. Discussion

In this prospective, observational study, we found that citrate accumulation due to PE occurred in all these patients, and it was still present in 29.6% of these patients at 1 hour after PE. However, all of the levels returned to normal the next morning. Hypocalcemia (ionized calcium) and mild metabolic alkalosis due to PE were the main alterations in calcium and acid-base status. An increase in citrate accumulation at 1 hour after PE was predicted by the presence of baseline levels of plasma lactate greater than or equal to 2.65 mmol/L.

The possible occurrence of citrate accumulation is the main issue for patients with liver failure who are receiving citrate anticoagulation. Several studies have focused on the safety and efficacy of RCA for blood purification in patients with liver failure and AKI and found that RCA seems safe and feasible in these patients [9–17]. Despite partial removal of the citrate by the dialyzer, a certain amount of citrate enters the systemic circulation. Citrate accumulation does occur in 2.3% to 23.2% sessions of CRRT for patients with liver failure and AKI [9, 11, 16, 17] and in 21.4% sessions of MARS therapy for patients with liver failure [10]. In our study, citrate was metabolized by the patients themselves,

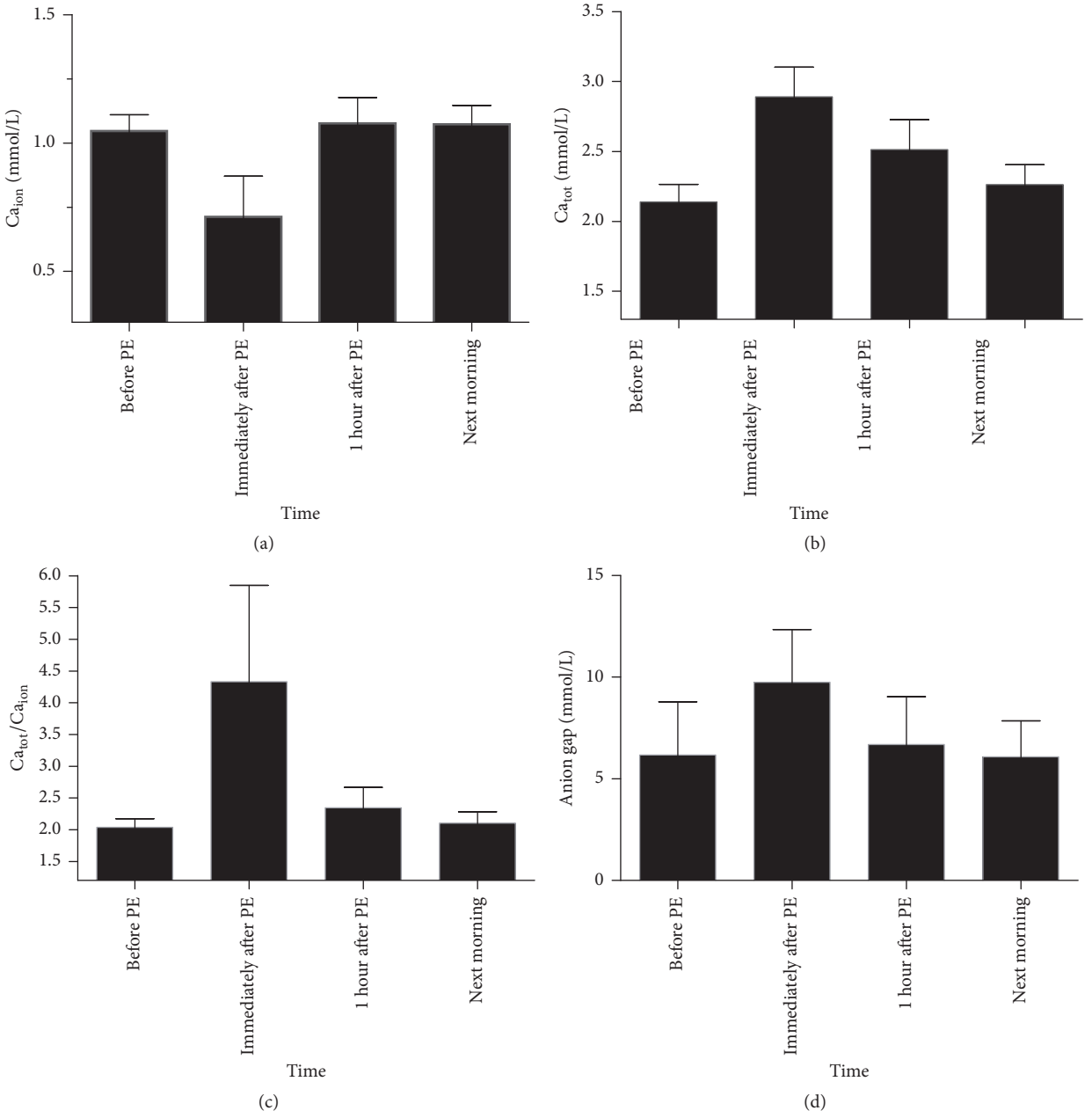

FIGURE 3: Trends of ionized calcium (Ca_{ion}, (a)), total calcium (Ca_{tot}, (b)), total-to-ionized calcium ratio (Ca_{tot}/Ca_{ion}, (c)), and anion gap (d) during and after ALSS therapy for patients with HBV-ACLF.

and citrate accumulation due to PE occurs in all the patients with HBV-ACLF. However, the main acid-base imbalance is mild metabolic alkalosis rather than metabolic acidosis in our study. A similar result was found in a study of patients with cirrhosis, in which the total body clearance of citrate was significantly reduced and the net metabolic changes were quantitatively similar to those in patients with no cirrhosis [29]. Another study of patients with acute liver failure found that citrate metabolism was severely impaired and the risk of adverse effects was high [19]. However, the results from acute liver failure were not necessarily the same as ACLF for there are big differences between them [20]. Our findings suggest that citrate accumulation is well tolerated with good safety among patients with HBV-ACLF, and these patients still have a certain ability to metabolize citrate.

The use of RCA could induce a lower activation of coagulation than both conventional and fractionated heparin, and this lower activation might contribute to an improvement of biocompatibility of extracorporeal circulation [30]. A meta-analysis conducted in adult critically ill patients with AKI who require CRRT reported that RCA is more efficacious in prolonging circuit lifespan and reducing the risk of bleeding with no difference in mortality between the RCA and heparin anticoagulation groups [31]. The benefits mentioned above

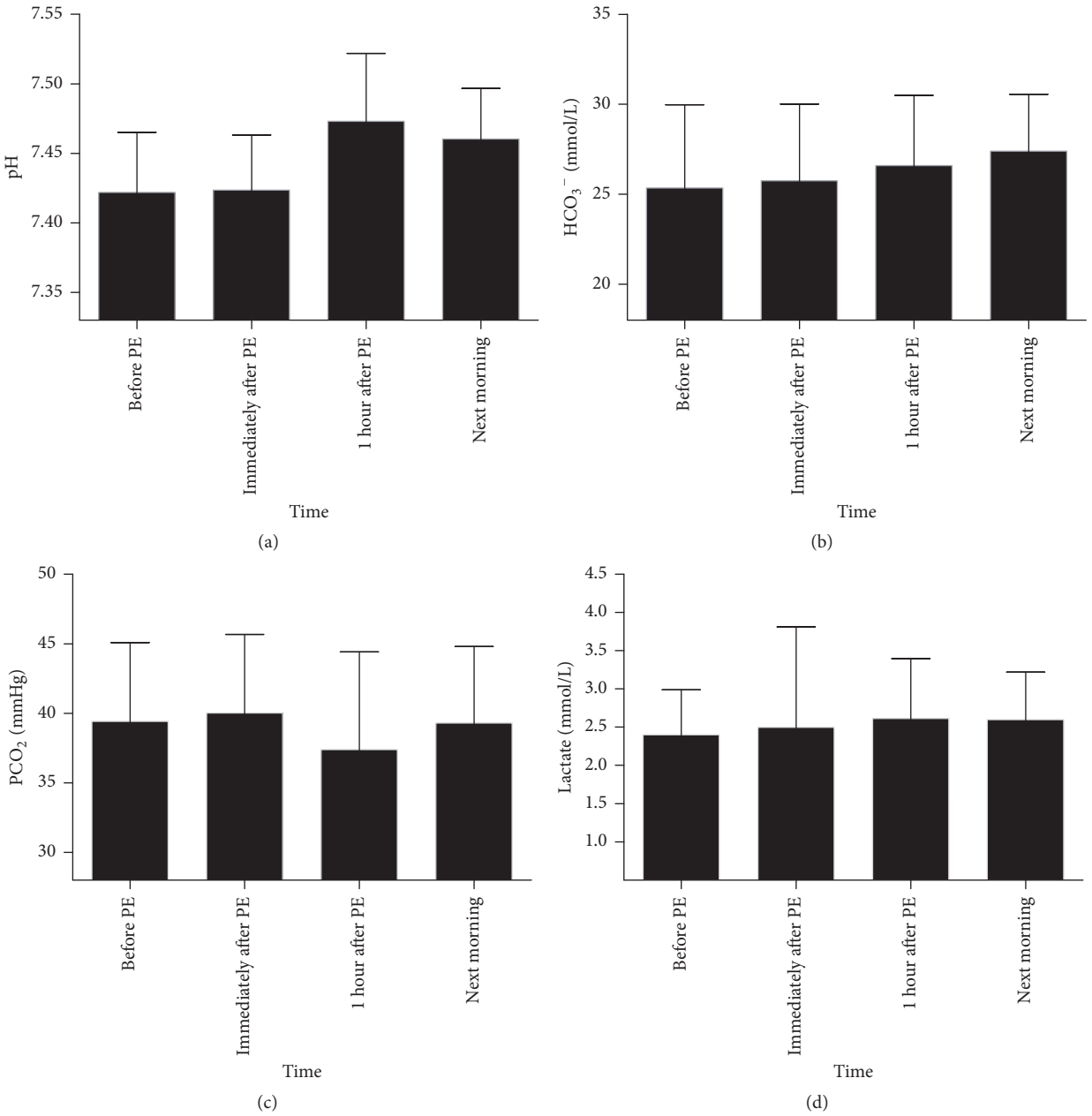

Figure 4: Trends of pH (a), standard bicarbonate (HCO_3^- (b)), partial pressure of carbon dioxide (PCO_2 (c)), and lactate (d) during and after ALSS therapy for patients with HBV-ACLF.

and good tolerance to citrate seem to somewhat dispel the idea that RCA is contraindicated in patients with liver failure [32]. We think that RCA might be particularly beneficial in patients with impaired coagulation due to liver failure. A similar study reporting dose adaptation and monitoring of Ca_{ion} has also suggested that RCA is feasible for patients with decompensated cirrhosis [29]. However, further research is needed to assess the safety and efficacy of RCA for PE-centered ALSS therapy in patients with HBV-ACLF who do not receive dialysis and filtration.

In this study, we found that gender, baseline lactate levels, and baseline Ca_{tot}/Ca_{ion} are independent predictors for citrate accumulation at 1 hour after PE therapy. Similar to our findings, 2 studies have reported that standard liver-function parameters show poor predictive capabilities regarding citrate accumulation in patients with cirrhosis and in patients with liver failure and AKI who received CVVHD [9, 29]. In our study, an increase in citrate accumulation at 1 hour after PE is predicted by the presence of baseline plasma lactate levels greater than or equal to 2.65 mmol/L (sensitivity 62.5%; specificity 84.2%). This finding is similar to that in patients with liver failure and AKI who received CVVHD, in which serum lactate levels greater than or equal to 3.4 mmol/L and prothrombin time activity less than or equal to 26% predict an increase in citrate accumulation with high sensitivity (86% for both lactate and prothrombin time

activity) and specificity (86% for lactate; 92% for prothrombin time) [9].

