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Original Article

Changes in the IL-18, IL-22, and T lymphocyte subset levels in patients with hepatitis B-related liver cirrhosis and their predictive values for hepatorenal syndrome

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Abstract: Objective: To investigate the changes in the interleukin (IL)-18, IL-22, and T lymphocyte subset levels in patients with hepatitis B-related liver cirrhosis and to determine their predictive values for hepatorenal syndrome (HRS). Methods: Clinical data of 70 healthy individuals (group A) and 84 patients with hepatitis B-related liver cirrhosis (group B) admitted to Hospital 989 of the PLA Joint Logistics Support Force were retrospectively collected. The serum levels of IL-18 and IL-22, concentrations of cluster of differentiation (CD)3^+, $CD4^+$, and $CD8^+$ cells, as well as the $CD4^+/CD8^+$ ratio in the peripheral blood T lymphocyte subsets were measured. Further, their predictive values for HRS were determined. Logistic regression analysis was employed to identify independent risk factors for HRS. Results: In group B, the posttreatment IL-18 and IL-22 levels and $CD8^+$ cell concentration significantly decreased after treatment, whereas the $CD3^+$ and $CD4^+$ cell concentrations and $CD4^+/CD8^+$ ratio increased. Notably, the serum IL-18 and IL-22 levels were higher in patients with HRS than in those without. Also, the $CD3^+$ and $CD4^+$ cell concentrations and $CD4^+/CD8^+$ ratio in the peripheral blood were lower in patients with HRS than in those without. The sensitivities of the serum IL-18 and IL-22 levels for predicting HRS were 90.32% and 80.65%, and the specificities were 71.70% and 77.36%, respectively. The sensitivities of $CD3^+$, $CD4^+$, and $CD8^+$ cell concentrations for predicting HRS were 77.42%, 90.32%, and 83.87%, and the specificity was 67.92%, 64.15%, and 52.83%, respectively. Moreover, the sensitivity and specificity of $CD4^+/CD8^+$ ratio for predicting HRS were 80.65% and 86.79%, respectively. Conclusions: IL-18, IL-22, and T lymphocyte subset levels may have significant implications in the progression of hepatitis B-related liver cirrhosis, and detecting these markers could aid in treatment, evaluation, and prediction of HRS in patients. Furthermore, IL-18 and IL-22 levels and the $CD4^+/CD8^+$ ratio were identified as independent risk factors for HRS.

Keywords: Hepatitis B-related liver cirrhosis, T lymphocyte subset, interleukin-18, interleukin-22

Introduction

Hepatitis B virus (HBV) is widespread in the human population and epidemiologically differs in various regions. According to the World Health Organization, approximately 2 billion people worldwide were infected with HBV during 1980-2007, and around 10% of these cases developed chronic infections [1]. Chronic HBV can lead to persistent viral presence in the liver, eventually leading to the development of cirrhosis over time [2]. Further, HBV infection can cause a clinically common condition, a diffuse liver disease [3]. Currently, to the best of our knowledge, there is no effective clinical treatment for cirrhosis, and antiviral therapy is the key to controlling its progression [4]. Notably, cirrhosis is influenced by various factors and can progress to hepatorenal syndrome (HRS), which is more difficult to treat and has a poor prognosis [5]. Therefore, the early diagnosis of cirrhosis-induced damage is of great significance.

HBV infection does not directly damage liver cells. The chronic infection-induced immune cell response leads to pathologic changes in liver tissue cells [6]. Further, HBV infection-

induced cirrhosis is often associated with an immunocompromised immune system and immune dysfunction [7]. T lymphocytes are known to mediate the immune cell response in the human body. Cellular immunity is responsible for maintaining normal immune defenses, regulating and monitoring bodily functions, and providing a warning of autoimmune diseases, infections, and tumors [8]. Cluster of differentiation (CD)4^+ and $CD8^+$ cells are the main functional subsets of T lymphocytes [9]. $CD4^+$ cells play a key role as helper T cells, whereas $CD8^+$ cells are known as killer T cells. Both of them play important roles in immune regulation and fighting infections [10, 11]. Interleukin (IL)-18 induces the secretion of interferon γ from immune cells, promotes T-cell proliferation and differentiation, and causes liver injury through Fas ligand-dependent hepatocyte apoptosis pathways [12]. In particular, T-helper (Th) 22 cells are a subset of $CD4^+$ cells that produce IL-22 [13]. IL-22 plays a vital part in various immune and infectious diseases, and it is closely related to the genesis and development of hepatitis and liver cancer [14, 15].

Several studies have investigated IL-18, IL-22, and T lymphocyte subsets in the peripheral blood of patients with hepatitis B-related liver cirrhosis [16-18]. However, their roles in concomitant HRS remain unexplored. In this study, we aimed to measure the levels of IL-18, IL-22, and T lymphocyte subsets in the peripheral blood and to analyze their importance in the diagnosis and treatment of HRS.

Materials and methods

Patient selection and data collection

In this study, clinical data of participants were retrospectively analyzed. The subjects were classified into two groups: group A, including 70 healthy individuals who underwent a physical examination at Hospital 989 of the PLA Joint Logistics Support Force, and group B, including 84 patients with hepatitis B-related liver cirrhosis who were admitted to the same hospital between June 2012 and February 2014. Patients in group B were classified according to the Child-Pugh score criteria, with 25 patients with grade A, 35 patients with grade B, and 24 patients with grade C [19]. The relevant clinical data, including IL-18, IL-22, T lymphocyte subset, HBV-DNA, and serum liver enzyme levels, were collected from the electronic medical records.

Inclusion and exclusion criteria for healthy individuals

Healthy individuals were included if they were aged ≤80 years and did not have cirrhosis or other abnormalities identified by physical examination. Individuals were excluded if they had been taking medications that affect immune function.

Inclusion and exclusion criteria for patients with hepatitis B-related liver cirrhosis

The inclusion criteria for patients were as follows: patients who met the diagnostic criteria for cirrhosis published by the American Society of Hepatology and the European Society of Hepatology [20]; patients who were ≤80 years; patients with HBV-DNA levels of $>10^4$ IU/mL; patients with jaundice, hypersplenism, and varying degrees of ascites; patients who underwent liver surgery, interventional therapy, or antiviral therapy; patients who were diagnosed with HRS based on the diagnostic criteria and literature report [21] of the International Ascites Club; patients with complete case records.

