Received: 5 August 2022 | Revised: 23 October 2022 | Accepted: 27 October 2022

DOI: 10.1002/jcla.24766

RESEARCH ARTICLE

WILEY

Lower level of IL-28A as a predictive index of the artificial liver support system in effective treatment of patients with HBV-ACLF

Yandi Huang[1,2,3] | Tao Ju[4] | Huafen Zhang[4] | Dan Cao[4] | Xuefen Li[1,2,3] | Jiezuan Yang[4] | Dong Yan[4]

[1]Department of Laboratory Medicine, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

[2]Key Laboratory of Clinical In Vitro Diagnostic Techniques of Zhejiang Province, Hangzhou, China

[3]Institute of Laboratory Medicine, Zhejiang University, Hangzhou, China

[4]State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, Zhejiang University School of Medicine, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Hangzhou, China

Correspondence
Dong Yan and Jiezuan Yang, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, Zhejiang University School of Medicine, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Hangzhou, 310003, China.
Email: yandonh@zju.edu.cn and yangyan@zju.edu.cn

Funding information
National Natural Science Foundation of China, Grant/Award Number: 82072357; National Science and Technology Major Project, Grant/Award Number: 2018ZX10101-001

Abstract

Background: HBV-related acute-on-chronic liver failure (HBV-ACLF) is the most common type of liver failure with high mortality. Artificial liver support system (ALSS) is an important mean to reduce the mortality of HBV-ACLF but lacking index to assess its effectiveness. The cytokines are closely related to the prognosis of HBV-ACLF patients with ALSS treatment, however, which is not fully understood.

Methods: One hundred forty-two patients with HBV-ACLF and 25 healthy donors were enrolled. The cytokine profile of peripheral blood was determined in the patients before and after ALSS treatment, and their relationship with effectiveness of ALSS treatment in HBV-ACLF was analyzed.

Results: Serum IL-28A levels were markedly lower in ALSS-effective patients than those in non-effective patients pre-ALSS treatment. Similarly, serum IL-6 was significantly lower in ALSS-effective patients. Furthermore, for patients with effective treatment, serum IL-28A levels were positively related with IL-6 levels post-ALSS ($r = 0.2413$, $p = 0.0383$). The ROC curve analysis showed that serum levels of IL-28A (AUC = 0.6959 when alone or 0.8795 when combined with total bilirubin, platelet count and INR, both $p < 0.0001$) and IL-6 (AUC = 0.6704, $p = 0.0005$) were useful indices for separating effective from non-effective ALSS treatment of HBV-ACLF patients. Multivariate logistic regression analysis demonstrated that lower level of IL-28A was independently associated with higher effective rate of ALSS treatments.

Conclusions: Lower level of IL-28A is a predictive biomarker for ALSS in effective treatment of HBV-ACLF patients and IL-28A may be potential target for the treatment of HBV-ACLF.

KEYWORDS
artificial liver support system, cytokine, HBV-ACLF, IL-28A, IL-6

Yandi Huang and Tao Ju equally contributed to this work and share first authorship.

1 | INTRODUCTION

HBV-related acute-on-chronic liver failure (HBV-ACLF) is a life-threatening disease whose progress is closely associated with the persistence of chronic hepatitis B (CHB) and popular in the Asia-Pacific and African regions.[1] It has become one of the great challenges of human death in the world due to its danger and rapid progress after its onset; moreover, the death rate of ACLF can reach 72% if the patients could not be timely and effectively treated.[2] Although liver transplantation is an effective treatment of advanced HBV-ACLF, this mean is not always available because of the shortage of organ sources.[3] Artificial liver support system (ALSS) is an adjuvant treatment of ACLF, which could produce conditions assisting hepatocyte regeneration by the way of temporary and partial replacement of the diseased liver function. A recent case–control matching study demonstrated that ALSS was an important means to reduce the 28-day liver transplant-free mortality of HBV-ACLF.[4] Nevertheless, approximately 50% of HBV-ACLF patients who received ALSS still cannot benefit from it. The ineffective treatment of ALSS not only leads to a waste of social medical resources but also delays the patient with HBV-ACLF from receiving other treatments means. Therefore, it is critical to explore a biomarker which could predict the outcomes of HBV-ACLF patients treated by ALSS.

Cumulative studies suggested that the innate immune and adaptive immune response be involved in the pathogenesis of HBV-ACLF.[5–7] Systemic inflammation assessed by cytokines indicated there was acutely exacerbated in ACLF patients compared with that in non-ACLF patients with decompensated cirrhosis, moreover, the systemic inflammation assess was strongly related to the severity and short-term mortality of ACLF.[8] Additionally, the prolonged expression of inflammatory cytokines is a common accompaniment to more severe viral diseases.[9–11] And ALSS had a satisfactory therapeutic effect on cytokine storm and inflammation of COVID-19 patients by means of peripheral plasma exchange, hemofiltration.[12,13] Therefore, profiling cytokines would be a useful way in evaluating the treatment effectiveness of ALSS of patients with HBV-ACLF.

Interleukin-28A (IL-28A) as a member of type III interferon is of antiviral and anti-inflammatory potential.[14] While interleukin-6 (IL-6) is a pro-inflammatory cytokine and sustained high level in the serum of patients with HBV-ACLF[15] and is independently associated with the risk of mortality in patients with HBV-ACLF.[16] Both the two inflammatory-related cytokines are proved to be key regulators of liver injury.[17,18] However, to date, little is known about the kinetic profile and diagnostic value of serum IL-28A and IL-6 levels in patients with HBV-ACLF during ALSS treatment. In the present study, we dynamically measured the levels of serum IL-28A, IL-6, and other multiple cytokines, including interleukin-4 (IL-4), IL-8, IL-18, CD40 ligand (CD40L), CC chemokine ligand-2 (CCL2), CCL4, CCL20, chemokine C-X-C ligand-10 (CXCL-10), tumor necrosis factor-α (TNF-α), transforming growth factor-beta 1 (TGFβ1), and TGFβ2 in HBV-ACLF patients before and after ALSS treatment and analyzed the relationship between the cytokines and clinical variables, moreover, explored the biomarkers that could be used to predict the prognosis of ALSS.