Our study has several limitations. First, we used Ca_{tot}/Ca_{ion} instead of direct measurement of plasma citrate concentration to reflect the citrate accumulation. However, citrate metabolism seems not to be restricted to the liver [17], and an upper normal or even toxic level of citrate in the blood is not well established [9]. Being a physiological metabolite, citrate might not be toxic itself but could induce metabolic disorders [9]. Second, we did not directly check the concentration of citrate in frozen plasma. Although we calculated it indirectly by summing up the amount of blood preservation solution the patients used, this might lead to erroneous estimation. Third, we performed blood gas analysis with venous blood samples instead of artery blood samples to reflect acid-base status because of poor clotting function and possible bleeding. However, venous gases are suitable for initial evaluation of acid-base status in critically ill patients [33].

In conclusion, in this prospective, observational study, we found that citrate accumulation due to PE occurred in all the patients with HBV-ACLF who received plasma adsorption and PE but did not receive filtration or dialysis, and it was well tolerated. Further research should be carried out to assess the safety and efficacy of RCA for PE-centered ALSS therapy in patients with HBV-ACLF who do not receive dialysis and filtration.

Conflicts of Interest

There are no conflicts of interest to delcare.

Authors' Contributions

Yuanji Ma and Yan Xu contributed equally to this work.

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References

[1] R. Hernaez, E. Solà, R. Moreau, and P. Ginès, "Acute-on-chronic liver failure: an update," *Gut*, vol. 66, no. 3, pp. 541–553, 2017.

[2] S. K. Sarin and A. Choudhury, "Acute-on-chronic liver failure: Terminology, mechanisms and management," *Nature Reviews Gastroenterology & Hepatology*, vol. 13, no. 3, pp. 131–149, 2016.

[3] G. Qin, J.-G. Shao, B. Wang et al., "Artificial liver support system improves short- and long-term outcomes of patients with HBV-associated acute-on-chronic liver failure: a single-center experience," *Medicine*, vol. 93, no. 28, p. e338, 2014.

[4] F. S. Larsen, L. E. Schmidt, C. Bernsmeier et al., "High-volume plasma exchange in patients with acute liver failure: an open randomised controlled trial," *Journal of Hepatology*, vol. 64, no. 1, pp. 69–78, 2016.

[5] A. Vaid, H. Chweich, E. M. Balk, and B. L. Jaber, "Molecular adsorbent recirculating system as artificial support therapy for liver failure: a meta-analysis," *ASAIO Journal*, vol. 58, no. 1, pp. 51–59, 2012.

[6] G. L. He, L. Feng, C. Y. Duan et al., "Meta-analysis of survival with the molecular adsorbent recirculating system for liver failure," *International Journal of Clinical and Experimental Medicine*, vol. 8, no. 10, pp. 17046–17054, 2015.

[7] E. Tsipotis, A. Shuja, and B. L. Jaber, "Albumin dialysis for liver failure: a systematic review," *Advances in Chronic Kidney Disease*, vol. 22, no. 5, pp. 383–390, 2015.

[8] Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group, "KDIGO clinical practice guideline for acute kidney injury," *Kidney International Supplements*, vol. 2, no. 1, pp. 1–138, 2012.

[9] C. Schultheiß, B. Saugel, V. Phillip et al., "Continuous veno-ovenous hemodialysis with regional citrate anticoagulation in patients with liver failure: a prospective observational study," *Critical Care*, vol. 16, no. 4, article R162, 2012.

[10] B. Meijers, W. Laleman, P. Vermeersch, F. Nevens, A. Wilmer, and P. Evenepoel, "A prospective randomized open-label crossover trial of regional citrate anticoagulation vs. anti-coagulation free liver dialysis by the molecular adsorbents recirculating system," *Critical Care*, vol. 16, no. 1, article R20, 2012.

[11] T. Slowinski, S. Morgera, M. Joannidis et al., "Safety and efficacy of regional citrate anticoagulation in continuous venovenous hemodialysis in the presence of liver failure: the liver citrate anticoagulation threshold (L-CAT) observational study," *Critical Care*, vol. 19, no. 1, article 349, 2015.

[12] T. Lahmer, M. Messer, S. Rasch et al., "Sustained low-efficiency dialysis with regional citrate anticoagulation in medical intensive care unit patients with liver failure: a prospective study," *Journal of Critical Care*, vol. 30, no. 5, pp. 1096–1100, 2015.

[13] E. Şentürk, F. Esen, P. E. Özcan et al., "The treatment of acute liver failure with fractionated plasma separation and adsorption system: experience in 85 applications," *Journal of Clinical Apheresis*, vol. 25, no. 4, pp. 195–201, 2010.

[14] A. Dyla, W. Mielnicki, J. Bartczak, T. Zawada, and P. Garba, "Effectiveness and safety assessment of citrate anticoagulation during albumin dialysis in comparison to other methods of anticoagulation," *Artificial Organs*, vol. 41, no. 9, pp. 818–826, 2017.

[15] P. Faybik, H. Hetz, G. Mitterer et al., "Regional citrate anticoagulation in patients with liver failure supported by a molecular adsorbent recirculating system," *Critical Care Medicine*, vol. 39, no. 2, pp. 273–279, 2011.

[16] H. U. Meier-Kriesche, J. Gitomer, K. Finkel, and T. DuBose, "Increased total to ionized calcium ratio during continuous venovenous hemodialysis with regional citrate anticoagulation," *Critical Care Medicine*, vol. 29, no. 4, pp. 748–752, 2001.

[17] M. Klingele, T. Stadler, D. Fliser, T. Speer, H. V. Groesdonk, and A. Raddatz, "Long-term continuous renal replacement therapy and anticoagulation with citrate in critically ill patients with severe liver dysfunction," *Critical Care*, vol. 21, no. 1, article 294, 2017.

[18] D. Khadzhynov, C. Schelter, I. Lieker et al., "Incidence and outcome of metabolic disarrangements consistent with citrate accumulation in critically ill patients undergoing continuous venovenous hemodialysis with regional citrate anticoagulation," *Journal of Critical Care*, vol. 29, no. 2, pp. 265–271, 2014.

[19] R. Apsner, M. Schwarzenhofer, K. Derfler, C. Zauner, K. Ratheiser, and A. Kranz, "Impairment of citrate metabolism in

acute hepatic failure," *Wiener Klinische Wochenschrift*, vol. 109, no. 4, pp. 123–127, 1997.

[20] S. K. Sarin, C. K. Kedarisetty, Z. Abbas et al., "Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) 2014," *Hepatology International*, vol. 8, no. 4, pp. 453–471, 2014.

[21] J. Schwartz, J. L. Winters, A. Padmanabhan et al., "Guidelines on the use of therapeutic apheresis in clinical practice—evidence-based approach from the writing committee of the american society for apheresis: the sixth special issue," *Journal of Clinical Apheresis*, vol. 28, no. 3, pp. 145–284, 2013.

[22] L. J. Li, Y. M. Zhang, X. L. Liu et al., "Artificial liver support system in china: a review over the last 30 years," *Therapeutic Apheresis and Dialysis*, vol. 10, no. 2, pp. 160–167, 2006.

[23] P.-Q. Zhou, S.-P. Zheng, M. Yu, S.-S. He, and Z.-H. Weng, "Prognosis of acute-on-chronic liver failure patients treated with artificial liver support system," *World Journal of Gastroenterology*, vol. 21, no. 32, pp. 9614–9622, 2015.

[24] J. Chen, J. Huang, Y. Chen, Y. Lu, and L. Li, "A clinical study on the treatment of severe hepatitis by a combined artificial liver," *Hepato-Gastroenterology*, vol. 59, no. 119, pp. 2273–2275, 2012.

[25] P. S. Kamath, R. H. Wiesner, M. Malinchoc et al., "A model to predict survival in patients with end-stage liver disease," *Hepatology*, vol. 33, no. 2, pp. 464–470, 2001.

[26] A. Link, M. Klingele, T. Speer et al., "Total-to-ionized calcium ratio predicts mortality in continuous renal replacement therapy with citrate anticoagulation in critically ill patients," *Critical Care*, vol. 16, no. 3, article R97, 2012.

[27] A. G. Schneider, D. Journois, and T. Rimmelé, "Complications of regional citrate anticoagulation: accumulation or overload?" *Critical Care*, vol. 21, no. 1, article 281, 2017.

[28] D. J. Kutsogiannis, I. Mayers, W. D. N. Chin, and R. T. N. Gibney, "Regional citrate anticoagulation in continuous venovenous hemodiafiltration," *American Journal of Kidney Diseases*, vol. 35, no. 5, pp. 802–811, 2000.

[29] L. Kramer, E. Bauer, C. Joukhadar et al., "Citrate pharmacokinetics and metabolism in cirrhotic and noncirrhotic critically ill patients," *Critical Care Medicine*, vol. 31, no. 10, pp. 2450–2455, 2003.

[30] R. Hofbauer, D. Moser, M. Frass et al., "Effect of anticoagulation on blood membrane interactions during hemodialysis," *Kidney International*, vol. 56, no. 4, pp. 1578–1583, 1999.

[31] C. Liu, Z. Mao, H. Kang, J. Hu, and F. Zhou, "Regional citrate versus heparin anticoagulation for continuous renal replacement therapy in critically ill patients: a meta-analysis with trial sequential analysis of randomized controlled trials," *Critical Care*, vol. 20, no. 1, article 144, 2016.

[32] S. Patel and J. Wendon, "Regional citrate anticoagulation in patients with liver failure—time for a rethink?" *Critical Care*, vol. 16, no. 5, article 153, 2012.

[33] C. M. Martin and F. Priestap, "Agreement between venous and arterial blood gas analysis of acid-base status in critical care and ward patients: a retrospective cohort study," *Canadian Journal of Anesthesia*, vol. 64, no. 11, pp. 1138–1143, 2017.

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Research Article

Transcriptome Analysis of Porcine PBMCs Reveals the Immune Cascade Response and Gene Ontology Terms Related to Cell Death and Fibrosis in the Progression of Liver Failure

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Background. The key gene sets involved in the progression of acute liver failure (ALF), which has a high mortality rate, remain unclear. This study aims to gain a deeper understanding of the transcriptional response of peripheral blood mononuclear cells (PBMCs) following ALF. *Methods*. ALF was induced by D-galactosamine (D-gal) in a porcine model. PBMCs were separated at time zero (baseline group), 36 h (failure group), and 60 h (dying group) after D-gal injection. Transcriptional profiling was performed using RNA sequencing and analysed using DAVID bioinformatics resources. *Results*. Compared with the baseline group, 816 and 1,845 differentially expressed genes (DEGs) were identified in the failure and dying groups, respectively. A total of five and two gene ontology (GO) term clusters were enriched in 107 GO terms in the failure group and 154 GO terms in the dying group. These GO clusters were primarily immune-related, including genes regulating the inflammasome complex and toll-like receptor signalling pathways. Specifically, GO terms related to cell death, including apoptosis, pyroptosis, and autophagy, and those related to fibrosis, coagulation dysfunction, and hepatic encephalopathy were enriched. Seven Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, cytokine-cytokine receptor interaction, hematopoietic cell lineage, lysosome, rheumatoid arthritis, malaria, and phagosome and pertussis pathways were mapped for DEGs in the failure group. All of these seven KEGG pathways were involved in the 19 KEGG pathways mapped in the dying group. *Conclusion*. We found that the dramatic PBMC transcriptome changes triggered by ALF progression was predominantly related to immune responses. The enriched GO terms related to cell death, fibrosis, and so on, as indicated by PBMC transcriptome analysis, seem to be useful in elucidating potential key gene sets in the progression of ALF. A better understanding of these gene sets might be of preventive or therapeutic interest.