The exclusion criteria were as follows: patients with concomitant other systemic autoimmune diseases, connective tissue diseases, hematopoietic dysfunction, malignant tumors, other types of hepatitis, or fungal infections; patients who had received antibacterial, anti-inflammatory, or immunosuppressive therapy in the past month; patients with dementia or primary mental disorders; patients with dysfunctions of heart, kidney, liver or other important organs; patients with a history of schistosomiasis or hepatitis; patients with incomplete case records.

This study was approved by the Ethics Committee of Hospital 989 of PLA Joint Logistics Support Force.

Methods

Patients with cirrhosis received conventional liver protective and symptomatic treatment. First, 1.2 g of reduced glutathione (Shanghai Fudan Fuhua Pharmaceutical Co., Ltd., No.

H20031265) was dissolved in 250 mL of 0.9% sodium chloride solution and infused intravenously once a day. Besides, 30 mg of liver hydrolyzed peptide (Jilin Huinan Changlong Biochemical Pharmaceutical Co., Ltd., No. H22025368) was dissolved in 500 mL of 10% glucose solution and infused intravenously once a day. The treatment included two cycles, with each cycle lasting for 7 days. Additionally, oral lamivudine [GlaxoSmithkline Pharmaceuticals (Suzhou) Co., Ltd., No. H20030581] and adefovir dipivoxil (Zhengda Tianqing Pharmaceutical Group Co., Ltd., No. H20060666) were administered at the rates of 100 mg/day and 10 mg/day, respectively, for 48 weeks. Glutathione and nucleoside analogs are established for the treatment of cirrhosis. However, since tenofovir alafenamide was first marketed in 2017, it was also included in our study.

Enzyme-linked immunosorbent assay (ELISA) protocols

In the mornings of the days before and after admission, 5 mL of the peripheral blood sample was obtained in ethylenediaminetetraacetic acid (EDTA) and anticoagulant vacuum tubes. In the morning of the days after admission, serum levels of IL-18 and IL-22 were measured using the HR-801 enzyme labeling analyzer (Shenzhen Huakerui Technology Co., Ltd.) and analyzed in accordance with the manufacturers' instructions for human IL-18 (Cat. No. ZK-H249; Wuhan HealthCare Biotechnology Co., Ltd.) and IL-22 (Cat. No. PI595; Shanghai Enzyme Biotechnology Co., Ltd.) using ELISA test kits.

Glucose detection protocols

Glucose was measured using the glucose kit (glucose oxidase method) (Cat. No. A154-1-1; Nanjing Jiancheng Bioengineering Institute, Nanjing, China) in accordance with the manufacturer's instructions. After mixing, the samples were heated in a water bath at 100°C for 5-6 min. Subsequently, they were cooled in a cold-water bath. Absorbance was measured at 620 nm within 30 min using a spectrophotometer. The test samples and kits were removed from the refrigerator 30 min before the experiment for temperature balancing. Reaction, standard, and blank wells were set up. No reagent was added to the blank well, and 50 μL of the sample or different dilutions of the standard were added to the remaining wells.

Flow cytometry analysis

In the morning of the day after admission, 5 mL of the peripheral blood sample was obtained in EDTA and anticoagulant vacuum tubes. Subsequently, 50 μL of the biotin-labeled antibodies (CD3-FITC [Cat. No. 11-0037-42], CD4-FITC [Cat. No. 11-0041-82], and CD8-PE-Texas Red [Cat. No. MHCD0817] Monoclonal Antibodies) purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd. were added. The membrane was covered and incubated at 37°C for 1 h. The $CD4^+/CD8^+$ ratio was measured using a FACSCalibur automatic flow cytometer (BD Biosciences). After centrifuging the peripheral blood at 500×g for 5 min at 4°C, the upper plasma was discarded, and 2 mL of the supernatant suspension was taken into a 10-mL centrifuge tube. Subsequently, 8 mL of the red blood cell lysate was added to the cells and incubated at room temperature for 10 min. This suspension was centrifuged again at 500×g for 5 min at 4°C. The upper layer was discarded, and 1 mL of phosphate-buffered saline (PBS) was added. Further, the cells were transferred to 1.5-mL EP tubes. Moreover, red blood cell lysate was added after centrifuging the EP tubes at 500×g for 5 min at 4°C. Subsequently, 500 μL of PBS was added to the precipitate after lysis and centrifugation at 500×g for 5 min at 4°C. Finally, 100 μL of this solution was transferred to each EP tube. After staining the cells with antibodies at room temperature, the concentrations of $CD3^+$, $CD4^+$, and $CD8^+$ and $CD4^+/CD8^+$ ratio were determined using the BD FACSCalibur automatic flow cytometer and analyzed using FlowJo (version 10; FlowJo LLC).

Measurements of the serum liver enzyme levels

Blood samples were obtained for assessing liver function parameters, including alanine aminotransferase (ALT; Cat. No. C009-2-1), aspartate aminotransferase (AST; Cat. No. C010-2-1), albumin (ALB; Cat. No. A028-2-1), alkaline phosphatase (ALP; Cat. No. A059-2-2) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), and total bilirubin (TBIL; Cat. No. KA1614) (AmyJet Scientific, Inc.) levels. The

serum levels were measured according to the manufacturer's instructions at baseline (before treatment) and after two cycles of treatment. The median values were considered as the cutoffs.

Outcome measures

The primary outcome measures were changes in the peripheral blood levels of IL-18, IL-22, and T lymphocyte subsets before and after treatment as well as changes in HBV-DNA levels before and after treatment. The secondary outcome measures were the predictive values of IL-18, IL-22, and T lymphocyte subsets in the treatment response and the correlation of these indicators with clinical characteristics and other factors.

The levels of IL-18, IL-22, T lymphocyte subsets, and HBV-DNA were examined before and after treatment. The HBV-DNA levels were measured in accordance with the instructions of the HBV-DNA quantitative detection kit (Shanghai Yaji Biotechnology Co., Ltd., 0343P). Then, lysate (50 μL) was collected in the centrifuge tube and mixed with 50 μL of serum for further centrifugation. Subsequently, 2 μL of the processed sample and standard substance were used for polymerase chain reaction (PCR) amplification. The conditions were as follows: 2 min predenaturation at 93°C, followed by 40 cycles of 30 s at 93°C and 30 s at 55°C. The results of HBV-DNA quantitative analysis were read using an ASAP (version 1.17.2; Tim Clark - Clark IT).