2 | MATERIALS AND METHODS

2.1 | Subjects

From December 2018 to July 2019, we enrolled 142 patients who had been treated in the First Affiliated Hospital, Zhejiang University School of Medicine since they had suffered from HBV-ACLF; additionally, 25 healthy donors (HD) were also enrolled from the physical examination center of this hospital as control group. HBV-ACLF was diagnosed following the criteria of the Chinese Group on the Study of Severe Hepatitis B (COSSH).[2] Patients with the following situations were excluded from the study: age less than 18 or more than 80 years, suffering from hepatocellular carcinoma or any other type of cancer, having experience in organ transplantation, with human immunodeficiency virus or other virus infection, usage of immunosuppressant.

All the enrolled subjects received 2–3 courses ALSS treatment (plasma exchange combined with hemofiltration) during their hospitalization. The therapeutic effects of 90 days after the patients natively subjected to ALSS admission was obtained by electronic medical records or follow-up by telephone. HBV-ACLF patients with ALSS treatment were classified as effective and non-effective ALSS treatment group who died or underwent liver transplantation after ALSS treatment. The patients who had survived within 90 days after admission were classified as effective ALSS treatment group. The study protocol conformed to the principles of the Declaration of Helsinki and its approval number from the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine was 2017(674).

2.2 | Data and sample collection

Morning blood samples were obtained from the HBV-ACLF patients before the time when the patient received the first ALSS treatment and after the patients received the last ALSS treatment. The blood samples were left standing at room temperature for 30 min before they were centrifuged at 1000 × g for 15 min. The isolated serum was aliquoted and stored in −30°C for standby. Clinical biochemical indices and blood routine parameters were obtained through electronic medical records in our affiliation before ALSS treatment. Further, clinical parameters including age, sex, body mass index (BMI), hospitalization days, C-reactive protein, procalcitonin, erythrocyte sedimentation rate (ESR), alpha fetoprotein (AFP), endotoxin, HBV DNA load, and plasma ammonia were also recorded.

2.3 | Cytokines measurement

The levels of cytokines (IL-28A, IL-6, IL-4, IL-8, IL-18, CD40L, CCL2, CCL4, CCL20, CXCL-10, TNF-α, TGFβ1, and TGFβ2) were measured in a Luminex 200 System (Merck, Germany) using a Human multicytokine detection kit (R&D Systems, cat.no.LXSAHM-27 and FCSTM17-03). Cytokine determined and analyzed was performed in accordance with the manufacturer's guideline.

2.4 | Statistical analysis

Continuous variables were expressed as means ± standard deviations (SD) or medians ± interquartile ranges (IQR), according to the data were normal or non-normal distribution, alternatively. Comparison of non-normal distribution data used the Mann–Whitney non-parametric *U* test (two groups) or the Kruskal–Wallis test (more than two groups). Categorical data were presented as frequency (percentage) and evaluated using a chi-squared test or Fisher's exact test, as appropriate. Spearman correlation analysis was performed to identify correlation between IL-28A, IL-6 levels, and other clinical parameters. Multivariate analyses were performed to explore the association of clinical variables with effectiveness of ALSS treatment of HBV-ACLF patients. The predictive value of IL-28A and IL-6 levels of pre-ALSS was measured using receiver operating characteristics (ROC) analysis. All statistical analyses were two-tailed and carried out using SPSS version 24.0 (IBM Corp., Armonk, NY) and GraphPad Prism 9.0 (San Diego, CA, USA). Statistical significance was set up as a value of $p < 0.05$.

3 | RESULTS

3.1 | Baseline characteristics of the subject enrolled

The demographic and clinical characteristics of the enrolled HBV-ACLF patients were presented in Table 1. The majority enrolled patients were male (119/142, 83.8%), and their average age was 48 ± 13 years. All the HBV-ACLF patients subjected to 2–3 courses of ALSS treatment, finally, 81 survived subjects without liver transplant were classified as ALSS treatment effective group, while 61 subjects who had died or received liver transplant were classified as ALSS treatment non-effective group. In addition, there were no significant differences in age, sex, BMI, hospitalization days and AST, total protein, C-reactive protein, procalcitonin, AFP, endotoxin, blood ammonia, HBV DNA load in serum between the effective, and non-effective group. Compared with the effective group, non-effective group had higher baseline levels of ALT ($p = 0.031$), total bilirubin ($p = 0.003$), white blood cell count ($p = 0.012$), neutrophils percentage ($p = 0.012$), creatinine ($p = 0.039$), and blood urea nitrogen ($p = 0.002$) but lower levels of albumin ($p = 0.020$), lymphocytes percentage ($p = 0.013$), platelet count ($p = 0.002$), and erythrocyte sedimentation rate ($p = 0.022$).

3.2 | Comparison of cytokines between the ALSS-effective and non-effective groups

The 142 HBV-ACLF patients subjected to 2–3 courses of ALSS treatment and there were significant changes in 9 out of 13 cytokines before and after ALSS treatment. The levels of IL-8, CCL4 and CCL20 with significant reduction were found only in the effective group (Figure 1A, all $p < 0.01$). Moreover, the levels of IL-6 [4.36 (2.60–8.4) pg/ml to 1.68 (0.64–3.76) pg/ml, 8.99 (4.36–15.59) pg/ml to 6.00 (1.26–14.22) pg/ml, $p < 0.05$], IL-18 [1013.2 (624.8–1507.1) pg/ml to 622.8 (404.1–1079.2) pg/ml, 926.1 (639.5–1395.5) pg/ml to 509.1 (359.1–927.0), $p < 0.05$], CD40L [5700.7 (3768.2–7771.6) pg/ml to 3330.7 (1296.2–6435.7) pg/ml, 4609.1 (3211.1–6584.5) pg/ml to 1372.2 (451.0–2671.1) pg/ml, $p < 0.05$], CCL2 [275.6 (183.2–355.1) pg/ml to 109.9 (73.2–204.7) pg/ml, 294.9 (179.2–435.7) pg/ml to 143.6 (78.7–274.2) pg/ml, $p < 0.05$], TGFβ1 [1182.5 (1120.4–3676.1) pg/ml to 1234.4 (654.2–3115.5) pg/ml, 2088.9 (1166.0–3596.6) pg/ml to 841.3 (507.8–1360.4) pg/ml, $p < 0.05$] and TGFβ2 [49.7 (31.1–64.7) pg/ml to 31.1 (15.5–49.3) pg/ml, 58.4 (33.3–70.8) pg/ml to 35.4 (14.7–56.3) pg/ml, $p < 0.05$] presented significant reduction both in the effective and non-effective group (Figure 1B). However, the differences between the levels of IL-28A, TNF-α (Figure 1C), IL-4, and CXCL-10 (Figure S1)pre- and post-ALSS were significant neither in the effective nor in the non-effective groups. And IL-28A was the only cytokine increased after ALSS treatment in serum of HBV-ACLF patients, although the difference was not statistically significant.