1. Introduction

Acute liver failure (ALF) is a severe syndrome characterised by hepatic encephalopathy and coagulation dysfunction, which can lead to multiorgan failure and death [1–3]. High morbidity and mortality following ALF are major problems worldwide [2, 3]. Thus, a thorough understanding of key genes or gene sets that regulate the progression of ALF is required.

The development of second-generation sequencing, particularly RNA-sequencing (RNA-Seq), has made it possible to perform global analysis of changes in gene expression during the course of a disease [4–6].

Taking biopsy samples during an ALF flare places the patient at high risk for lethal bleeding. More importantly, biopsy would influence the progression of ALF.

Analysis of the transcriptome of peripheral blood mononuclear cells (PBMCs) has successfully elucidated the

mechanisms of numerous complex diseases and vaccination models [7–10]. These studies showed that analysing the PBMC transcriptome is helpful in identifying key genes and gene sets that control disease progression.

Here, we performed a comparative analysis of PBMC transcriptome in a porcine model of D-galactosamine- (D-gal-) induced ALF to identify candidate genes and gene sets that play important roles in the progression of ALF.

2. Materials and Methods

2.1. Porcine Model of D-gal-Induced ALF. A D-gal-induced ALF porcine model was used as previously described by our group [11]. Briefly, male Bama experimental miniature pigs were used and 1.3 g/kg body weight D-gal (Hanhong Chemical, Shanghai, China) was intravenously injected to induce ALF. Blood samples were collected at baseline (time zero) and 36 and 60 h after D-gal injection. Pigs were sacrificed after blood sample collection at 60 h. The general medical condition of the experimental pigs was monitored throughout the experiment.

All animal experiments were conducted in the Department of Experimental Animals, Zhejiang Academy of Traditional Chinese Medicine, China, and approved by the Animal Care Ethics Committee of the Academy. All experimental animals were treated humanely.

2.2. Clinical Parameters following D-gal-Induced Porcine ALF. At 0, 36, and 60 h, parameters to quantify the severity of liver failure were collected including the international normalization ratio (INR), and alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transpeptidase, total bilirubin, and creatinine levels.

Blood ammonia was measured using an ammonia test kit (ARKRAY, Tokyo, Japan) with a detection range between 10 and 400 μg/dL. INR was quantified using STA-R (Diagnostic Stago, Asnieres, France) in the emergency laboratory at the First Affiliated Hospital, College of Medicine, Zhejiang University. Serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transpeptidase, total bilirubin, and creatinine levels were measured using an automated biochemical analyser (Abbott Aeroset; Abbott Laboratories, Chicago, IL, USA) in the same laboratory.

2.3. PBMC Isolation and RNA Extraction. PBMCs were isolated using Ficoll-Histopaque (Sigma Aldrich, St. Louis, MO, USA) immediately after blood sample collection. Subsequently, total RNA was extracted using RNeasy Mini kits (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. All RNA samples were stored at −80°C for future analysis.

2.4. mRNA Library Construction, RNA-Sequencing, and Data Analysis. Total RNA (1 μg) was thawed to create a library using TruSeq Stranded RNA LT Guide (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. An Agilent 2100 bioanalyser (Santa Clara, CA, USA) was used to evaluate the concentration and size distribution of complementary DNA (cDNA) in the library before sequencing with the Illumina HiSequation 2500 system. The high-throughput sequencing was performed according to the manufacturer's instructions (Illumina HiSequation 2500 User Guide).

The raw data were filtered by FASTX (ver. 0.0.13) before mapping to the genome using TopHat (ver. 2.0.9). Gene fragments were counted using HTSeq followed by trimmed mean of M values (TMM) normalization. Significantly differentially expressed genes (DEGs) were identified using Cufflinks (ver. 2.2.1) [12]. DEGs were then submitted to Visualisation and Integrated Discovery analysis (DAVID; ver. 6.8) [13] for gene ontology (GO) term enrichment and clustering and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway mapping using default parameters, except for an EASE score setting of 0.05.

2.5. Validation of RNA-Seq Data by qRT-PCR. Quantitative RT-PCR was performed on selected genes to validate the data obtained from mRNA sequencing. Briefly, total RNA was reverse-transcribed into cDNA using the Fast Quant RT kit (Tiangen, Beijing, China). All qRT-PCR was conducted using SYBR Green SuperReal PreMix Plus (FP205; Tiangen) on an ABI 7900HT (Applied Biosystems, Foster City, CA, USA). Experimental conditions included a 3-min cycle at 94°C followed by 40 cycles of 20 s at 94°C, 20 s at 58°C, and 20 s at 72°C.

Each qRT-PCR run was performed in triplicate with two biological replicates. Beta-2-microglobulin (B2M) was used as the reference gene for data normalization, as previously described. A correlation analysis of the fold change of selected genes between qRT-PCR and RNA-Seq was performed.

2.6. Statistical Analysis. RNA-seq data analyses were described previously in Section 2.4. Other statistical analyses were performed by Graphpad Prism (Version 5.0, GraphPad Software, San Diego, United States). Biochemical parameters in the progress of ALF were compared using Student's t-test. Linear regression was performed in validation of RNA-Seq data by qRT-PCR. A p value less than 0.05 was considered significant.

3. Results

3.1. Clinical Features and Biochemical Parameters of D-gal-Induced ALF in Pigs. All animals enrolled in this experiment were healthy, with a good appetite and response to the D-gal injection at time zero (baseline). The ALF model was successfully established in all the animals at 36 h (failure) after D-gal injection. The pigs stopped eating and became obviously restless, with yellow urine. At 60 h post-injection (dying), the pigs showed ataxia and symptoms of hepatic encephalopathy, with no reaction to painful stimuli.

The biochemical parameters as ALF progressed are listed in Table 1. Liver failure was identified by the progressive increase in liver enzymes, bilirubin, blood ammonia, and the international normalization ratio in both the failure and dying groups as compared to the baseline group. A deviation of bilirubin and liver enzymes, or elevated total bilirubin with decreased liver enzymes, was observed in the dying group but not in the failure group.

Table 1: Biochemical parameters in a porcine model of ALF.

Parameters	Baseline	Failure	Dying
International normalization ratio	0.9 ± 0.05	2.7 ± 0.2**	4.8 ± 0.8**
Ammonia (μg/dl)	22.3 ± 3.1	76.5 ± 8.7**	225.5 ± 47.4**
Alanine aminotransferase (U/L)	56.3 ± 8.0	311.5 ± 65.0*	230.3 ± 46.5*
Aspartate aminotransferase (U/L)	36.0 ± 3.3	5023.8 ± 1034.6*	1788.5 ± 263.6**
Alkaline phosphatase (U/L)	72.8 ± 16.9	232.8 ± 53.4*	564.0 ± 82.6**
γ-Glutamyl transpeptidase (U/L)	64.5 ± 9.6	77.0 ± 5.1	96.3 ± 5.0*
Total bilirubin (μmol/L)	2.3 ± 0.3	40.8 ± 5.7**	70.8 ± 7.6**
Creatinine (mmol/L)	58.0 ± 2.1	59.3 ± 6.4	49.5 ± 3.3

Data are means ± SEM. * $p < 0.05$, ** $p < 0.01$ versus baseline.

Table 2: Qualitative analysis of PBMC RNA-Seq data in a porcine model of ALF.

Sample name	Raw reads	Q20 value	Clean reads	Mapped reads	Genic reads	Percentage of genic reads	Expressed gene number
Baseline-1	80,876,352	94.80%	77,508,054	63,736,589	51,530,237	80.80%	15,249
Baseline-2	122,478,648	95.40%	117,994,584	64,288,782	47,627,719	74.10%	14,990
Baseline-3	97,633,918	95.10%	93,252,338	76,290,007	48,400,655	63.40%	15,470
Baseline-4	91,203,498	95.30%	87,519,976	70,759,871	54,612,207	77.20%	15,642
Dying-1	106,182,528	95.20%	101,728,266	83,553,821	57,816,273	69.20%	15,563
Dying-2	93,997,436	95.20%	90,503,598	74,471,193	60,365,900	81.10%	15,101
Dying-3	118,901,914	94.90%	91,408,790	73,952,809	57,686,330	78.00%	15,712
Dying-4	84,848,674	95.30%	81,623,206	67,004,468	55,869,637	83.40%	15,343
Failure-1	80,262,120	95.20%	76,885,638	63,014,127	49,808,658	79.00%	15,297
Failure-2	100,438,952	95.30%	100,291,397	82,963,827	68,021,072	82.00%	15,812
Failure-3	86,394,661	94.90%	66,295,638	54,080,154	38,129,055	70.50%	15,228
Failure-4	99,697,480	94.80%	95,927,678	79,312,204	60,637,999	76.50%	15,404

3.2. Statistical Analysis of PBMC Transcriptome Data. RNA-Seq was performed in a total of 12 samples, with 4 samples in each group (baseline, failure, and dying). More than 9 Gb sequence data was the yield in each sample. Overall, 80.3–122.5 million raw reads per sample were generated with the quality of over 94.8% Q20, in which 66.3–118.0 million were clean reads.

A total of 54.1–83.5 million reads were mapped to the porcine genome, in which 63.4–83.4% fell in genic regions while the remaining were in intergenic regions. 14,990 to 15,812 expressed genes were identified (fragments per kilobase of exon per million mapped reads [FPKM] > 0) in each sample, respectively. Detailed information is presented in Table 2.

3.3. Differential Expression of Genes Associated with the Progression of D-gal-Induced ALF. DEGs during progression of D-gal-induced ALF were identified using Cufflinks (ver. 2.2.1). Genes were identified as significantly different with a false discovery rate (FDR) when the adjusted p value was (<0.05) and a greater than twofold log change was evident. Compared to the baseline group, 816 DEGs (Supplementary Table 1) were identified in the failure group and 1,845 DEGs (Supplementary Table 2) were identified in the dying group. A total of 590 identified genes overlapped between the two groups. Details are presented in Figure 1.

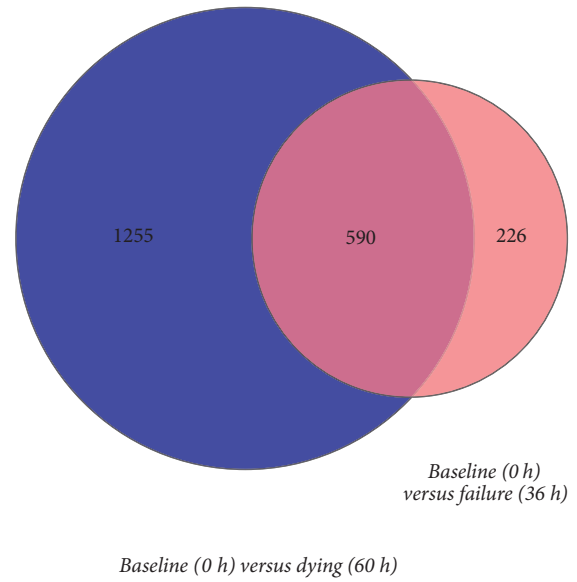

Figure 1: Differential expression of genes involved in the progression of acute liver failure (ALF).

3.4. Progression of D-gal-Induced ALF: GO Analysis. GO enrichment analysis and term clustering were performed to identify DEGs in the failure and dying groups as compared

to the baseline group. In total, 107 GO terms were enriched for DEGs identified in the failure group, of which 76 were within the biological process (BP) category, 15 were within the cellular component (CC) category, and 16 were within the molecular function (MF) category. Among these GO terms, 26 were grouped into five independent clusters. The GO terms in the five clusters were related to positive regulation of the inflammatory response, the inflammasome complex, the toll-like receptor (TLR) signalling pathway, cell chemotaxis, and semaphorin receptor activity. With the exception of predominantly innate immune-related terms, important GO terms related to cell death were also enriched for processes such as apoptosis and pyroptosis. GO terms related to autophagy, another type of programmed cell death, were also identified. These terms are the regulation of autophagy, phagocytic vesicles, and lysosomes. Terms related to the process of liver fibrosis included gene sets important in the negative regulation of the fibroblast growth factor receptor signalling pathway and semaphorin receptor activity, and so on. Moreover, GO terms related to coagulation dysfunction and hepatic encephalopathy were also enriched, such as blood coagulation, astrocyte development, and the semaphorin-plexin signalling pathway involved in axon guidance, branchiomotor neuron axon guidance, and so on.