Statistical analysis

All statistical analyses were performed using SPSS (version 18.0; SPSS Inc., Chicago, IL, USA) and visualized using GraphPad Prism (version 7; GraphPad Software, Inc.). Counted data were presented as n (%) and evaluated using χ^2 test. Measured data were presented as mean ± standard deviation (mean ± SD) and analyzed using unpaired *t*-test for intergroup comparisons and paired *t*-test for comparisons before and after treatment in group B. Pearson's coefficient was calculated for correlation analysis. The receiver operating characteristic (ROC) curve was used to evaluate the predictive values of IL-18, IL-22, and T lymphocyte subsets for HRS. To identify independent risk factors for HRS, logistic regression analysis was performed using the following variables: IL-18 level, IL-22 level, $CD3^+$ cell concentration, $CD4^+$ cell concentration, $CD8^+$ cell concentration, and $CD4^+/CD8^+$ ratio. $P<0.05$ was considered significant.

Results

General data

There were no significant differences between the two groups in general data, including sex, age, body mass index, history of smoking and drinking, place of residence, blood glucose levels, and white blood cell counts ($P>0.05$). However, there were significant differences in the levels of ALT, AST, TBIL, ALB, and ALP between the two groups ($P<0.001$; **Table 1**).

Serum IL-18 and IL-22 concentrations before treatment

The serum concentrations of IL-18 (403.16 ± 48.37 vs. 76.43 ± 19.25 pg/mL) and IL-22 (419.26 ± 89.57 vs. 113.26 ± 31.69 pg/mL) in group B were significantly higher than those in group A before treatment ($P<0.001$; **Figure 1**).

T lymphocyte subset concentrations before treatment

The concentrations of $CD3^+$ cells (67.23 ± 6.74% vs. 57.20 ± 6.18%) and $CD4^+$ cells (42.53 ± 5.16% vs. 31.37 ± 5.53%) as well as $CD4^+/CD8^+$ ratio (1.78 ± 0.54 vs. 1.26 ± 0.37) in the peripheral blood were significantly higher in group A than in group B ($P<0.001$). The concentration of $CD8^+$ cells (21.35 ± 6.24% vs. 23.97 ± 7.08%) in the peripheral blood was significantly lower in group A than in group B ($P<0.05$; **Figure 2**).

Correlation among the IL-18, IL-22, and T lymphocyte subset levels and Child-Pugh score

Correlations among the IL-18, IL-22, and T lymphocyte subset levels determined by ELISA and Child-Pugh score were analyzed. Flow cytometry results revealed that with the increase of Child-Pugh scores, the concentrations of IL-18, IL-22, and $CD8^+$ increased, whereas those of $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ gradually decreased. Scores of 1, 2, and 3 were assigned to Child-Pugh grades A, B, and C, respectively, and their correlations with the IL-18, IL-22, $CD3^+$, $CD4^+$, and $CD8^+$ levels and the $CD4^+/CD8$ ratio were analyzed. Pearson's cor-

Table 1. Demographic data and patient characteristics

Category	Group A (n = 70)	Group B (n = 84)	t/χ^2	*P*-value
Sex			0.177	0.674
Male	41 (58.57)	52 (61.90)		
Female	29 (41.43)	32 (38.10)		
Age (years)	44.28 ± 5.31	45.73 ± 5.18	1.710	0.089
BMI (kg/m^2)	23.51 ± 3.64	22.47 ± 3.18	1.892	0.060
History of smoking			1.226	0.268
Yes	38 (54.29)	53 (63.10)		
No	32 (45.71)	31 (36.90)		
Drinking history			2.063	0.151
Yes	33 (47.14)	30 (35.71)		
No	37 (52.86)	54 (64.29)		
Place of residence			1.155	0.282
City	53 (75.71)	57 (67.86)		
Rural	17 (24.29)	27 (32.14)		
WBC (10^9/L)	6.57 ± 1.67	6.61 ± 1.03	0.182	0.856
Glu (mmol/L)	5.49 ± 1.22	5.67 ± 0.93	1.038	0.301
ALT (U/L)	23.41 ± 12.83	173.61 ± 82.16	15.130	<0.001
AST (U/L)	19.61 ± 7.13	152.23 ± 60.47	18.230	<0.001
TBIL (μmol/L)	10.51 ± 7.19	26.31 ± 15.48	7.859	<0.001
ALB (g/L)	38.61 ± 8.61	20.23 ± 9.41	12.540	<0.001
ALP (U/L)	70.62 ± 10.37	316.31 ± 253.69	8.093	<0.001
Child-Pugh			-	-
Class A	-	25 (29.76)		
Class B	-	35 (41.67)		
Class C	-	24 (28.57)		

WBC, white blood cells; Glu, glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; ALB, albumin; ALP, alkaline phosphatase. Data are presented as n (%) or mean ± standard deviation.

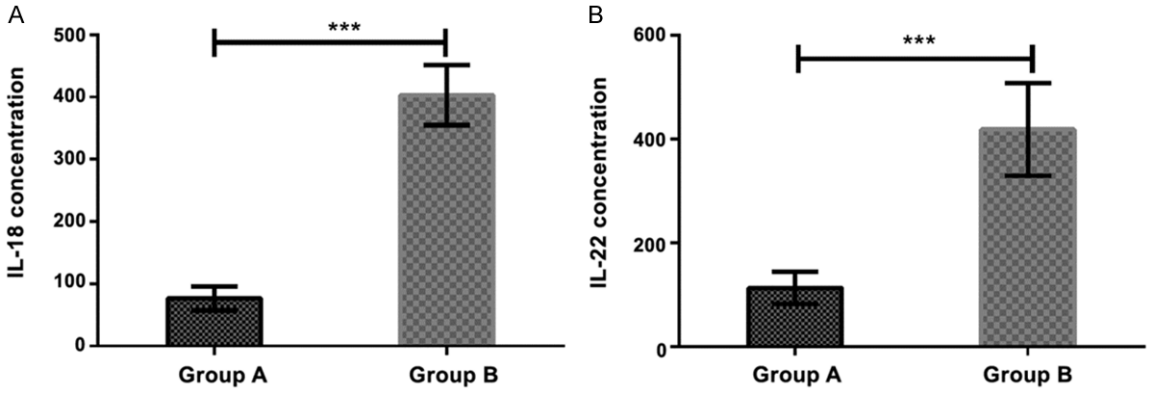


Figure 1. Comparison of the serum concentrations of IL-18 and IL-22 between the two groups before treatment. Group A exhibited significantly lower serum concentrations of (A) IL-18 and (B) IL-22 than group B. ***P<0.001. IL, interleukin.

relation analysis revealed that the IL-18, IL-22, and CD8$^+$ levels were positively correlated with the Child-Pugh score, whereas the CD3$^+$, CD4$^+$, and CD4$^+$/CD8$^+$ levels were negatively correlated with the score (P<0.001; **Figure 3**).