Additionally, at baseline, 11 out of 13 cytokines of HBV-ACLF patients were significantly different from these in HD, the detail was that serum levels of IL-6, IL-8, IL-18, CCL2, CCL20, CXCL-10, and CD40L were increased in HBV-ACLF patients, while IL-28A, IL-4, TGFβ1, and TGFβ2 were decreased (Table S1). However, no significant differences in serum levels of CCL4 or TNF-α were observed between HBV-ACLF patients and HD (Table S1). Furthermore, at baseline, the median of IL-28A levels in effective group (103.8 pg/ml) was significantly lower ($p = 0.003$) than that in non-effective group (182.3 pg/ml), and the median of IL-6 levels in effective group (4.36 pg/ml) was significantly lower ($p = 0.001$) than that in non-effective group (8.99 pg/ml) (Figure 2).

3.3 | Correlation of serum IL-28A or IL-6 levels with clinical variables

Using Spearman correlation analysis, we found that there was a significantly positive relationship between baseline IL-28A and white blood cell count, procalcitonin, blood urea nitrogen, INR, and a negative relationship between IL-28A and albumin. Simultaneously, the baseline IL-6 level was positively associated with serum ALT, total bilirubin, C-reactive protein, procalcitonin levels, and INR, but negatively correlated with serum levels of total protein and albumin (Table 2).

3.4 | Serum IL-28A level at post-ALSS positively associated with IL-6 in effective group

The Relationship between the serum IL-28A and IL-6 levels of ACLF patients with effective ALSS treatment in pre-ALSS (Figure 3A) or post-ALSS (Figure 3B), and with non-effective ALSS treatment in pre-ALSS (Figure 3C) or post-ALSS (Figure 3D) were analyzed using

TABLE 1 Clinical characteristics of the study population

Characteristic	Patients ($n = 142$)	Effective ($n = 81$)	Non-Effective ($n = 61$)	p-Value
Age (years)	48 ± 13	47 ± 13	50 ± 12	0.125
Male, n (%)	119 (83.8)	65 (80.2)	54 (88.5)	0.251
BMI (kg/m^2)	24 ± 3	24 ± 3	24 ± 3	0.535
Hospitalization days	20 ± 11	20 ± 11	19 ± 12	0.224
ALT (U/L)	195 (101–353)	165 (94–294)	239 (128–467)	**0.031**
AST (U/L)	129 (85–231)	124 (75–216)	136 (97–245)	0.135
Total protein (g/dl)	58 ± 7	59 ± 7	57 ± 6	0.151
Albumin (g/dl)	32 ± 4	33 ± 5	31 ± 3	**0.020**
total Bilirubin (μmol/L)	345 ± 114	318 ± 93	380 ± 129	**0.003**
White blood cell count (×10^9/L)	7.3 ± 3.6	6.6 ± 2.8	8.3 ± 4.3	**0.012**
Neutrophils percentage (%)	68 ± 12	65 ± 12	70 ± 11	**0.012**
Lymphocytes percentage (%)	20 ± 9	21 ± 9	17 ± 9	**0.013**
Platelet count (×10^9/L)	110 ± 55	124 ± 61	92 ± 38	**0.002**
C-reactive protein (mg/L)	12 (8–20)	12 (7–19)	11 (9–22)	0.775
Procalcitonin (ng/ml)	0.59 (0.41–0.91)	0.58 (0.38–0.90)	0.62 (0.44–0.99)	0.287
Erythrocyte sedimentation Rate (mm/h)	5 (2–10)	6 (2–11)	2 (2–8)	**0.022**
Creatinine (μmol/L)	62 (53–75)	61 (51–71)	65 (57–90)	**0.039**
Blood Urea Nitrogen (mmol/L)	4.0 (2.9–5.8)	3.5 (2.7–4.9)	4.2 (3.1–7.0)	**0.002**
INR	2.0 ± 0.7	1.6 ± 0.4	2.4 ± 0.8	**<0.001**
AFP (ng/ml)	107 (27–240)	100 (20–250)	115 (32–222)	0.967
Endotoxin (EU/ml)	0.87 (0.32–1.78)	0.82 (0.31–1.75)	0.91 (0.33–1.89)	0.827
HBV DNA ($\log_{10}$IU/ml)	6.1 ± 2.1	5.8 ± 1.9	6.5 ± 2.2	0.052
Plasma ammonia (μmol/L)	51 (32–76)	45 (28–65)	55 (36–78)	0.052

Note: Data are expressed as mean ± SD, median (25th and 75th percentiles), or percentage (frequency).
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; INR, international normalized ratio.
Bold values indicates $p < 0.05$.

Spearman correlation analysis. Interestingly, there was only a statistically positive correlation between IL-28A and IL-6 in effective group at post-ALSS treatment ($r = 0.2413$, $p = 0.0383$, Figure 3B), but there were no significant correlation in effective group at pre-ALSS or in non-effective groups.

3.5 | Predictive value of serum IL-28A and IL-6 levels in ALSS effectiveness

To evaluate the value for serum IL-28A or IL-6 levels to predict ALSS treatment effectiveness, receiver operating characteristic (ROC) curves were obtained (Figure 4). The AUC was 0.6959 for IL-28A and 0.6704 for IL-6 (both $p < 0.001$). The optimal cut-off value for IL-28A (170.4 pg/ml) and IL-6 (7.335 pg/ml) were determined using ROC analysis.