In total, 154 GO terms were enriched for the DEGs identified in the dying group, which included 104 BP terms, 25 CC terms, and 25 MF terms. Overall, 20 out of 154 GO terms were included in two clusters. The representative GO terms in these clusters were related to regulation of the inflammatory response and the inflammasome complex. Most enriched GO terms were predominantly immune-related. Other important GO terms were related to cell death, including apoptotic processes, apoptotic-signalling pathways, negative regulation of apoptotic processes, pyroptosis, autophagy, lysosomal membrane, and lysosomal lumen. Fibrosis-related terms such as collagen catabolic processes and collagen binding were also enriched; hepatic encephalopathy-related GO term, astrocyte development, was also enriched.

Details of GO enrichment and clustering are presented in Figure 2.

3.5. KEGG Pathways Involved in the Progression of D-gal-Induced ALF. KEGG pathway mapping was used to study the molecular interactions and relation networks of the identified DEGs participating in metabolism, cellular processes and so on following D-gal-induced ALF. A total of seven KEGG pathways were mapped from DEGs identified in the failure group, all of which overlapped with the 19 identified KEGG pathways in the dying group. The seven KEGG pathways that were common to both included cytokine-cytokine receptor interaction, hematopoietic cell lineage, lysosome, rheumatoid arthritis, malaria, phagosome, and pertussis pathways. The remaining 12 KEGG pathways identified in the dying group were predominantly immune-related pathways, such as the NF-kappa B signalling pathway, the tumour necrosis factor (TNF) signalling pathway, and the complement and coagulation cascade pathways. KEGG pathways of diseases characterised by impaired liver function, such as Chagas disease (American trypanosomiasis), *Salmonella* infection, and Legionellosis, were also mapped using KEGG pathway mapping. Details are presented in Table 3.

3.6. Validation of RNA-Seq Data by qRT-PCR Analysis. To validate the RNA-Seq data, qRT-PCR of 12 selected genes was performed. The forward and reverse pairs of qRT-PCR primers for each gene are listed in Supplementary Table 3. Linear correlation analysis was conducted between the RNA-Seq and qRT-PCR results, which showed that the fold changes were significantly concordant between RNA-Seq and qRT-PCR data ($r = 0.95$, $p < 0.0001$). Results are shown in Figure 3 and Supplementary Table 4.

4. Discussion

ALF is a syndrome characterised by severe coagulopathy due to liver dysfunction and altered consciousness as a result of hepatic encephalopathy [3]. These features of ALF can be revealed at the PBMC level by transcriptome analysis, with the enriched GO term of blood coagulation and the mapped KEGG pathway of complement and coagulation cascades. Vemuganti et al. reported that, in association with hepatic encephalopathy, axon guidance micro-RNA levels changed in the cerebral cortex of a rat model of ALF [14]. In this study, three GO terms—branchiomotor neuron axon guidance, the semaphorin-plexin signalling pathway involved in axon guidance, and the cortical cytoskeleton—were identified. KEGG mapping analysis also identified disease-related KEGG pathways characterised by liver dysfunction, such as malaria, Chagas disease (American trypanosomiasis), *Salmonella* infection, and Legionellosis. The ability of PBMCs to migrate in a transendothelial manner and establish a dialogue between cells in solid organs has been reported previously [15–17]. These findings may explain the transcriptome changes observed in PBMCs that parallel the changes observed in solid organs, such as the liver and brain.

Previous studies have revealed extensive differential gene expression detected in the liver during the progression of ALF [18]. In this study, compared to the baseline group, the number of DEGs identified in the dying group was more extensive than in the failure group (1845 and 816 genes, resp.), which suggests that the cascades identified by PBMC transcriptome analysis change as ALF progresses. In addition, seven common KEGG pathways were identified for DEGs in both the failure and dying groups, which showed that the key pathways triggered by ALF result in further cascades at the transcriptome level.

Systemic inflammatory responses play an important role in the progression of ALF. Several key innate and adaptive immune mechanisms of ALF have been described previously, including acquired neutrophil dysfunction [19, 20], TLR function [21–23], and the important actions of chemokine and cytokine storms [24, 25]. All of these immune-related changes were identified in our GO enrichment and KEGG pathway mapping studies, which included genes involved in neutrophil chemotaxis, the TLR signalling pathway, and the TNF signalling pathway, among others.

Cell death plays an important role in ALF [26, 27]. Apoptosis is a form of hepatocyte death that contributes to

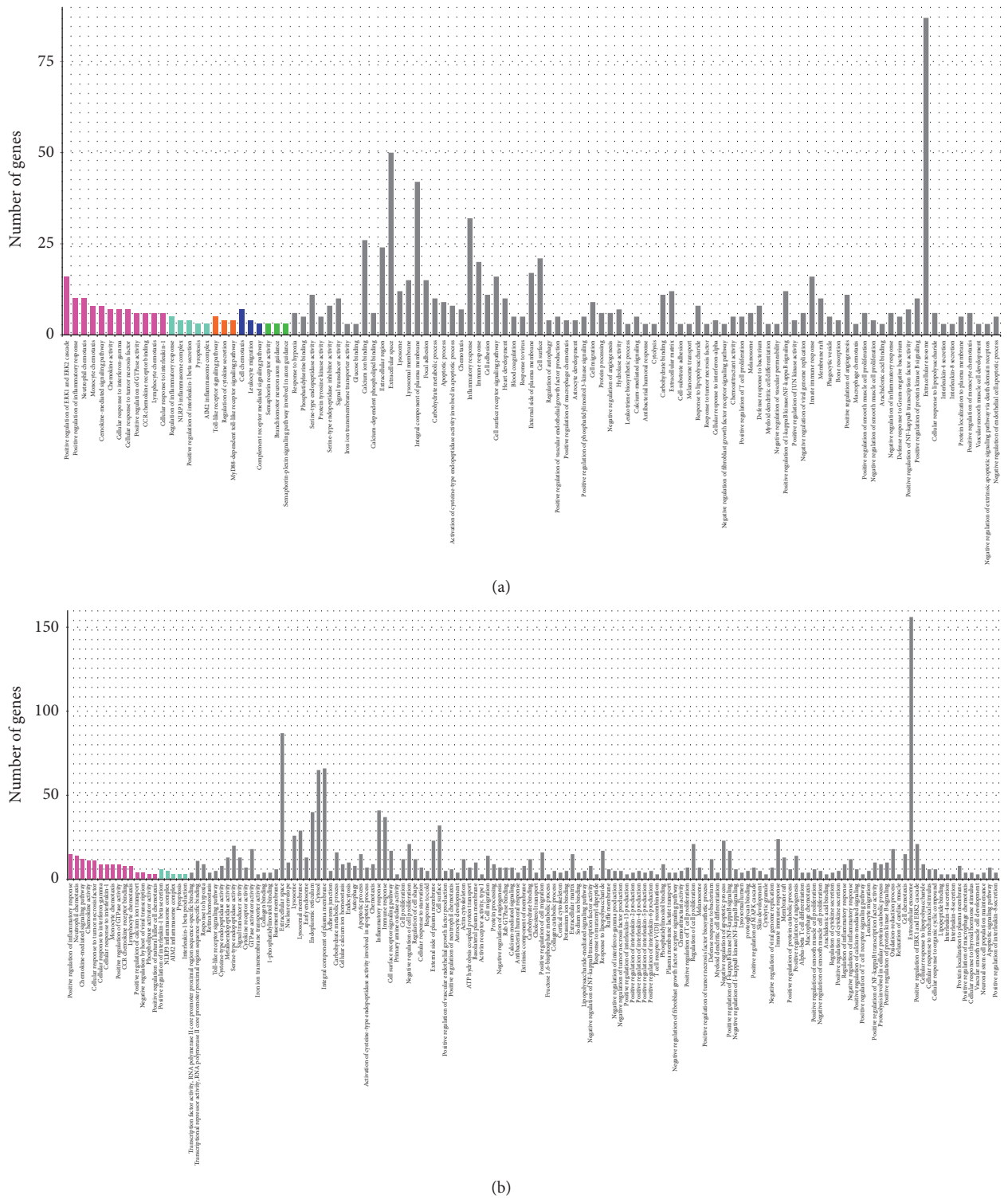

FIGURE 2: (a) Gene ontology (GO) terms of differentially expressed genes (DEGs) in the failure versus baseline group. (b) GO terms of DEGs in the dying versus baseline group.

ALF [28, 29]. Evidence of apoptotic pathways was identified in our transcriptome analysis. Two GO terms, the apoptotic process and apoptotic-signalling pathways, were enriched. Apart from apoptosis, recent research has focused on a new form of proinflammatory cell death known as pyroptosis [30]. Until now, studies on pyroptosis in ALF have been limited [31, 32]. Furthermore, to the best of our knowledge, a role for pyroptosis in drug-induced ALF has not been reported previously. The enrichment of pyroptosis GO terms following D-gal-induced ALF suggests that pyroptosis is an important

Table 3: KEGG pathways involved in the progression of ALF.

Name	Failure versus baseline			Dying versus baseline		
	Mapped genes	Fold enrichment	FDR adjusted p	Mapped genes	Fold enrichment	FDR adjusted p
Cytokine-cytokine receptor interaction	25	3.0	$4.3E-04$	38	2.5	$3.0E-05$
Hematopoietic cell lineage	13	4.4	$3.8E-03$	15	2.7	$2.1E-02$
Lysosome	14	3.2	$2.1E-02$	34	4.2	$2.9E-10$
Rheumatoid arthritis	12	3.7	$2.3E-02$	21	3.5	$7.0E-05$
Malaria	9	4.6	$2.6E-02$	14	3.9	$1.4E-03$
Phagosome	15	2.7	$3.9E-02$	26	2.5	$1.1E-03$
Pertussis	10	3.7	$4.4E-02$	20	3.9	$4.0E-05$
NF-kappa B signaling pathway				19	3.1	$1.3E-03$
Transcriptional misregulation in cancer				26	2.4	$1.6E-03$
Chagas disease (American trypanosomiasis)				20	2.7	$3.3E-03$
Leishmaniasis				14	3.3	$6.6E-03$
TNF signaling pathway				19	2.5	$8.5E-03$
Salmonella infection				16	2.8	$8.8E-03$
Complement and coagulation cascades				14	2.8	$2.1E-02$
Mineral absorption				10	3.4	$3.1E-02$
Osteoclast differentiation				20	2.2	$3.2E-02$
Legionellosis				12	2.8	$4.4E-02$
Pentose phosphate pathway				7	4.6	$4.6E-02$
Histidine metabolism				7	4.6	$4.6E-02$

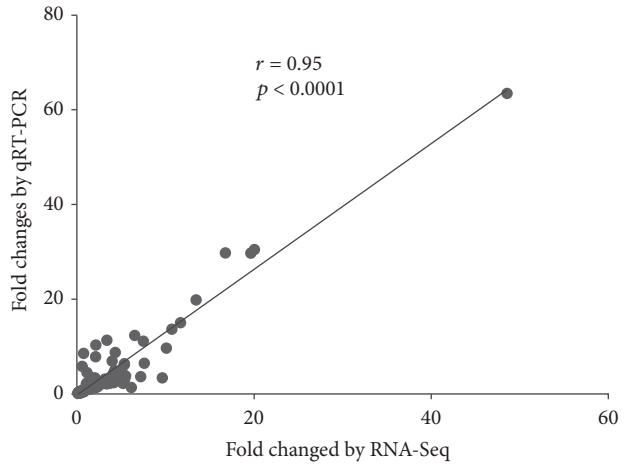

Figure 3: Correlation of gene fold changes between RNA-sequencing (RNA-Seq) and qRT-PCR analysis.

route to cell death in a model of drug-induced ALF and therefore merits further study. In addition to pyroptosis, necrapoptosis, also known as aponecrosis or apoptotic necrosis, is an important proinflammatory cell death pattern that shared common features and pathways with both apoptosis and necrosis [33, 34]. Also, this cell death pattern was found in liver injury [35, 36]. The necrapoptosis GO term or KEGG pathway was not enriched or mapped in this study. The possible reason might be that this cell demise pattern has not been annotated in databases of GO (http://geneontology.org/) and KEGG (http://www.kegg.jp/), for we cannot retrieve it in either of two databases so far. However, GO term, the adenosine triphosphate (ATP) hydrolysis coupled proton transport, was enriched in this study. ATP has been proved as a key factor to determine the way out of necrapoptosis [34]. This might verify from another aspect in transcriptome level that necrapoptosis is an important cell death pattern involved in the progression of ALF.