Clinical indicators before and after treatment

PCR revealed that the HBV-DNA level after treatment (3.12 ± 0.86 IU/mL) was significantly lower than that before treatment (5.32 ± 3.16

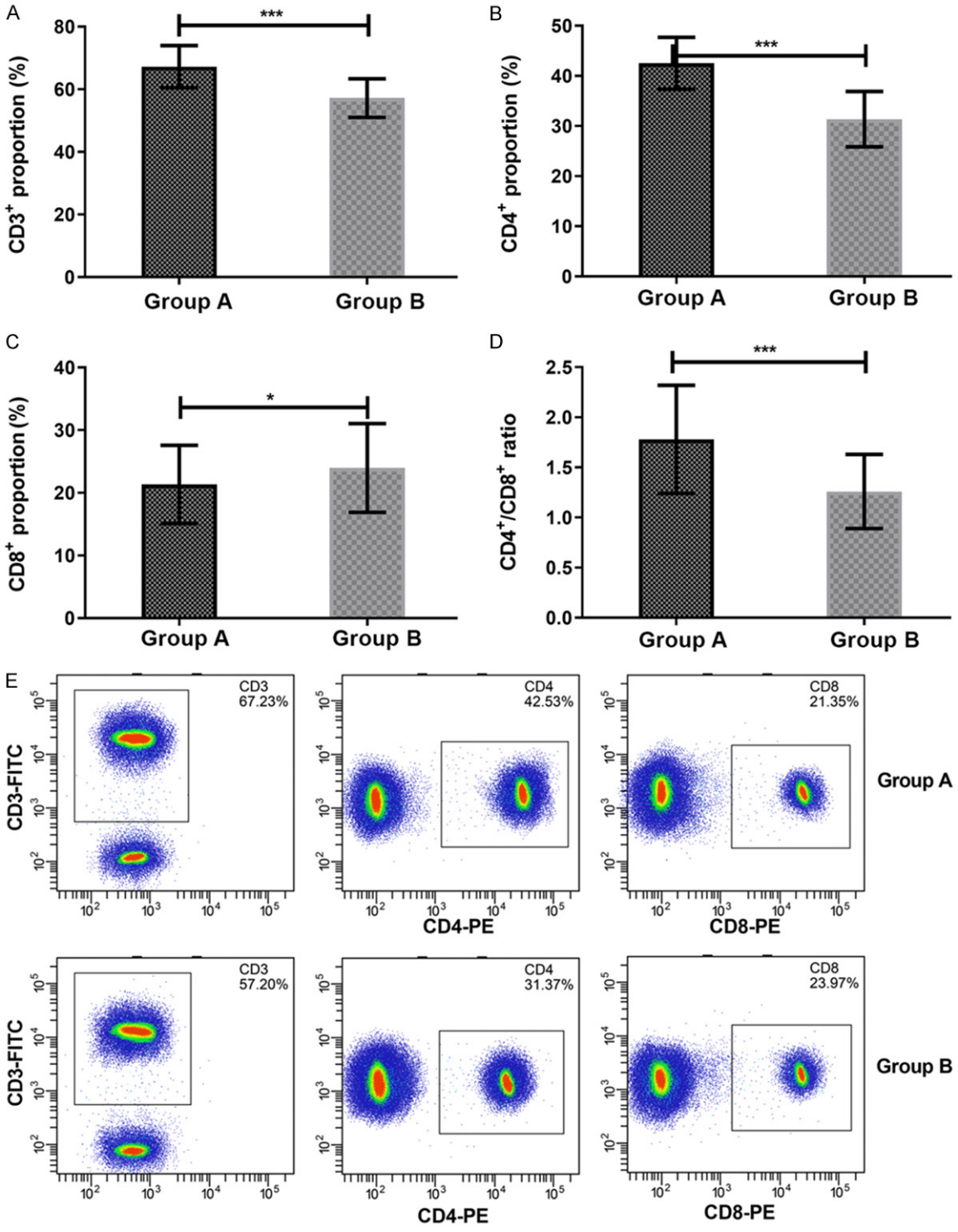
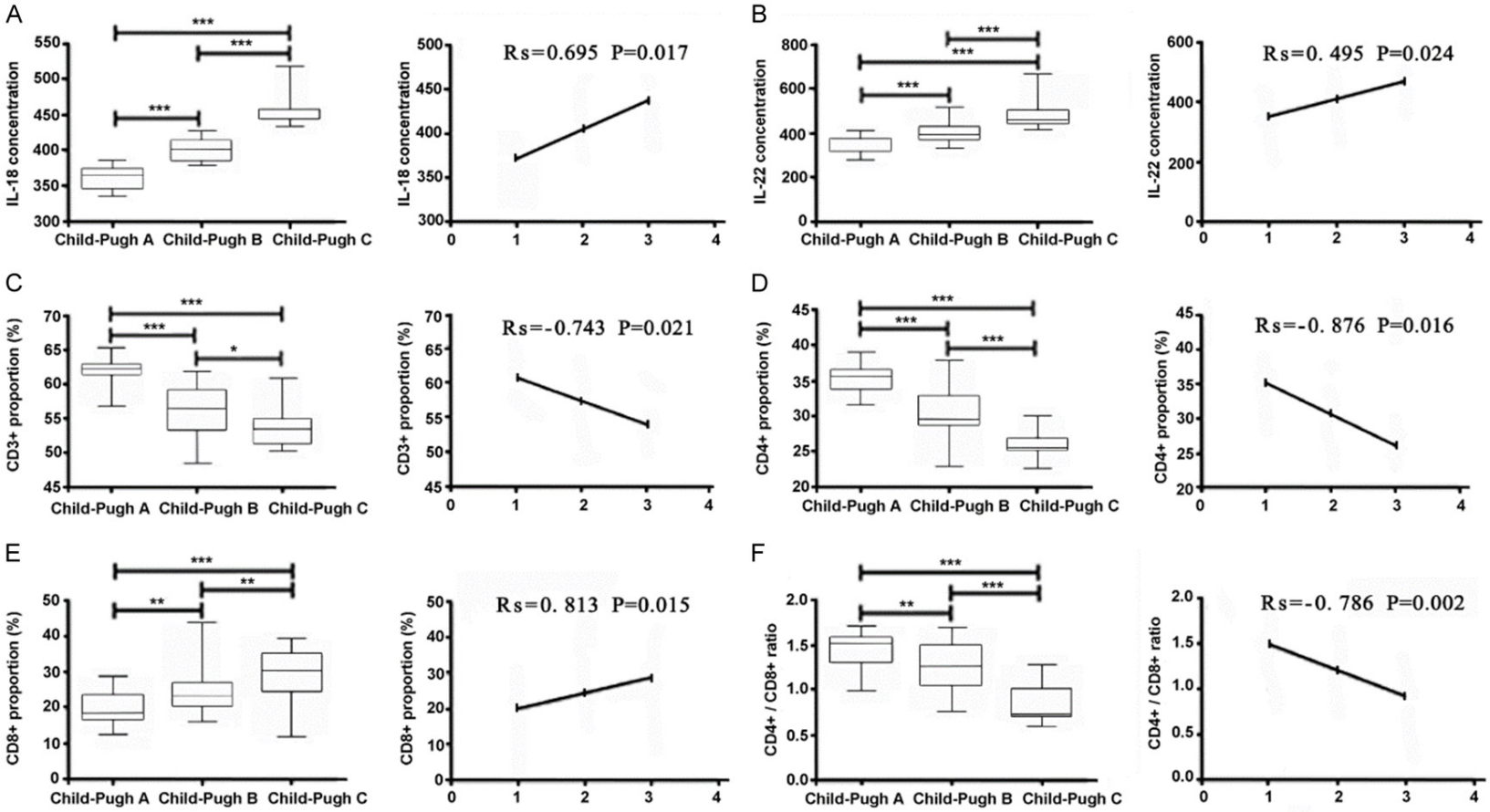