Variables (albumin, total bilirubin, white blood cell count, neutrophils percentage, lymphocytes percentage, platelet count, creatinine, blood urea nitrogen, INR, HBV DNA, plasma ammonia, IL-6, and IL-28A) that showed significant difference between ALSS treatment effective and non-effective groups were put into multivariate analyses. The results demonstrated that total bilirubin, platelet count, INR, and IL-28A were independent predictor of the ALSS being effective in the treatment of HBV-ACLF patients (Table 3). The effective rate of ALSS treatment of HBV-ACLF patients with low IL-28A (<170.4 pg/ml) were significantly higher than that in patients with high IL-28A (>170.4 pg/ml, OR = 4.854, 95% CI: 1.690–13.945, $p = 0.003$). And the AUC of the model built by IL-28A, total bilirubin, platelet count, and INR was 0.8795 (Figure 4C).

4 | DISCUSSION

In the present study, the effective rate of ALSS treatment of HBV-ACLF patients is of 57.0% (81/142) and the higher levels of serum IL-28A or IL-6 in non-effective group is compared with that in effective group. By establishing a ROC curve, we found that the level of IL-28A or IL-6 could be used to predict the effectiveness for ALSS treatment of HBV-ACLF patients. Multivariate logistic regression analysis showed that lower level of IL-28A was independently associated with higher effective rate of ALSS treatment of HBV-ACLF patients.

HBV-ACLF patients could demonstrate a seriously high mortality if lack of effective and timely therapies, such as liver transplantation.

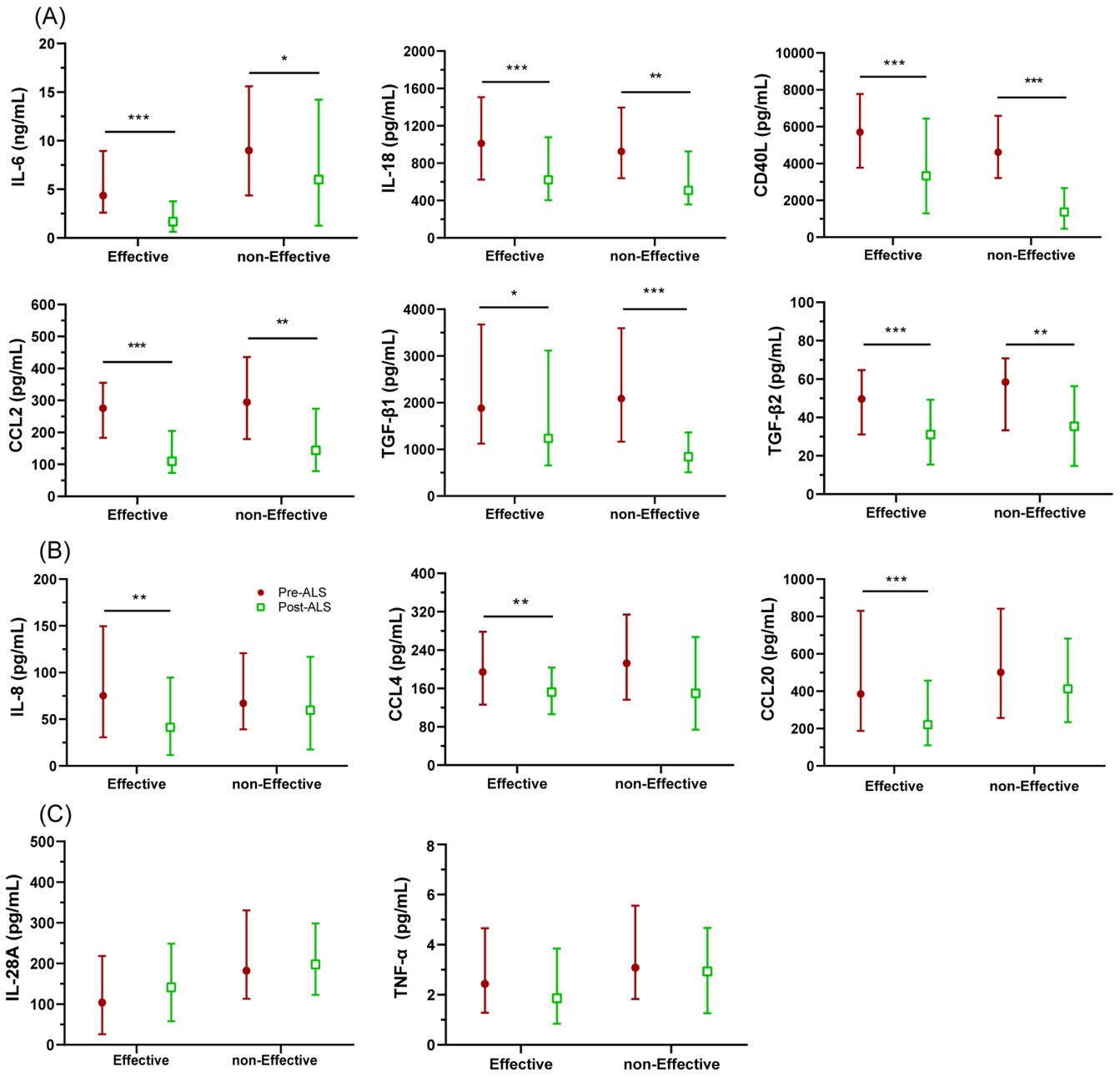


FIGURE 1 Serum cytokine levels in ACLF patients pre- and post-ALSS analyzed. Pre-ALSS, post-ALSS represented the cytokine levels in 142 ACLF patients before, after the 2~3 courses of ALSS treatment. In all, 9 out of 13 cytokines exhibited significant changes due to ALSS treatment. (A) The levels of 6 cytokines showed significant reduction both in the effective and non-effective groups at post-ALSS. (B) The levels of 3 cytokines showed significant reduction in the effective group at post-ALSS. (C) The levels of IL-28A and TNF-α showed significant difference neither in the effective nor in the non-effective group sat post-ALSS. The data were expressed as Median with interquartile range (IQR), the middle solid circle (pre-ALSS) or square (post-ALSS) in the each panel presented as median values. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