Autophagy is a lysosomal pathway tasked with the process of self-degradation of cellular components by the sequestration of these components in double-membrane autophagosomes [37]. It has been widely reported that autophagy plays an important role in cancer and other chronic diseases of the organs [38–42]. Autophagy is an important current research topic in models of liver disease [43–45]. However, currently there are limited data on the role of autophagy in the progression of ALF [46, 47]. In this study, GO terms such as autophagy, regulation of autophagy, and phagocytic-vehicle were all enriched. Two KEGG pathways of lysosomal and phagosome regulation were mapped. These results provide another potential avenue of transcriptome-level research on the influence of autophagy on the progression of ALF.

The role of fibrosis in the progression of chronic liver disease has been widely studied. Although fibrosis is observed in ALF [48], an understanding of the underlying mechanisms remains limited. GO terms related to the collagen-related component of liver fibrosis, such as collagen catabolic processes and collagen binding, were also enriched in this study. Semaphorin families are regulators of the progression of fibrosis in chronic liver diseases [49, 50]. However, the role of semaphorin families in ALF remains unknown. Semaphorin receptor activity GO terms were also found in this study, which constitutes another interesting avenue for research.

In conclusion, this study identified dramatic changes in the PBMC transcriptome predominantly related to immune responses in ALF. Enriched GO terms related to coagulation dysfunction, hepatic encephalopathy, and mapped KEGG pathways of diseases characterised by liver injury demonstrated that the PBMC transcriptome reflects the features of ALF. The enrichment of GO terms related to cell death and fibrosis indicates that PBMC transcriptome analysis is a useful method to elucidate potential key gene sets involved in ALF progression. Thus, a better understanding of the gene sets identified in this study may contribute to ALF prevention or treatment.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

YiMin Zhang and Li Shao contributed equally to this paper; LanJuan Li and YiMin Zhang designed the study; YiMin Zhang, Ning Zhou, JianZhou Li, Yu Chen, and Juan Lu performed experiments; Li Shao, ErMei Chen, Jie Wang, and ZhongYang Xie collected the data. YiMin Zhang and Li Shao analysed the data and wrote the paper.

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Supplementary Materials

Supplementary Table 1: list of differentially expressed genes identified in failure group; Supplementary Table 2: list of differentially expressed genes identified in dying group; Supplementary Table 3: product sizes and the primers used for qRT-PCR; Supplementary Table 4: gene fold change tested by qRT-PCR and RNA-Seq. *(Supplementary Materials)*

References

[1] J. Polson and W. M. Lee, "AASLD position paper: The management of acute liver failure," *Hepatology*, vol. 41, no. 5, pp. 1179–1197, 2005.

[2] S. L. Flamm, Y.-X. Yang, S. Singh et al., "American Gastroenterological Association Institute Guidelines for the Diagnosis and Management of Acute Liver Failure," *Gastroenterology*, vol. 152, no. 3, pp. 644–647, 2017.

[3] J. Wendon, J. Cordoba, A. Dhawan et al., "EASL clinical practical guidelines on the management of acute (fulminant) liver failure," *Journal of Hepatology*, vol. 66, no. 5, pp. 1047–1081, 2017.

[4] T. A. Skvortsov, D. V. Ignatov, K. B. Majorov, A. S. Apt, and T. L. Azhikina, "Mycobacterium tuberculosis transcriptome profiling in mice with genetically different susceptibility to tuberculosis," *Acta Naturae*, vol. 5, no. 17, pp. 62–69, 2013.

[5] H. Kalam, M. F. Fontana, and D. Kumar, "Alternate splicing of transcripts shape macrophage response to Mycobacterium tuberculosis infection," *PLoS Pathogens*, vol. 13, no. 3, Article ID e1006236, 2017.

[6] C. Rippe, B. Zhu, K. K. Krawczyk et al., "Hypertension reduces soluble guanylyl cyclase expression in the mouse aorta via the Notch signaling pathway," *Scientific Reports*, vol. 7, no. 1, article no. 1334, 2017.

[7] J. Chesne, R. Danger, K. Botturi et al., "Systematic analysis of blood cell transcriptome in EndStage chronic respiratory diseases," *PLoS ONE*, vol. 9, no. 10, Article ID e109291, 2014.

[8] S. Tattermusch, J. A. Skinner, D. Chaussabel et al., "Systems biology approaches reveal a specific interferon-inducible signature in HTLV-1 associated myelopathy," *PLoS Pathogens*, vol. 8, no. 1, Article ID e1002480, 2012.

[9] T. D. Querec, R. S. Akondy, E. K. Lee et al., "Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans," *Nature Immunology*, vol. 10, no. 1, pp. 116–125, 2009.

[10] J. S. Tsang, P. L. Schwartzberg, Y. Kotliarov et al., "Global analyses of human immune variation reveal baseline predictors of postvaccination responses," *Cell*, vol. 157, no. 2, pp. 499–513, 2014.

[11] N. Zhou, J. Li, Y. Zhang et al., "Efficacy of coupled low-volume plasma exchange with plasma filtration adsorption in treating pigs with acute liver failure: a randomised study," *Journal of Hepatology*, vol. 63, no. 2, pp. 378–387, 2015.

[12] C. Trapnell, A. Roberts, L. Goff et al., "Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks," *Nature Protocols*, vol. 7, no. 3, pp. 562–578, 2012.

[13] D. W. Huang, B. T. Sherman, and R. A. Lempicki, "Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources," *Nature Protocols*, vol. 4, no. 1, pp. 44–57, 2009.

[14] R. Vemuganti, V. R. Silva, S. L. Mehta, and A. S. Hazell, "Acute liver failure-induced hepatic encephalopathy is associated with changes in microRNA expression profiles in cerebral cortex of the rat," *Metabolic Brain Disease*, vol. 29, no. 4, pp. 891–899, 2014.

[15] R. B. Meeker, D. C. Bragg, W. Poulton, and L. Hudson, "Transmigration of macrophages across the choroid plexus epithelium in response to the feline immunodeficiency virus," *Cell and Tissue Research*, vol. 347, no. 2, pp. 443–455, 2012.

[16] Y. Huang, X.-Y. Zhu, M.-R. Du, and D.-J. Li, "Human trophoblasts recruited T lymphocytes and monocytes into decidua by secretion of chemokine CXCL16 and interaction with CXCR6 in the first-trimester pregnancy," *The Journal of Immunology*, vol. 180, no. 4, pp. 2367–2375, 2008.

[17] T. Zhang, L. Tian, G. Hu, K. Teng, and X. Mu, "Microvascular endothelial cells play potential immunoregulatory roles in the immune response to foot-and-mouth disease vaccines," *Cell Biochemistry & Function*, vol. 29, no. 5, pp. 394–399, 2011.

[18] F. Chen, H.-H. Zhu, L.-F. Zhou et al., "Genes related to the very early stage of ConA-induced fulminant hepatitis: A gene-chip-based study in a mouse model," *BMC Genomics*, vol. 11, no. 1, article no. 240, 2010.

[19] N. J. Taylor, A. Nishtala, G. K. Manakkat Vijay et al., "Circulating neutrophil dysfunction in acute liver failure," *Hepatology*, vol. 57, no. 3, pp. 1142–1152, 2013.

[20] P. E. Marques, S. S. Amaral, D. A. Pires et al., "Chemokines and mitochondrial products activate neutrophils to amplify organ injury during mouse acute liver failure," *Hepatology*, vol. 56, no. 5, pp. 1971–1982, 2012.

[21] S. Zhang, N. Yang, S. Ni et al., "Pretreatment of lipopolysaccharide (LPS) ameliorates D-GalN/LPS induced acute liver failure through TLR4 signaling pathway," *International Journal of Clinical and Experimental Pathology*, vol. 7, no. 10, pp. 6626–6634, 2014.

[22] N. Shah, M. Montes De Oca, M. Jover-Cobos et al., "Role of toll-like receptor 4 in mediating multiorgan dysfunction in mice with acetaminophen induced acute liver failure," *Liver Transplantation*, vol. 19, no. 7, pp. 751–761, 2013.

[23] R. Sehgal, S. Patra, P. David et al., "Impaired monocyte-macrophage functions and defective toll-like receptor signaling in hepatitis E virus-infected pregnant women with acute liver failure," *Hepatology*, vol. 62, no. 6, pp. 1683–1696, 2015.

[24] L. Collin, P. Moulin, M. Jungers, and A. P. Geubel, "Epstein-Barr virus (EBV)-induced liver failure in the absence of extensive liver-cell necrosis: A case for cytokine-induced liver dysfunction?" *Journal of Hepatology*, vol. 41, no. 1, pp. 174-175, 2004.

[25] J. Li, X. Zhu, F. Liu et al., "Cytokine and autoantibody patterns in acute liver failure," *Journal of Immunotoxicology*, vol. 7, no. 3, pp. 157–164, 2010.

[26] Z. Cao, F. Li, X. Xiang et al., "Circulating cell death biomarker: Good candidates of prognostic indicator for patients with hepatitis B virus related acute-on-chronic liver failure," *Scientific Reports*, vol. 5, Article ID 14240, 2015.

[27] H. Bantel and K. Schulze-Osthoff, "Mechanisms of cell death in acute liver failure," *Frontiers in Physiology*, vol. 3, article no. 79, 2012.

[28] J. An, C. Harms, G. Lättig-Tünnemann et al., "TAT-apoptosis repressor with caspase recruitment domain protein transduction rescues mice from fulminant liver failure," *Hepatology*, vol. 56, no. 2, pp. 715–726, 2012.

[29] P. Zhang, M. Zhang, M. Wan et al., "Tamoxifen Attenuates Lipopolysaccharide/Galactosamine-induced acute liver failure by antagonizing hepatic inflammation and apoptosis," *Immunological Investigations*, vol. 46, no. 3, pp. 284–294, 2017.

[30] K. R. Bortoluci and R. Medzhitov, "Control of infection by pyroptosis and autophagy: Role of TLR and NLR," *Cellular and Molecular Life Sciences*, vol. 67, no. 10, pp. 1643–1651, 2010.

[31] Y. Geng, Q. Ma, Y.-N. Liu et al., "Heatstroke induces liver injury via IL-1β and HMGB1-induced pyroptosis," *Journal of Hepatology*, vol. 63, no. 3, article no. 5648, pp. 622–633, 2015.

[32] Y. L. Chen, G. Xu, X. Liang et al., "Inhibition of hepatic cells pyroptosis attenuates CLP-induced acute liver injury," *American journal of translational research*, vol. 8, no. 12, pp. 5685-95, 2016.