Figure 2. Comparison of the T lymphocyte subsets in the peripheral blood between the two groups before treatment. The concentrations of (A) $CD3^+$ and (B) $CD4^+$ cells and (D) $CD4^+/CD8^+$ ratio in the peripheral blood of group A were significantly higher than those in the blood of group B, whereas (C) the concentration of $CD8^+$ cells in group A was significantly lower than that in group B. (E) Flow cytometry results. $^*P<0.05$, $^{***}P<0.001$. CD, cluster of differentiation.

IU/mL) in group B ($P<0.001$). After treatment, the serum levels of ALT, AST, TBIL, and ALP decreased, whereas the level of ALB increased significantly ($P<0.001$; **Table 2**).


A
IL-18 concentration
Child-Pugh A Child-Pugh B Child-Pugh C
Rs=0.695 P=0.017
B
IL-22 concentration
Rs=0. 495 P=0.024
C
CD3+ proportion (%)
Rs=-0.743 P=0.021
D
CD4+ proportion (%)
Rs=-0. 876 P=0.016
E
CD8+ proportion (%)
Rs=0. 813 P=0.015
F
CD4+ / CD8+ ratio
Rs=-0. 786 P=0.002

G

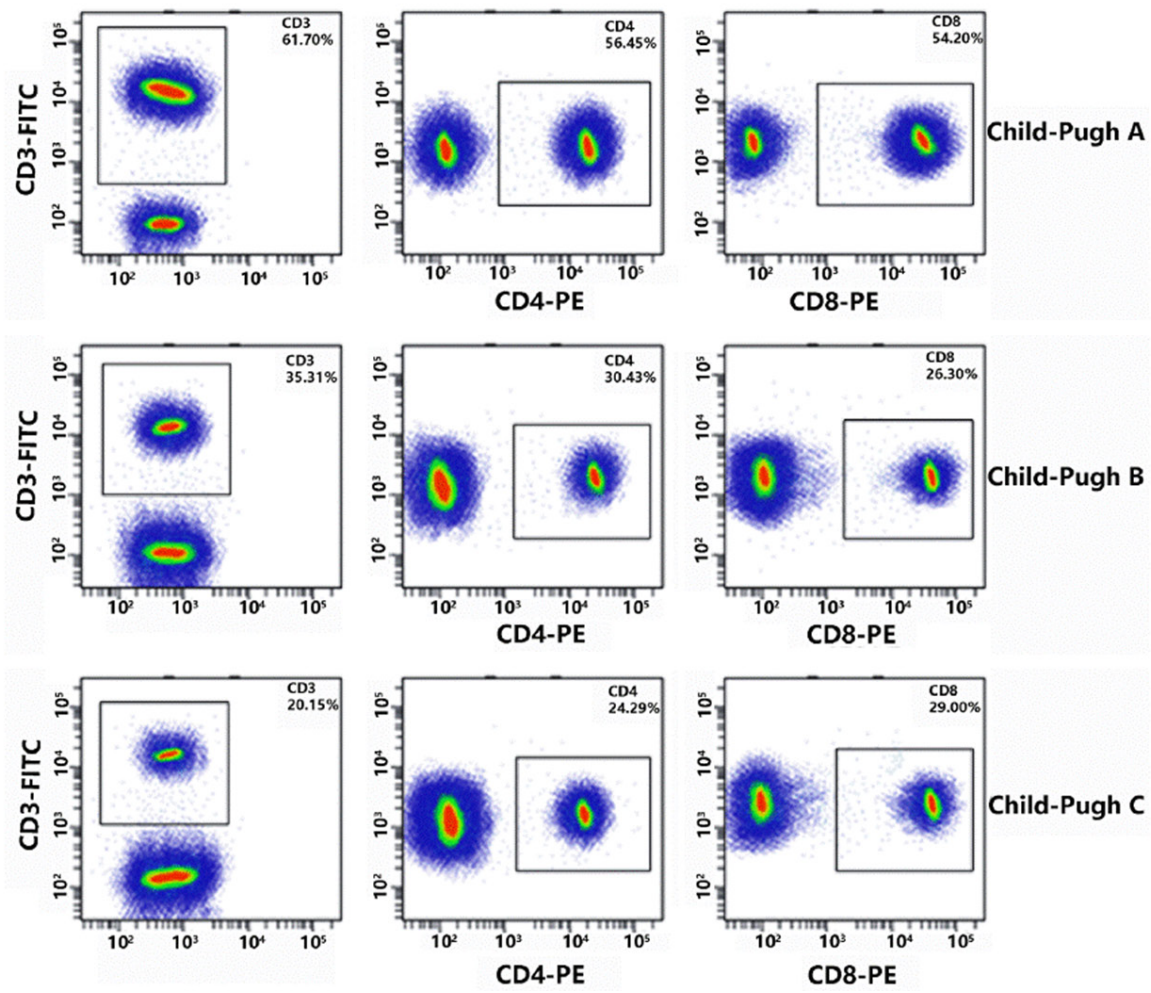


Figure 3. Correlation of the IL-18, IL-22, and T lymphocyte subset levels with the Child-Pugh score. The concentrations of (A) IL-18, (B) IL-22, and (E) $CD8^+$ increased as the Child-Pugh score increased (positive correlation). The (C) $CD3^+$, (D) $CD4^+$, and (F) $CD4^+/CD8^+$ decreased as the Child-Pugh score increased (negative correlation). (G) Flow cytometry results. $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$. IL, interleukin; CD, cluster of differentiation.