ALSS is widely used to bridge patients with liver failure to liver regeneration or liver transplantation when they were waiting liver source and reported to be effective in removing toxins of patients.[19] The 90-day survival probability of HBV-ACLF patients treated by ALSS was approximately 50.0%–60.0%,[4,20,21] which is consistent with our result (57%). And more and more studies succeeded to demonstrate that ALSS can improve survival rate of HBV-ACLF patients.[4,21,22] Early prediction of the treatment effect of ALSS on ACLF patients is important for clinicians to make timely and accurate therapeutic decision. Novel models[20,23] comprising four (number of complications, age, scores of the model for end-stage liver disease and type of ALSS) or five (liver cirrhosis, total bilirubin, INR, infection, and hepatic encephalopathy) variables were constructed to discriminate survival in ACLF patients treated with ALSS. The former was related to ACLF patients with HBV as prevailing etiology and the latter focused on HBV-ACLF only. Another study[24] developed a classification and regression tree constituted of three factors (hepatic encephalopathy, prothrombin time, and total bilirubin level) to identify subgroups of patients with

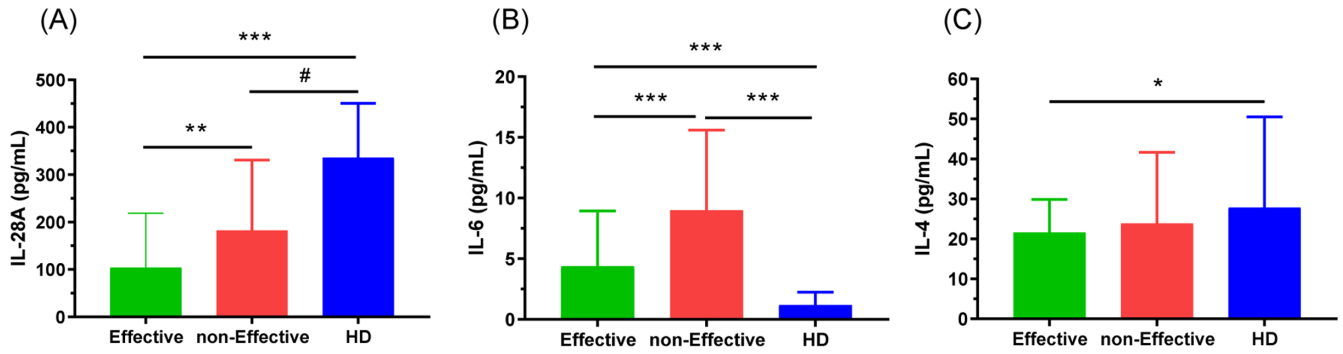


FIGURE 2 Comparison of serum cytokine levels between the effective and non-effective group at pre-ALSS treatment. (A) IL-28A levels are significantly lower in ALSS treatment-effective patients than those in ALSS non-effective group and healthy donors (HD). (B) IL-6 levels are significantly lower in ALSS-effective patients compared with that in non-effective patients. However, IL-6 levels are significantly higher in both effective and non-effective patients than those in HD. (C) IL-4 levels have no significant difference between ALSS-effective and non-effective patients. Data are expressed as bars and boxes (Median with IQR), in which the height of boxes is median, and the up-error bars presents the 75th percentiles. $^{\#}p > 0.05$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

TABLE 2 Correlations between serum IL-28A or IL-6 level of pre-ALSS and clinical variables in HBV-ACLF patients

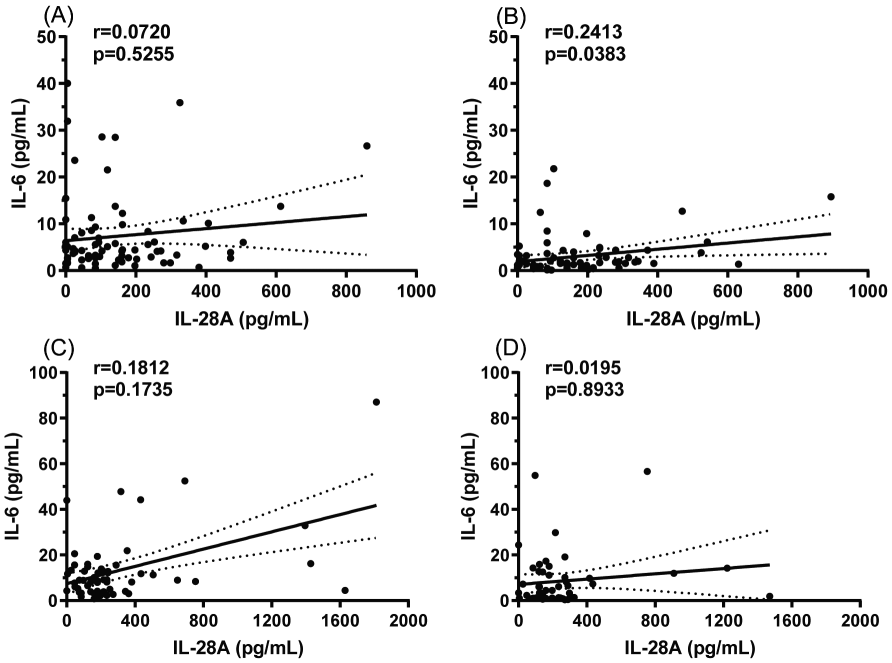
	IL-28A		IL-6	
Variables	Spearman correlation	*p* Value	Spearman correlation	*p* Value
Age (years)	0.126	0.134	0.297	0.263
BMI (kg/m^2)	0.066	0.450	0.098	0.257
Hospitalization days	−0.084	0.322	−0.030	0.720
ALT (U/L)	0.138	0.101	−0.085	0.314
AST (U/L)	0.123	0.146	**0.171**	**0.042**
Total protein (g/dl)	−0.011	0.895	**−0.187**	**0.026**
Albumin (g/dl)	**−0.228**	**0.006**	**−0.211**	**0.012**
Total bilirubin (μmol/L)	0.110	0.192	**0.257**	**0.002**
White blood cell count (×10^9/L)	**0.175**	**0.037**	0.164	0.051
Neutrophils percentage (%)	0.147	0.080	0.100	0.238
Lymphocytes percentage (%)	−0.151	0.073	−0.164	0.052
Platelet count (×10^9/L)	−0.117	0.166	−0.108	0.200
C-reactive protein (mg/L)	0.077	0.369	**0.289**	**0.001**
Procalcitonin (ng/ml)	**0.207**	**0.020**	**0.276**	**0.002**
Erythrocyte sedimentation Rate (mm/h)	−0.027	0.768	−0.016	0.863
Creatinine (μmol/L)	0.164	0.051	0.064	0.451
Blood Urea Nitrogen (mmol/L)	**0.238**	**0.004**	0.134	0.111
INR	**0.256**	**0.002**	**0.384**	**<0.001**
Alpha fetoprotein (ng/ml)	−0.092	0.281	−0.023	0.793
Endotoxin (EU/ml)	−0.077	0.377	−0.010	0.913
HBV DNA ($\log_{10}$IU/ml)	0.087	0.324	0.069	0.429
Plasma ammonia (μmol/L)	0.092	0.341	0.073	0.449