[33] L. Formigli, L. Papucci, A. Tani et al., "Aponecrosis: morphological and biochemical exploration of a syncretic process of cell death sharing apoptosis and necrosis," *Journal of Cellular Physiology*, vol. 182, no. 1, pp. 41–49, 2000.

[34] J. J. Lemasters, T. Qian, L. He et al., "Role of mitochondrial inner membrane permeabilization in necrotic cell death, apoptosis, and autophagy," *Antioxidants & Redox Signaling*, vol. 4, no. 5, pp. 769–781, 2002.

[35] A. M. El-Gibaly, C. Scheuer, M. D. Menger, and B. Vollmar, "Improvement of rat liver graft quality by pifithrin-α-mediated inhibition of hepatocyte necrapoptosis," *Hepatology*, vol. 39, no. 6, pp. 1553–1562, 2004.

[36] H. Jaeschke and J. J. Lemasters, "Apoptosis versus oncotic necrosis in hepatic ischemia/reperfusion injury," *Gastroenterology*, vol. 125, no. 4, pp. 1246–1257, 2003.

[37] C. He and D. J. Klionsky, "Regulation mechanisms and signaling pathways of autophagy," *Annual Review of Genetics*, vol. 43, pp. 67–93, 2009.

[38] Z. Zhong, E. Sanchez-Lopez, and M. Karin, "Autophagy, inflammation, and immunity: a troika governing cancer and its treatment," *Cell*, vol. 166, no. 2, pp. 288–298, 2016.

[39] J. Moscat and M. T. Diaz-Meco, "p62 at the crossroads of autophagy, apoptosis, and cancer," *Cell*, vol. 137, no. 6, pp. 1001–1004, 2009.

[40] Z. V. Wang, B. A. Rothermel, and J. A. Hill, "Autophagy in hypertensive heart disease," *The Journal of Biological Chemistry*, vol. 285, no. 12, pp. 8509–8514, 2010.

[41] T. Kimura, Y. Isaka, and T. Yoshimori, "Autophagy and kidney inflammation," *Autophagy*, vol. 13, no. 6, pp. 997–1003, 2017.

[42] K. Mizumura, S. Cloonan, and M. E. Choi, "Autophagy: Friend or Foe in Lung Disease?" *Annals of the American Thoracic Society*, vol. 13, 1, pp. S40–S47, 2016.

[43] X.-M. Yin, W.-X. Ding, and W. Gao, "Autophagy in the liver," *Hepatology*, vol. 47, no. 5, pp. 1773–1785, 2008.

[44] C. Zhang and A. M. Cuervo, "Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function," *Nature Medicine*, vol. 14, no. 9, pp. 959–965, 2008.

[45] P.-E. Rautou, A. Mansouri, D. Lebrec, F. Durand, D. Valla, and R. Moreau, "Autophagy in liver diseases," *Journal of Hepatology*, vol. 53, no. 6, pp. 1123–1134, 2010.

[46] F. Ren, L. Zhang, X. Zhang et al., "Inhibition of glycogen synthase kinase 3beta promotes autophagy to protect mice from acute liver failure mediated by peroxisome proliferator-activated receptor alpha," *Cell death & disease*, vol. 7, article e2151, 2016.

[47] L. Jin, H. Gao, J. Wang et al., "Role and regulation of autophagy and apoptosis by nitric oxide in hepatic stellate cells during acute liver failure," *Liver International*, vol. 37, no. 11, pp. 1651–1659, 2017.

[48] Y. He, L. Jin, J. Wang, Z. Yan, T. Chen, and Y. Zhao, "Mechanisms of fibrosis in acute liver failure," *Liver International*, vol. 35, no. 7, pp. 1877–1885, 2015.

[49] T. Yagai, A. Miyajima, and M. Tanaka, "Semaphorin 3E secreted by damaged hepatocytes regulates the sinusoidal regeneration and liver fibrosis during liver regeneration," *The American Journal of Pathology*, vol. 184, no. 8, pp. 2250–2259, 2014.

[50] S. De Minicis, C. Rychlicki, L. Agostinelli et al., "Semaphorin 7A contributes to TGF-β-mediated liver fibrogenesis," *The American Journal of Pathology*, vol. 183, no. 3, pp. 820–830, 2013.

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Research Article

Bacterial Infection and Predictors of Mortality in Patients with Autoimmune Liver Disease-Associated Acute-On-Chronic Liver Failure

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Objective. To date, few studies are available on autoimmune liver disease-associated acute-on-chronic liver failure (ACLF). The aim of this study is to investigate bacterial infection and predictors of mortality in these patients. *Methods*. We retrospectively studied patients with autoimmune liver disease from August 2012 to August 2017. Clinical data of the patients were retrieved for analysis. *Results*. There were 53 ACLF patients and 53 patients without ACLF in this study. The ACLF group had a higher prevalence of complications ($P < 0.05$). The 28-day and 90-day mortality rates were also obviously high in patients with ACLF (38.3% and 74.5%, resp.) ($P < 0.05$). No predictor was significantly associated with 28-day and 90-day transplant-free mortality. In 53ACLF patients, 40 (75.5%) patients showed bacterial infection. ACLF patients with bacterial infection showed high Child-Pugh score, MELD score, CLIF-SOFA score, 28-day mortality, and 90-day mortality ($P > 0.05$). Moreover, C-reactive protein (CRP) using 12.15 mg/L cut-off value proved to be more accurate than procalcitonin in identifying patients with infection. *Conclusions*. Autoimmune liver disease-associated ACLF showed more complications and high mortality. Bacterial infection patients displayed a more severe condition than those without infection. Elevated CRP is an accurate marker for diagnosing bacterial infection in autoimmune liver disease-associated ACLF patients.

1. Introduction

In recent years, acute-on-chronic liver failure (ACLF) as a specific clinical form of liver failure has attracted increasing attention. In fact, ACLF is considered a syndrome that occurs on the background of chronic liver disease, and previously diagnosed cirrhosis is not required, which is characterized by acute hepatic decompensation resulting in liver failure (jaundice and prolongation of the international normalized ratio [INR]) and one or more extrahepatic organ failures that are associated with increased mortality within a period of 28 days and up to three months from onset [1, 2]. Moreover, ACLF can rapidly progress, requiring an urgent need for assessment and referral for liver transplantation [3]. Therefore, recognition and intervention of the predictors of mortality in ACLF patients can prevent or reverse the process and improve the survival rate.

Several patients with ACLF have been recently reported [3–7]. Moreau et al. found that bacterial infection is the trigger of 33% ACLF and is the most commonly identifiable trigger of this syndrome [4]. Some studies considered hepatic encephalopathy, low-serum sodium, and high INR as predictors of poor outcome in ACLF patients [3, 5]. However, few studies about autoimmune liver disease-induced ACLF patients are available to date. Except for the known predictors, possible risk factors have also received less attention. Moreover, bacterial infection in the population is not largely known. The aim of this study is, therefore, to collect data

about autoimmune liver disease-associated ACLF patients to investigate bacterial infection and predictors of mortality to reduce the mortality in this population.

2. Materials and Methods

2.1. Study Population and Data Collection. We retrospectively analyzed the data of all patients admitted at the infectious disease wards of the First Affiliated Hospital, College of Medicine, Zhejiang University, China, and diagnosed with autoimmune liver disease-associated ACLF from August 2012 to August 2017. Patients with autoimmune liver disease who satisfied the 13th Asia-Pacific Congress of Clinical Microbiology and Infection (APCCMI) Consensus Guidelines for diagnosis and treatment of liver failure [8], but not diagnostic criteria of the European Association for the Study of the Liver (EASL) [4], were as control group (i.e., no ACLF group). All of the patients in our study had oral care after they admitted to hospital. Patients showing the following were excluded: coinfections with other viruses, including hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, and human immunodeficiency virus; concomitant liver diseases, such as Wilson's disease; coexisting liver cancer or extrahepatic malignancy; usage of hepatotoxic drugs. We used the composite of death or liver transplantation as our endpoint.

The research protocol was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University. The need for consent was waived because the study was retrospective and data were analyzed anonymously.

The following data were collected from hospital information system and medical documents: age, gender, diabetes, coexisting other autoimmune diseases, steroid exposure, laboratory findings, symptoms, the presence of bacterial infection at admission or during hospitalization, and mortality at 28 and 90 days. The chronic liver failure-sequential organ failure assessment (CLIF-SOFA) score, Child-Pugh score, and model for end-stage liver disease (MELD) score were calculated from the collected data.

2.2. Definitions. Diagnostic criteria of primary biliary cholangitis (PBC), autoimmune hepatitis (AIH), and primary sclerosing cholangitis (PSC) were defined by American Association for the Study of Liver Diseases (AASLD) [9–11]. Diagnosis of AIH-PBC overlap syndrome referenced the standard which was proposed by Chazouillères et al. in 1998 [12].

Diagnostic criteria and grades of ACLF were defined according to EASL definition [4], as follows.

ACLF Grade 1. This group includes 3 subgroups: (1) patients with single kidney failure, (2) patients with single failure of the liver, coagulation, circulation, or respiration who had a serum creatinine level ranging from 1.5 to 1.9 mg/dL and/or mild to moderate hepatic encephalopathy, and (3) patients with single cerebral failure who had a serum creatinine level ranging from 1.5 and 1.9 mg/dL.

ACLF Grade 2. This group includes the patients with 2 organ failures.

ACLF Grade 3. This group includes the patients with 3 organ failures or more.

The patients as control group satisfied the following criteria specified by the 13th APCCMI Consensus Guidelines for diagnosis and treatment of liver failure [8] as follows.

Patients with chronic liver diseases have acute or subacute deterioration of liver function. ACLF usually exhibits the following symptoms: (a) fatigue with gastrointestinal tract symptoms; (b) rapidly deepening jaundice, with total bilirubin 10 times higher than the upper limit of normal or a daily increase $\geq 17.1\,\mu$mol/L; (c) hemorrhagic tendency with INR ≥ 1.5 or prothrombin activity $\leq 40\%$ and other causes which have been excluded; (d) progressive reduction in liver size; and (e) hepatic encephalopathy occurrence.

Bacterial infection in parts of the body was defined as follows [13]. Bacterial pneumonia was defined as the association of clinical and radiological signs of lung infection observed in chest radiographs. Spontaneous bacterial peritonitis was diagnosed when ascites culture was positive or polymorphonuclear count was no less than 250 cells/μL in ascites, excluding other inflammations such as pancreatitis, peritoneal carcinosis, tuberculosis, and bloody ascites. Urinary tract infection was diagnosed using bacterial culture positive or urine leukocyte count >15 cells/high power field and $>10^6$ bacteria/μL. Fever and cellulitis associated with leukocytosis were used to diagnose skin and soft tissue infection. Septicemia was defined as clinical signs of infection and two consecutive blood cultures yielding the same organism, when a blood culture yielding an organism was considered as bacteremia. Patients considered for bacterial infections but without positive culture or evidence of organ involvement were considered as undetermined infection.

2.3. Statistical Analyses. Statistical analysis was performed using SPSS version 18.0 (SPSS, Chicago, IL, USA). Categorical variables were expressed in percentages and frequencies. Continuous variables were expressed as means and standard deviation. Continuous variables were analyzed with independent-sample t-test when they in line with the normal distribution otherwise Mann–Whitney U test was used. The chi-square test was performed to analyze categorical variables. The 90-day mortality prediction was carried out with univariate and multivariate logistic regression. P value < 0.05 was considered statistically significant.

3. Result

3.1. Characteristics of the Study Cohort. During the study period, 53 patients with autoimmune liver disease-associated ACLF who were admitted to the infectious disease ward of our hospital were included in this study. Fifty-three patients were included as control group. The clinical features and laboratory results of the ACLF patients and no ACLF patients are shown in Table 1. From these 53 ACLF patients, 30 patients (56.6%) showed PBC, 21 (39.6%) displayed AIH, 1 (1.9%) had PSC, and one (1.9%) patients had AIH-PBC overlap

Table 1: Comparison of clinical features and laboratory results among the overall study collective.