Table 2. Changes in clinical indicators in group B

Indicator	Before treatment	After treatment	*t*	*P*-value
HBV-DNA (IU/ml)	5.32 ± 3.16	3.12 ± 0.86	6.157	<0.001
ALT (U/L)	173.61 ± 82.16	30.52 ± 10.17	15.840	<0.001
AST (U/L)	152.23 ± 60.47	39.45 ± 13.52	16.680	<0.001
TBIL (μmol/L)	26.31 ± 15.48	19.15 ± 11.69	3.383	<0.001
ALB (g/L)	20.23 ± 9.41	31.52 ± 4.53	9.908	<0.001
ALP (U/L)	316.31 ± 253.69	204.13 ± 158.05	3.440	<0.001

HBV, Hepatitis B virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; ALB, albumin; ALP, alkaline phosphatase. Data are presented as mean ± SD.

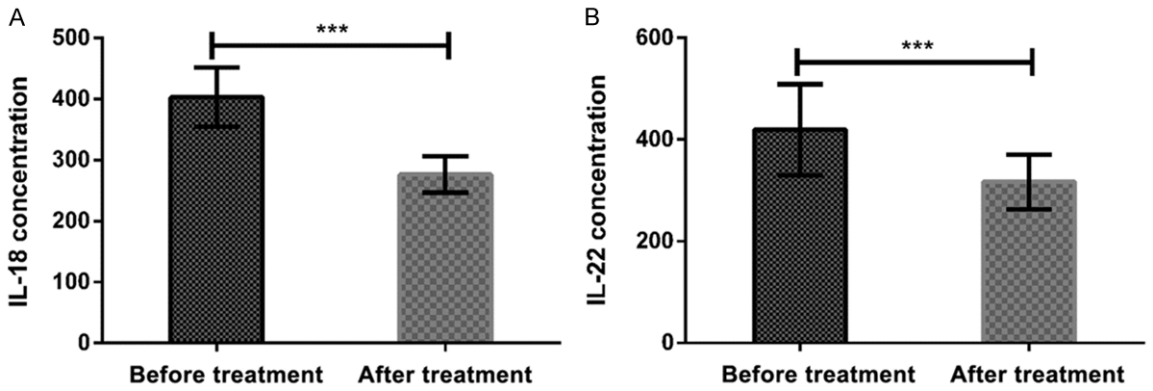


Figure 4. Concentrations of IL-18 and IL-22 in the peripheral blood of group B before and after treatment. After treatment, the serum (A) IL-18 and (B) IL-22 levels were significantly decreased compared with those before treatment. ***P<0.001. IL, interleukin.

Peripheral blood IL-18 and IL-22 concentrations before and after treatment

ELISA revealed that the serum levels of IL-18 (276.53 ± 29.51 pg/mL) and IL-22 (316.54 ± 53.71 pg/mL) after treatment were significantly lower than those before treatment in group B (P<0.001; **Figure 4**).

T lymphocyte subsets in the peripheral blood before and after treatment

Flow cytometry results indicated that the concentrations of $CD3^+$ (61.29 ± 5.32%) and $CD4^+$ (36.15 ± 4.94%) cells and the $CD4^+/CD8^+$ ratio (1.53 ± 0.24) in the peripheral blood were higher after treatment than before treatment in group B (P<0.05). However, the concentration of $CD8^+$ cells (20.31 ± 4.23%) was significantly lower after treatment than before treatment (P<0.001; **Figure 5**).

Predictive values of the peripheral blood IL-18, IL-22, and T lymphocyte levels for HRS

Patients in group B were followed up for 3 years. At follow-up, 31 and 53 patients had and had not developed HRS, respectively. The serum levels of IL-18 and IL-22 were significantly higher in patients with HRS than in those without (P<0.001). Also, the concentrations of $CD3^+$ and $CD4^+$ cells and the $CD4^+/CD8^+$ ratio in the peripheral blood were lower in patients with HRS than in those without (P<0.001). The concentration of $CD8^+$ cells was higher in patients with HRS than in those without (**Table 2**). The area under the curve (AUC) of serum concentration of IL-18 for HRS diagnosis was 0.825 (95% confidence interval [CI]: 0.730-0.921; sensitivity: 90.32%, specificity: 71.70%, optimal cutoff: 420.10). The AUC of serum concentration of IL-22 for HRS diagnosis was 0.811 (95% CI: 0.703-0.918; sensitivity: 80.65%, specificity: 77.36%, optimal cutoff: 427.10). The AUC of the concentration of $CD3^+$ cells in the peripheral blood for HRS diagnosis was 0.734 (95% CI: 0.625-0.843; sensitivity: 77.42%, specificity: 67.92%, optimal cutoff: 55.96). The AUC of the concentration of $CD4^+$ cells in the peripheral blood for HRS diagnosis was 0.797 (95% CI: 0.690-0.905; sensitivity: 90.32%, specificity: 64.15%, optimal cutoff: 27.69). The AUC of the concentration of $CD8^+$