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; INR, international normalized ratio.

Bold values indicates $p < 0.05$.

HBV-ACLF who may benefit from ALSS therapy. However, none of these studies was associated with cytokines that was related not only to the prognosis of patients with HBV-ACLF but also to the effectiveness of ALSS treatment of HBV-ACLF.

Cytokine storm was first reported in graft-versus-host disease[25] and referred to the phenomenon that immune cells in grafts attack host cells as foreign bodies. From then on, researchers gradually found that cytokine storm can also result from certain infections such as bacteria[26] and virus.[27,28] And cytokine storm produced by enhanced immune reaction against hepatitis B virus is considered to be an important incentive of ACLF derived from chronic hepatitis B.[29] In HBV-ACLF, a variety of cytokines, such as



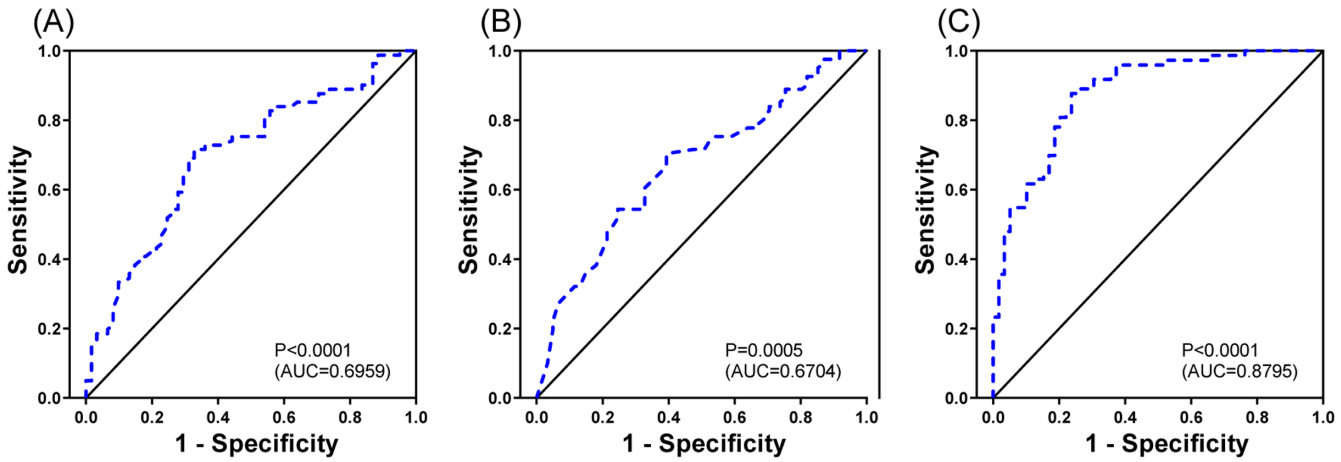


FIGURE 3 Relationship between the IL-28A and IL-6 levels in ACLF patients with effective ALSS treatment at pre-ALSS (A) or post-ALSS (B) and with non-effective ALSS treatment at pre-ALSS (C) or post-ALSS (D)

FIGURE 4 Receiver operating characteristic (ROC) curves. The ROC curves of the IL-28A (A), IL-6 (B), and the model built by IL-28A, total bilirubin, platelet count, and INR (C) after 2–3 courses of ALSS treatment for separating the HBV-ACLF patients with effective from non-effective treatment

IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, and IFN-γ, were of high levels in serum and could be removed by ALSS.[30,31] However, we found no significant difference in TNF-α between HBV-ACLF patients and HD. And there were no obvious changes in IL-4, TNF-α in HBV-ACLF patients after ALSS. The different results may be related to the subjects in different population and different assays used to detect cytokines.

Similarly, our data showed elevated levels of serum IL-6 in patients with HBV-ACLF and decreased levels of IL-6 after ALSS treatment compared with that at before ALSS. Moreover, it was the first time to profile the level of serum IL-28A, which is a member of type III interferons (IFN-λ) and controls the inflammatory response,[32] in HBV-ACLF patients before and after ALSS treatment. Among multitudinous cytokines detected, only IL-6 and IL-28A levels had ability to distinguish effectives from non-effective ALSS treatment of HBV-ACLF patients. Interestingly, the relationship between IL-6 and IL-28A is significantly positive in effective group at post-ALSS treatment, but there was no significant correlation in effective group at pre-ALSS or in non-effective groups. This finding will contribute to predicting effectiveness of ALSS treatment of HBV-ACLF patients.