Characteristics	No ACLF (n = 53)	ACLF (n = 53)	P value	ACLF		P value
				Infection group (n = 40)	Noninfection group (n = 13)	
Age	56.34 ± 11.64	58.23 ± 11.86	0.410	58.88 ± 13.18	56.23 ± 6.29	0.336
Female	46 (86.8%)	47 (88.7%)	0.767	34 (85.0%)	13 (100%)	0.317
Diabetes mellitus	9 (17.0%)	6 (11.3%)	0.403	5 (12.5%)	1 (7.7%)	0.635
Coexisting other autoimmune diseases	7 (13.2%)	7 (13.2%)	1.000	4 (10.0%)	3 (23.1%)	0.460
Steroid exposure	8 (15.1%)	11 (20.8%)	0.447	7 (17.5%)	4 (30.8%)	0.528
Complications						
Ascites	25 (47.2%)	41 (77.4%)	0.001	32 (80%)	9 (69.2%)	0.710
Gastrointestinal bleeding	3 (5.7%)	12 (14.0%)	0.026	8 (20%)	4 (30.8%)	0.671
Hepatic encephalopathy	2 (3.8%)	29 (54.7%)	≤0.001	21 (52.5%)	8 (61.5%)	0.570
Hepatorenal syndrome	2 (3.8%)	16 (30.2%)	0.001	15 (37.5%)	1 (7.7%)	0.092
Bacterial infection	27 (50.9%)	40 (75.5%)	0.005			
Laboratory data						
Leukocyte counts (×10 ∗ 9/L)	6.32 ± 3.93	10.43 ± 6.40	≤0.001	12.20 ± 6.34	4.97 ± 2.01	≤0.001
Hemoglobin (g/L)	99.06 ± 21.09	89.32 ± 19.51	0.015	86.30 ± 19.91	98.62 ± 15.39	0.047
Platelet (×10 ∗ 9/L)	108.83 ± 65.32	87.25 ± 52.30	0.063	89.70 ± 56.43	79.69 ± 37.68	0.472
CRP (mg/L)	19.25 ± 15.71	25.51 ± 19.05	0.071	30.49 ± 18.83	8.89 ± 5.93	≤0.001
PCT (ng/ml)	0.43 ± 0.44	1.25 ± 1.65	0.007	1.44 ± 1.76	0.33 ± 0.14	0.136
ALT (U/L)	227.06 ± 302.30	258.34 ± 331.79	0.613	177.15 ± 210.97	508.15 ± 492.74	0.034
AST (U/L)	288.42 ± 352.18	310.58 ± 290.45	0.724	270.48 ± 244.16	434.00 ± 387.24	0.172
AKP (U/L)	197.77 ± 105.19	1879.09 ± 104.54	0.361	176.05 ± 100.90	188.46 ± 118.91	0.714
GGT (U/L)	185.15 ± 199.38	143.72 ± 165.20	0.247	161.15 ± 183.58	90.08 ± 67.81	0.045
Total bilirubin (mg/dL)	20.44 ± 8.21	26.68 ± 8.20	0.005	26.16 ± 8.49	21.37 ± 6.21	0.067
Albumin (g/L)	27.53 ± 5.98	26.71 ± 3.30	0.372	26.59 ± 3.61	26.97 ± 2.22	0.723
Creatinine (mg/dL)	0.65 ± 0.19	1.22 ± 0.88	≤0.001	1.39 ± 0.91	0.72 ± 0.56	0.004
Fasting blood glucose (mmol/L)	4.68 ± 1.52	3.99 ± 1.99	0.119	3.80 ± 0.90	4.57 ± 3.74	0.471
Serum sodium (mmol/L)	136.60 ± 5.12	133.81 ± 5.59	0.009	133.03 ± 5.88	136.23 ± 3.79	0.072
INR	1.76 ± 0.29	3.04 ± 1.23	≤0.001	3.33 ± 1.54	2.95 ± 1.11	0.328
Child-Pugh score	9.53 ± 1.19	11.79 ± 1.46	≤0.001	11.06 ± 1.49	10.47 ± 1.85	0.055
MELD score	18.72 ± 3.15	29.60 ± 8.72	≤0.001	29.47 ± 7.06	27.37 ± 5.35	0.109
CLIF-SOFA score	7.43 ± 0.77	10.74 ± 2.22	≤0.001	11.03 ± 2.38	10.23 ± 1.54	0.266
ACLF grade						
Grade 1	42 (79.2%)	6 (11.3%)	0.186	4 (20.0%)	2 (15.4%)	0.678
Grade 2	0	30 (56.6%)	≤0.001	22 (55.0%)	8 (61.5%)	0.003
Grade 3	0	17 (32.1%)	≤0.001	14 (35.0%)	3 (23.1%)	0.008
28-day transplant-free mortality	4 (8.9%)	18 (38.3%)	0.001	15 (39.5%)	3 (33.3%)	0.733
90-day transplant-free mortality	6 (13.3%)	35 (74.5%)	≤0.001	27 (71.1%)	5 (55.6%)	0.618

CRP, C-reactive protein; PCT, procalcitonin; ALT, alanine transaminase; AST, aspartate aminotransferase; AKP, alkaline phosphatase; GGT, gamma glutamyl transpeptidase; INR, international normalized ratio; MELD, model for end-stage liver disease; CLIF-SOFA, chronic liver failure-sequential organ failure assessment.

Table 2: Significant univariate and multivariate logistic regression analyses of 28-day transplant-free mortality.

Variable	Univariate			Multivariate		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Gastrointestinal bleeding	5.000	1.225–20.409	0.025	3.406	0.687–16.894	0.134
Hepatic encephalopathy	3.683	1.036–13.100	0.044	2.349	0.461–11.972	0.304
CLIF-SOFA score	1.362	1.023–1.812	0.034	1.093	0.741–1.613	0.653

CLIF-SOFA, chronic liver failure-sequential organ failure assessment.

Table 3: Significant univariate and multivariate logistic regression analyses of 90-day transplant-free mortality.

Variable	Univariate			Multivariate		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Hepatic encephalopathy	8.800	2.024–38.253	0.004	36.714	0.085–15810.528	0.244
Leukocyte counts	1.160	1.003–1.342	0.046	1.328	0.962–1.832	0.085
Hemoglobin	0.964	0.930–1.000	0.048	0.912	0.808–1.029	0.135
AST	0.997	0.995–1.000	0.021	0.998	0.992–1.004	0.477
Creatinine	5.426	1.362–21.612	0.016	160.487	0.049–527959.376	0.219
INR	2.935	1.192–7.224	0.019	50.782	0.415–6206.850	0.109
MELD score	1.232	1.070–1.418	0.004	0.664	0.309–1.430	0.295
Child-Pugh score	2.003	1.169–3.430	0.011	0.595	0.114–2.457	0.473
CLIF-SOFA score	2.936	1.532–5.630	0.001	1.578	0.350–7.117	0.553

AST, aspartate aminotransferase; INR, international normalized ratio; MELD, model for end stage liver disease; CLIF-SOFA, chronic liver failure-sequential organ failure assessment.

syndrome. The control group included 26 (49.1%) patients of PBC, 24 (45.3%) patients of AIH, and 3 (5.7%) cases of PSC. Compared to patients without ACLF, the ACLF group had a higher prevalence of complications ($P < 0.05$), such as hepatic encephalopathy (54.7% and 3.8%, $P \leq 0.001$) and bacterial infection (75.5% and 50.9%, $P = 0.005$). The 28-day and 90-day mortality rates were also obviously high in patients with ACLF ($P < 0.05$).

3.2. Risk Factors Associated with 28-Day and 90-Day Mortality. In the study, 6 (11.3%) patients underwent liver transplantation. In the remaining 47 patients, 28-day mortality and 90-day mortality were 38.3% (18) and 74.5% (35), respectively. For the 28-day transplant-free mortality, in univariate analysis, gastrointestinal bleeding ($P = 0.025$), hepatic encephalopathy ($P = 0.044$), and CLIF-SOFA score ($P = 0.034$) were significant factors (Table 2). However, in multivariate analysis, there was no predictor associated with increased mortality. In addition, for the 90-day transplant-free mortality, in univariate analysis, hepatic encephalopathy ($P = 0.004$), leukocyte counts ($P = 0.046$), hemoglobin ($P = 0.048$), aspartate aminotransferase (AST) ($P = 0.021$), serum creatinine ($P = 0.016$), serum sodium ($P = 0.017$), INR ($P = 0.019$), MELD score ($P = 0.004$), Child-Pugh score ($P = 0.011$), and CLIF-SOFA score ($P = 0.001$) were significant factors (Table 3). In multivariate logistic regression analysis, no predictor was significantly associated with 90-day transplant-free mortality (Table 3).

3.3. Clinical Features of Bacterial Infection in Patients with Autoimmune Liver Disease-Associated ACLF. In our study, 40 (75.5%) patients had bacterial infection, and 14 (35.0%) patients were diagnosed with bacterial coinfection in the first 72 h of admission. The demographic and clinical characteristics of ACLF patients with and without bacterial infection are detailed in Table 1.

ACLF patients with bacterial infection showed high Child-Pugh score, MELD score, CLIF-SOFA score, 28- day mortality, and 90-day mortality. Meanwhile, no statistical significance was observed ($P > 0.05$). However, in laboratory findings, bacterial infection patients displayed high leukocyte counts and C-reactive protein (CRP) ($P < 0.05$). High serum levels of serum creatinine and gamma glutamyl transpeptidase (GGT), low levels of hemoglobin, and alanine transaminase (ALT) were also observed in bacterial infection patients ($P < 0.05$) (Table 1).

In 40 ACLF patients with bacterial infection, the most common site of bacterial infection was in the respiratory tract (17, 42.5%), followed by the peritoneum, bloodstream, biliary tract, urinary tract, intestinal tract, skin and soft tissue, and undetermined site with percentage values of 22.5% (9), 7.5% (3), 7.5% (3), 5.0% (2), 5.0% (2), 2.5% (1), and 7.5% (3), respectively. The bacteriological evidence of infection was 7 cases (17.5%). The pathogens that caused bacterial infections were *Escherichia coli* in three cases, two of which produced extended spectrum β lactamase (ESBL); *Klebsiella pneumoniae* in two cases; *Staphylococcus aureus* in two cases, one of which involved methicillin-resistant *S. aureus*; and *Enterococcus faecalis* in one case.

3.4. Comparison of CRP and PCT in ACLF Patients with Bacterial Infection. CRP and PCT levels were obtained in 52 and 37 patients, respectively, among 53 patients. Thirty-seven patients had both biomarkers measured simultaneously.

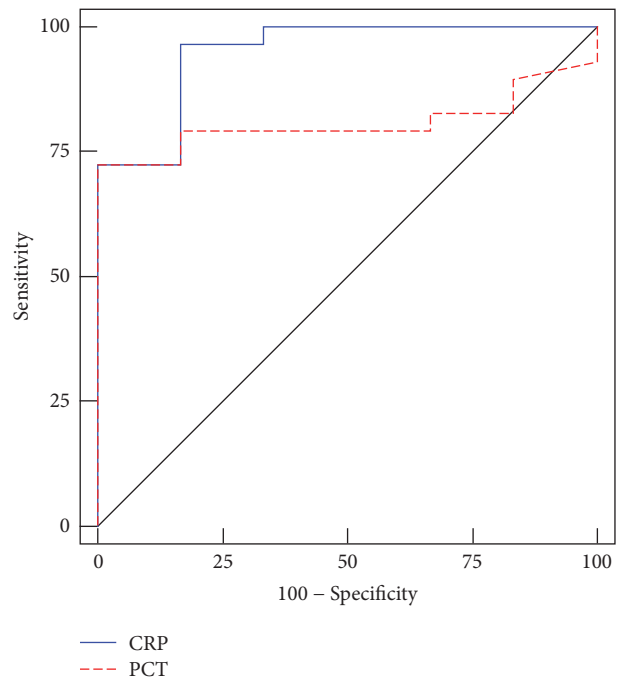

Figure 1: Using receiver operating characteristic analysis, comparison of C-reactive protein (CRP) and procalcitonin (PCT) in acute-on-chronic liver failure patients with bacterial infection.