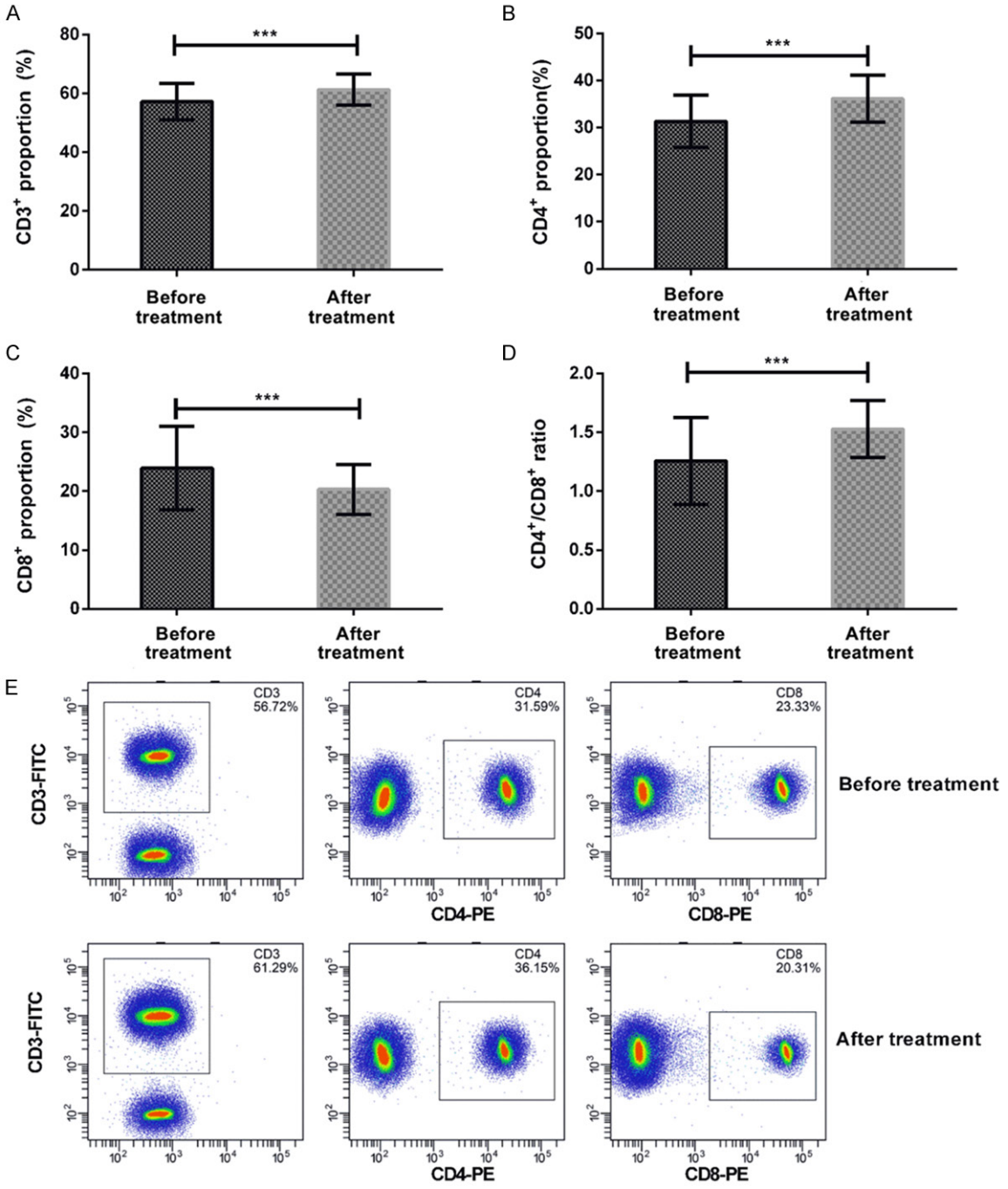


Figure 5. Comparison of the T lymphocyte subset levels in the peripheral blood of group B before and after treatment. Based on the results of flow cytometry, (A) $CD3^+$, (B) $CD4^+$, and (D) $CD4^+/CD8^+$ increased significantly, whereas (C) $CD8^+$ cells significantly decreased in the peripheral blood of group B. (E) Flow cytometry results. $^{***}P<0.001$. CD, cluster of differentiation.

cells in the peripheral blood for HRS diagnosis was 0.713 (95% CI: 0.601-0.824; sensitivity: 83.87%, specificity: 52.83%, cutoff value: 25.91). The AUC of the peripheral blood $CD4^+/CD8^+$ ratio for HRS diagnosis was 0.862 (95% CI: 0.775-0.948; sensitivity: 80.65%, specificity: 86.79%, cutoff value: 1.18) (**Tables 3** and **4**; **Figure 6**).

Table 3. Comparison of IL-18, IL-22, and T lymphocyte subset levels in the peripheral blood between patients with and without HRS

Index	Patients with HRS (n = 31)	Patients without HRS (n = 53)	*t*	*P*-value
IL-18 (pg/mL)	457.54 ± 48.37	381.94 ± 39.74	7.758	<0.001
IL-22 (pg/mL)	478.62 ± 73.58	374.28 ± 64.59	6.784	<0.001
$CD3^+$ (%)	52.24 ± 5.92	61.37 ± 6.09	6.698	<0.001
$CD4^+$ (%)	27.51 ± 5.27	32.65 ± 5.03	4.441	<0.001
$CD8^+$ (%)	27.36 ± 4.97	22.99 ± 5.81	3.503	<0.001
$CD4^+/CD8^+$	0.81 ± 0.71	1.43 ± 0.83	3.479	<0.001

IL, interleukin; CD, cluster of differentiation; HRS, hepatorenal syndrome. Data are presented as mean ± SD.

Logistic regression analysis of risk factors

Logistic regression analysis revealed that the IL-18 (odds ratio [OR] = 1.34, 95% CI = 1.05-1.71, $P = 0.019$) and IL-22 (OR = 1.65, 95% CI = 1.28-2.13, $P = 0.001$) levels as well as the $CD4^+/CD8^+$ ratio (OR = 0.54, 95% CI = 0.35-0.83, $P = 0.005$) were independent risk factors for HRS in patients with hepatitis B-related liver cirrhosis (**Table 5**).

Discussion

HBV infection-induced liver cirrhosis is considered one of the main infectious diseases in humans [22]. During the progression of chronic hepatitis B to cirrhosis, a hepatic immune cell response is induced by HBV. Further, immune cells gradually recognize and eliminate HBV, causing inflammation and liver damage [23]. Subsequently, fibrosis and cirrhosis occur because of the immunocompromised state of the patients [24]. Moreover, hepatitis B-related liver cirrhosis can lead to infection of the surrounding normal tissues, including lung infections, urinary tract infections, and spontaneous peritonitis, eventually resulting in cancer [25, 26]. Immune cells and their secretory factors play an important role in this process; therefore, they deserve further investigation [27].