Additionally, the lower level of IL-28A or IL-6 was associated with higher effective rate of ALSS treatment of HBV-ACLF patients. Moreover, ROC curve was established to define the optimal cutoff value of IL-28A or IL-6 for distinguishing effective ALSS treatment of HBV-ACLF patients from non-effectiveness. Multivariate analysis showed that lower level of IL-28A was independently associated with higher effective rate of ALSS treatment of HBV-ACLF patients. And the AUC of the model built by IL-28A, total bilirubin, platelet count, and INR was 0.8795. Collectively, IL-28A may be a potential index of the ALSS-effective treatment of HBV-ACLF patients. As mentioned above, IL-28A is a member of type III interferons with

TABLE 3 Multivariate analyses for effectiveness of ALSS treating patients with HBV-ACLF

Variables	OR (95% CI)	*p* Value
ALT (U/L)		0.528
Albumin (g/dl)		0.677
Total bilirubin (μmol/L)	0.993 (0.987–0.999)	**0.016**
White blood cell count ($\times 10^9$/L)		0.107
Neutrophils percentage (%)		0.083
Lymphocytes percentage (%)		0.084
Platelet count ($\times 10^9$/L)	1.018 (1.005–1.031)	**0.005**
Erythrocyte sedimentation Rate (mm/h)		0.389
Creatinine (μmol/L)		0.271
Blood Urea Nitrogen (mmol/L)		0.177
INR	0.050 (0.014–0.177)	**<0.001**
IL-6 (IL-6 < 7.335 pg/ml vs. IL-6 ≥ 7.335 pg/ml)		0.317
IL-28A (IL-28A < 170.4 pg/ml vs. IL-28A ≥ 170.4 pg/ml)	4.854 (1.690–13.945)	**0.003**

Abbreviations: ALT, alanine aminotransferase; INR, international normalized ratio.

Bold values indicates $p < 0.05$.

antiviral, anti-cancer, and immune-regulatory functions.[33–35] It can be restrictively produced by hepatocytes, airway epithelial cells, plasmacytoid, and myeloid DCs. Once these cells are injured, serum levels of IL-28A will be downregulated. For example, a strong link between low expression of IL-28A and more severity of the disease has been described among patients with COVID-19.[36] It is reasonable to doubt that the low IL-28A level in HBV-ACLF patients is as a result of seriously injured hepatocytes. And ALSS is believed to be conducive to the regeneration of hepatocytes.[37] This may be one of the reasons why IL-28A is lower in HBV-ACLF patients who respond to ALSS treatment. In fact, IL-28A was the only cytokine increased after ALSS treatment in serum of HBV-ACLF patients, and the raised IL-28A levels may suggest potential hepatic recovery.

There are some limitations in this study. First, the limited sample size of our study may cause potential bias of ability cytokines identifying effectiveness and non-effectiveness of ALSS treating HBV-ACLF patients. Second, subjects of this study are from the same center, and the generalizability of our results to other populations needs further verification.

In summary, the study presents that the changing profile of cytokines in peripheral blood of ACLF patients before and after ALSS treatment, and the lower level of IL-28A is an index of the ALSS-effective treatment of HBV-ACLF patients. Detection of serum level of IL-28A could contribute to early estimate HBV-ACLF patients who can exactly benefit from ALSS or not, and the clinician timely and accurately make therapeutic decision, although their roles in the pathogenesis of ALSS effectiveness need to be confirmed in the future.

AUTHOR CONTRIBUTIONS

DY, JY, and YH were principal investigators, designed, and supervised the study. YH, HZ, and JT had roles in recruitment, data collection, and clinical management. YH, JT, and DC did clinical laboratory testing and analysis. YH and JY wrote the first draft. DY and XL obtained funding and revised the article. All authors gave final approval and agreed to be accountable for all aspects of the work.

ACKNOWLEDGMENTS

None.

FUNDING INFORMATION

The work was supported by the National Science and Technology Major Project (2018ZX10101-001) and National Natural Science Foundation of China (grant number 82072357).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available for reasonable requirements of readers.

ORCID

Yandi Huang https://orcid.org/0000-0002-6364-1839
Xuefen Li https://orcid.org/0000-0002-2989-3477

REFERENCES

1. Sarin SK, Choudhury A, Sharma MK, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update[J]. *Hepatol Int*. 2019;13(4):353-390.
2. Wu T, Li J, Shao L, et al. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure[J]. *Gut*. 2018;67(12):2181-2191.
3. Arroyo V, Moreau R, Jalan R. Acute-on-chronic liver failure[J]. *N Engl J Med*. 2020;382(22):2137-2145.
4. Yang L, Wu T, Li J, et al. Artificial liver treatment improves survival in patients with hepatitis B virus-related acute-on-chronic liver failure: a case-control matched analysis[J]. *Hepatol Res*. 2020;50(6):656-670.
5. Claria J, Arroyo V, Moreau R. The acute-on-chronic liver failure syndrome, or when the innate immune system goes astray[J]. *J Immunol*. 2016;197(10):3755-3761.
6. Rueschenbaum S, Ciesek S, Queck A, et al. Dysregulated adaptive immunity is an early event in liver cirrhosis preceding acute-on-chronic liver failure[J]. *Front Immunol*. 2020;11:534731.
7. Li J, Liang X, Jiang J, et al. PBMC transcriptomics identifies immune-metabolism disorder during the development of HBV-ACLF[J]. *Gut*. 2022;71(1):163-175.
8. Claria J, Stauber RE, Coenraad MJ, et al. Systemic inflammation in decompensated cirrhosis: characterization and role in acute-on-chronic liver failure[J]. *Hepatology*. 2016;64(4):1249-1264.
9. Barathan M, Riazalhosseini B, Iyadorai T, et al. Comparative expression of pro-inflammatory and apoptotic biosignatures in chronic HBV-infected patients with and without liver cirrhosis[J]. *Microb Pathog*. 2021;161(Pt A):105231.