Using receiver operating characteristic analysis, area under curve (AUC) to diagnose bacterial infection was 0.948 (95% confidence interval [CI]: 0–1.000) for CRP ($P = 0.001$) compared with 0.807 (95% CI: 0.668–0.947) for PCT ($P = 0.019$) (Figure 1). For diagnosis of bacterial infection in CRP at a cut-off value of 12.15 mg/L, the sensitivity and specificity were 96.6% and 83.3%, respectively. For diagnosis of bacterial infection in PCT at a cut-off value of 0.57 ng/mL, the sensitivity and specificity were 72.4% and 100% respectively.

4. Discussion

ACLF is a hotspot issues; however, it is lack of uniform diagnostic criteria. Different diagnostic criteria could lead to different patient prognosis. In our study, ACLF patients satisfied the EASL definition, and patients who met APCCMI definition but not EASL definition were included as control group. The 28-day transplantation-free mortality of autoimmune liver disease-associated ACLF was 38.3%, and 90-day transplantation-free mortality was 74.5%. Of note, a great number of critically ill patients were included in the ACLF group; ACLF grades 2 and 3 (56.6% and 36.1%, resp.) were dominant. Moreover, similar to the CANONIC study [4], the ACLF group had a higher prevalence of complications and higher 28-day and 90-day mortality rates.

Several studies were available about predictors of mortality in ACLF patients [5, 14–19]. Yu et al. [14] found that age, etiology, serum sodium, and ascites are independently associated with mortality. Cárdenas et al. [5] considered that the presence of hyponatremia is an independent predictive factor of survival in patients with ACLF. In Mücke's study, infection-triggered ACLF was considered as an independent predictors associated with increased mortality [19]. In our study, no predictor was significantly associated with 28-day and 90-day transplant-free mortality. Small size of study population may cause the situation.

In this study, among the 53 patients with autoimmune liver disease-associated ACLF, 40 (75.5%) patients had bacterial infection. Bacterial infection in liver disease had been reported by previous documents, and the incidence was 24.4%–90% [13, 20–23]. However, study populations were different. Although bacterial infection in ACLF had been studied, autoimmune liver disease-associated ACLF received minimal attention.

In bacterial infection patients with ACLF, Child-Pugh score, MELD score, CLIF-SOFA score, 28-day mortality, and 90-day mortality were high in bacterial infection patients although no statistical significance was observed ($P > 0.05$). ACLF patients with bacterial infection showed a more severe condition than those without infection; this finding was consistent with that of previous study [13].

In recent years, serum CRP and PCT have been suggested for diagnosis and prediction of bacterial infection in chronic liver disease, with or without cirrhosis [13, 24–26]. Papp et al. [25] reported that CRP using a 10 mg/L cut-off value proved to be more accurate than PCT in identifying patients with infection (AUC: 0.93). In this study, we also revealed that CRP levels are more effective than PCT in the diagnosis of bacterial infection in autoimmune liver disease-associated ACLF. The optimal cut-off value of CRP for bacterial infection diagnosis is 12.15 mg/L (96.6% sensitivity and 83.3% specificity), with an AUC of 0.948, which is similar to previous report [25].

Several limitations were observed in this study. First, this work was a retrospective study at a single center. Some data collections were limited, and bacterial infection might be affected by specific circumstance of our institution. Second, some patients had been treated by antibacterial agents before admission, which might affect the accuracy of bacterial infection rate. Third, immunosuppressor except corticosteroid was not used in our study population. Thus, further studies are needed to investigate bacterial infection and predictors of mortality in autoimmune liver disease-associated ACLF patients using immunosuppressor.

5. Conclusions

In conclusion, autoimmune liver disease-associated ACLF displayed high mortality and had more complications. ACLF patients with bacterial infection showed a more severe condition than those without infection. CRP levels higher than 12.15 mg/L suggested bacterial infection in autoimmune liver disease-associated ACLF patients. No predictor was significantly associated with 28-day and 90-day transplant-free mortality. Further prospective and intervention studies on bacterial infection and predictors of mortality in autoimmune liver disease-associated ACLF patients with large sample numbers are thus needed.

Abbreviations

ACLF: Acute-on-chronic liver failure
PBC: Primary biliary cholangitis
AIH: Autoimmune hepatitis
PSC: Primary sclerosing cholangitis
CLIF-SOFA: Chronic liver failure-sequential organ failure assessment
APPCCMI: Asia-Pacific Congress of Clinical Microbiology and Infection
EASL: European Association for the Study of the Liver
CRP: C-reactive protein
PCT: Procalcitonin
GGT: Gamma glutamyl transpeptidase
ALT: Alanine transaminase
INR: International normalized ratio
MELD: End-stage liver disease
AUC: Area under curve
CI: Confidence interval.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xuan Zhang and Ping Chen contribute equally to the study.

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References

[1] R. Jalan, P. Gines, J. C. Olson et al., "Acute-on chronic liver failure," *Journal of Hepatology*, vol. 57, no. 6, pp. 1336–1348, 2012.

[2] R. Jalan, C. Yurdaydin, J. S. Bajaj et al., "Toward an improved definition of acute-on-chronic liver failure," *Gastroenterology*, vol. 147, no. 1, pp. 4–10, 2014.

[3] H. Garg, A. Kumar, V. Garg, P. Sharma, B. C. Sharma, and S. K. Sarin, "Clinical profile and predictors of mortality in patients of acute-on-chronic liver failure," *Digestive and Liver Disease*, vol. 44, no. 2, pp. 166–171, 2012.

[4] R. Moreau, R. Jalan, P. Gines et al., "Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis," *Gastroenterology*, vol. 144, no. 7, pp. 1426.e9–1437.e9, 2013.

[5] A. Cárdenas, E. Solà, E. Rodríguez et al., "Hyponatremia influences the outcome of patients with acute-on-chronic liver failure: an analysis of the CANONIC study," *Critical Care (London, England)*, vol. 18, no. 6, p. 700, 2014.

[6] R. Jalan, F. Saliba, M. Pavesi et al., "Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure," *Journal of Hepatology*, vol. 61, no. 5, pp. 1038–1047, 2014.

[7] Y. Shi, M. Zheng, and Y. Yang, "Increased delayed mortality in patients with acute-on-chronic liver failure who have prior decompensation," *Journal of Gastroenterology Hepatology*, vol. 30, no. 4, pp. 712–718, 2015.

[8] L.-J. Li, "13th Asia-Pacific congress of clinical microbiology and infection consensus guidelines for diagnosis and treatment of liver failure," *Hepatobiliary & Pancreatic Diseases International*, vol. 12, no. 4, pp. 346–354, 2013.

[9] E. J. Heathcote, "Management of primary biliary cirrhosis. The American Association for the Study of Liver Diseases practice guidelines," *Hepatology*, vol. 31, no. 4, pp. 1005–1013, 2000.

[10] M. P. Manns, A. J. Czaja, J. D. Gorham et al., "Diagnosis and management of autoimmune hepatitis," *Hepatology*, vol. 51, no. 6, pp. 2193–2213, 2010.

[11] R. Chapman, J. Fevery, A. Kalloo et al., "Diagnosis and management of primary sclerosing cholangitis," *Hepatology*, vol. 51, no. 2, pp. 660–678, 2010.

[12] O. Chazouillères, D. Wendum, L. Serfaty, S. Montembault, O. Rosmorduc, and R. Poupon, "Primary biliary cirrhosis-autoimmune hepatitis overlap syndrome: clinical features and response to therapy," *Hepatology*, vol. 28, no. 2, pp. 296–301, 1998.

[13] J. K. Park, C. H. Lee, I. H. Kim et al., "Clinical characteristics and prognostic impact of bacterial infection in hospitalized patients with alcoholic liver disease," *Journal of Korean Medical Science*, vol. 30, no. 5, pp. 598–605, 2015.

[14] J.-W. Yu, G.-Q. Wang, and S.-C. Li, "Prediction of the prognosis in patients with acute-on-chronic hepatitis using the MELD scoring system," *Journal of Gastroenterology and Hepatology*, vol. 21, no. 10, pp. 1519–1524, 2006.

[15] S. Alam, B. Lal, V. Sood, and et al., "Pediatric Acute-on-chronic Liver Failure in a Specialized Liver Unit: Prevalence, Profile, Outcome and Predictive Factors," *Journal of Pediatric Gastroenterology & Nutrition*, vol. 63, no. 4, pp. 400–405, 2016.

[16] C. Dominguez, E. Romero, J. Graciano, J. L. Fernandez, and L. Viola, "Prevalence and risk factors of acute-on-chronic liver failure in a single center from Argentina," *World Journal of Hepatology*, vol. 8, no. 34, pp. 1529–1534, 2016.

[17] T. Bruns, R. Nuraldeen, M. Mai et al., "Low serum transferrin correlates with acute-on-chronic organ failure and indicates short-term mortality in decompensated cirrhosis," *Liver International*, vol. 37, no. 2, pp. 232–241, 2017.

[18] K. A. Wlodzimirow, S. Eslami, A. Abu-Hanna, M. Nieuwoudt, and R. A. F. M. Chamuleau, "A systematic review on prognostic indicators of acute on chronic liver failure and their predictive value for mortality," *Liver International*, vol. 33, no. 1, pp. 40–52, 2013.

[19] M. M. Mücke, T. Rumyantseva, V. T. Mücke et al., "Bacterial infection-triggered acute-on-chronic liver failure is associated with increased mortality," *Liver International*, 2017.

[20] N. Rolando, F. Harvey, J. Brahm et al., "Prospective study of bacterial infection in acute liver failure: An analysis of fifty patients," *Hepatology*, vol. 11, no. 1, pp. 49–53, 1990.

[21] N. Rolando, J. Philpott-Howard, and R. Williams, "Bacterial and fungal infection in acute liver failure," *Seminars in Liver Disease*, vol. 16, no. 4, pp. 389–402, 1996.

[22] A. D. Zider, R. Zopey, R. Garg, X. Wang, T. S. Wang, and J. C. Deng, "Prognostic significance of infections in critically ill adult patients with acute liver injury: a retrospective cohort study," *Liver International*, vol. 36, no. 8, pp. 1143–1150, 2016.

[23] R. Moreau and V. Arroyo, "Acute-on-Chronic Liver Failure: A New Clinical Entity," *Clinical Gastroenterology and Hepatology*, vol. 13, no. 5, pp. 836–841, 2015.

[24] A. Tsiakalos, A. Karatzaferis, P. Ziakas, and G. Hatzis, "Acute-phase proteins as indicators of bacterial infection in patients with cirrhosis," *Liver International*, vol. 29, no. 10, pp. 1538–1542, 2009.

[25] M. Papp, Z. Vitalis, I. Altorjay et al., "Acute phase proteins in the diagnosis and prediction of cirrhosis associated bacterial infections," *Liver International*, vol. 32, no. 4, pp. 603–611, 2012.

[26] C. Lazzarotto, M. F. Ronsoni, L. Fayad et al., "Acute phase proteins for the diagnosis of bacterial infection and prediction of mortality in acute complications of cirrhosis.," *Annals of Hepatology*, vol. 12, no. 4, pp. 599–607, 2013.