10. Lowery SA, Sariol A, Perlman S. Innate immune and inflammatory responses to SARS-CoV-2: implications for COVID-19[J]. *Cell Host Microbe*. 2021;29(7):1052-1062.
11. To KK, Lau CC, Woo PC, et al. Human H7N9 virus induces a more pronounced pro-inflammatory cytokine but an attenuated interferon response in human bronchial epithelial cells when compared with an epidemiologically-linked chicken H7N9 virus[J]. *Virol J*. 2016;13:42.
12. Xia Q, Xu K, Yu L, Zhang H, Li L. Application value of artificial liver support system in the treatment of cytokine storm in patients with COVID-19[J]. *Int Immunopharmacol*. 2021;90:107120.
13. Guo J, Xia H, Wang S, et al. The artificial-liver blood-purification system can effectively improve Hypercytokinemia for COVID-19[J]. *Front Immunol*. 2020;11:586073.
14. Dolganiuc A, Kodys K, Marshall C, et al. Type III interferons, IL-28 and IL-29, are increased in chronic HCV infection and induce myeloid dendritic cell-mediated FoxP3+ regulatory T cells[J]. *PLoS One*. 2012;7(10):e44915.
15. Wu ZB, Zheng YB, Wang K, et al. Plasma Interleukin-6 level: a potential prognostic indicator of emergent HBV-associated ACLF[J]. *Can J Gastroenterol Hepatol*. 2021;2021:5545181.
16. Zhou C, Zhang N, He TT, et al. High levels of serum interleukin-6 increase mortality of hepatitis B virus-associated acute-on-chronic liver failure[J]. *World J Gastroenterol*. 2020;26(30):4479-4488.
17. Siebler J, Wirtz S, Weigmann B, et al. IL-28A is a key regulator of T-cell-mediated liver injury via the T-box transcription factor T-bet[J]. *Gastroenterology*. 2007;132(1):358-371.
18. Zhang JX, Li N, Xu QY, et al. Kupffer cell depletion attenuates IL-6/STAT3 mediates hepatocyte apoptosis in immunological liver injury of trichloroethylene sensitized mice[J]. *Int Immunopharmacol*. 2020;88:106897.
19. Kribben A, Gerken G, Haag S, et al. Effects of fractionated plasma separation and adsorption on survival in patients with acute-on-chronic liver failure[J]. *Gastroenterology*. 2012;142(4):782-789 e3.
20. Du L, Ma Y, Zhou S, et al. A prognostic score for patients with acute-on-chronic liver failure treated with plasma exchange-centered artificial liver support system[J]. *Sci Rep*. 2021;11(1):1469.
21. Xiao L-L, Xu X-W, Huang K-Z, et al. Artificial liver support system improves short-term outcomes of patients with HBV-associated acute-on-chronic liver failure: a propensity score analysis[J]. *Biomed Res Int*. 2019;2019:1-8.
22. Liu H, Zhang Q, Liu L, et al. Effect of artificial liver support system on short-term prognosis of patients with hepatitis B virus-related acute-on-chronic liver failure[J]. *Artif Organs*. 2020;44(10):E434-E447.
23. Zhou PQ, Zheng SP, Yu M, He SS, Weng ZH. Prognosis of acute-on-chronic liver failure patients treated with arti fi cial liver support system[J]. *World J Gastroenterol*. 2015;21(32):9614-9622.
24. Huang K, Ji F, Xie Z, et al. Artificial liver support system therapy in acute-on-chronic hepatitis B liver failure: classification and regression tree analysis[J]. *Sci Rep*. 2019;9(1):16462.
25. Ferrara JL, Abhyankar S, Gilliland DG. Cytokine storm of graft-versus-host disease: a critical effector role for interleukin-1[J]. *Transplant Proc*. 1993;25(1 Pt 2):1216-1217.
26. Bisno AL, Brito MO, Collins CM. Molecular basis of group a streptococcal virulence[J]. *Lancet Infect Dis*. 2003;3(4):191-200.
27. Leisman DE, Ronner L, Pinotti R, et al. Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes[J]. *Lancet Respir Med*. 2020;8(12):1233-1244.
28. Huang KJ, Su IJ, Theron M, et al. An interferon-gamma-related cytokine storm in SARS patients[J]. *J Med Virol*. 2005;75(2):185-194.
29. Liu Q. Role of cytokines in the pathophysiology of acute-on-chronic liver failure[J]. *Blood Purif*. 2009;28(4):331-341.
30. Mao WL, Chen Y, Chen YM, Li LJ. Changes of serum cytokine levels in patients with acute on chronic liver failure treated by plasma exchange[J]. *J Clin Gastroenterol*. 2011;45(6):551-555.
31. Guo LM, Liu JY, Xu DZ, et al. Application of molecular adsorbents recirculating system to remove NO and cytokines in severe liver failure patients with multiple organ dysfunction syndrome[J]. *Liver Int*. 2003;23(Suppl 3):16-20.
32. Zanoni I, Granucci F, Broggi A. Interferon (IFN)-lambda takes the helm: immunomodulatory roles of type III IFNs[J]. *Front Immunol*. 2017;8:1661.
33. Xu F, Song H, Xiao Q, et al. Type III interferon-induced CBFbeta inhibits HBV replication by hijacking HBx[J]. *Cell Mol Immunol*. 2019;16(4):357-366.
34. Numasaki M, Tagawa M, Iwata F, et al. IL-28 elicits antitumor responses against murine fibrosarcoma[J]. *J Immunol*. 2007;178(8):5086-5098.
35. Li B, Xie C, Lin X, Su SB. Interleukin-28A promotes IFN-gamma production by peripheral blood mononuclear cells from patients with Behcet's disease[J]. *Cell Immunol*. 2014;290(1):116-119.
36. Fukuda Y, Homma T, Inoue H, et al. Downregulation of type III interferons in patients with severe COVID-19[J]. *J Med Virol*. 2021;93(7):4559-4563.
37. MacDonald AJ, Karvellas CJ. Emerging role of extracorporeal support in acute and acute-on-chronic liver failure: recent developments[J]. *Semin Respir Crit Care Med*. 2018;39(5):625-634.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Huang Y, Ju T, Zhang H, et al. Lower level of IL-28A as a predictive index of the artificial liver support system in effective treatment of patients with HBV-ACLF. *J Clin Lab Anal*. 2022;36:e24766. doi: 10.1002/jcla.24766