T lymphocyte subsets are important immune cells in the human body and have relatively stable concentrations in the peripheral blood. Thus, changes in the absolute number and ratio of T lymphocytes usually indicate changes in immune function [28]. $CD3^+$ cells are mainly found on the cell surface and are common markers of T lymphocytes [29]. $CD4^+$ cells are effector cells that assist the cellular and humoral immune response. $CD8^+$ cells are cytotoxic T cells that recognize and kill HBV-infected cells [30]. T lymphocyte subsets are the main indicators of immune function in the body. Abnormal immune functions indicate an imbalance among the T lymphocyte subsets. Changes in the $CD4^+/CD8^+$ ratio indicate changes in the state of immune function, which plays a vital part in the determination of disease outcomes [31]. In the present study, the concentrations of $CD3^+$ and $CD4^+$ T cells and the $CD4^+/CD8^+$ ratio in the peripheral blood were significantly lower in group B (after treatment) than in group A. This finding indicates that patients with hepatitis B-related liver cirrhosis have abnormal immune functions and weakened immunity, making their immune system unable to fight against the HBV infection. Li et al. [32] reported that the concentration of T lymphocyte subsets was dysregulated in patients with HBV infection. Our study suggests that disruption of T lymphocyte subsets in chronic HBV infection could result in decreased auxiliary humoral immune function, leading to decreased antibody production and cytotoxicity.

During prolonged HBV infection, the liver undergoes continuous damage, resulting in cirrhosis. IL-18 stimulates the proliferation and differentiation of T cells. Moreover, it synergistically acts with IL-12 to induce the differentiation of $CD4^+$ T cells into Th1 cells, leading to sustained inflammatory reactions and specific immune responses. These immune responses eliminate the viruses and attack the HBV antigens, damaging liver cells [33]. IL-22 is mainly secreted by $CD4^+$ T cells and distributed in hepatic stellate, intestinal epithelial, and other tissue cells. Moreover, IL-22 has several effects on liver diseases [34]. In the present study, the serum levels of IL-18 and IL-22 were significantly higher in patients with hepatitis B-related liver cirrhosis than in the healthy individuals, suggesting that they are involved in the development and progression of hepatitis B-related liver cirrhosis. Lee et al. [35] reported that the X-protein of HBV may cause liver damage by inducing IL-18 expression. Zhao et al. [36] reported that in

Table 4. Predictive value of the peripheral blood IL-18, IL-22, and T lymphocyte subset values for HRS

Predictive indicator	AUC	95% CI	Standard error	Cutoff	Sensitivity (%)	Specificity (%)
IL-18	0.825	0.730-0.921	0.049	420.10	90.32	71.70
IL-22	0.811	0.703-0.918	0.055	427.10	80.65	77.36
$CD3^+$	0.734	0.625-0.843	0.056	55.96	77.42	67.92
$CD4^+$	0.797	0.690-0.905	0.055	27.69	90.32	64.15
$CD8^+$	0.713	0.601-0.824	0.057	25.91	83.87	52.83
$CD4^+/CD8^+$	0.862	0.775-0.948	0.044	1.18	80.65	86.79

IL, interleukin; HRS, hepatorenal syndrome; AUC, area under the curve; CI, confidence interval; CD, cluster of differentiation.

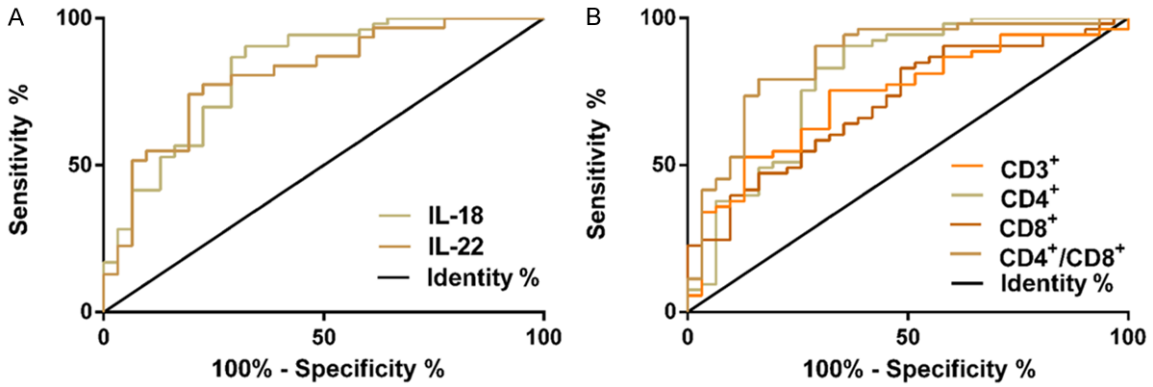


Figure 6. ROC curves of the peripheral blood levels of IL-18, IL-22, and T lymphocytes subsets for the diagnosis of HRS. The ROC curve of (A) serum IL-18 and IL-22 concentrations and (B) the peripheral blood T lymphocyte subsets for the diagnosis of HRS. ROC, receiver operating characteristic; IL, interleukin; CD, cluster of differentiation, HRS, hepatorenal syndrome.

Table 5. Logistic regression analysis for risk factors of HRS

Variable	Odd ratio	95% CI	*P*-value
IL-18	1.34	1.05-1.71	0.019
IL-22	1.65	1.28-2.13	0.001
$CD3^+$ cell concentration	1.12	0.92-1.36	0.256
$CD4^+$ cell concentration	0.87	0.70-1.08	0.199
$CD8^+$ cell concentration	1.26	0.96-1.64	0.094
$CD4^+/CD8^+$ ratio	0.54	0.35-0.83	0.005

HRS, hepatorenal syndrome; IL, interleukin; CI, confidence interval; CD, cluster of differentiation.

patients with chronic HBV hepatitis, IL-22 recruited Th17 cells to aggravate fibrosis.

The present study revealed a positive correlation of the IL-18 and IL-22 levels with the Child-Pugh score and a negative correlation of the $CD3^+$ and $CD4^+$ concentrations and $CD4^+/CD8^+$ ratio with the Child-Pugh score. A previous study indicated that the concentration of Th22 cells was significantly increased in patients with liver cirrhosis and was positively correlated with the ALT and AST levels and the Child-Pugh score; furthermore, the expression of IL-22, IL-22R1, and aryl hydrocarbon receptor were increased in such cases [37]. This finding may be attributed to the fact that HBV infection causes sustained liver inflammation and inflammatory stress in the body. Further, inflammatory stress can affect immune regulation, including the humoral and cellular immunity. In addition, inflammatory stress and abnormal immune function may promote the development of cirrhosis.

Because the extent of HBV replication is closely related to the prognosis of patients with cirrhosis, antiviral intervention is the key to the treatment [38]. Nucleoside analogs are commonly used antiviral drugs to inhibit the reverse transcriptase activity of HBV in a clinical setting [39]. Among them, amivudine and adefovir dipivoxil are commonly used in clinical practice. Lamivudine blocks HBV replication by infiltrating the HBV-DNA strand and preventing HBV

replication. These drugs are commonly used to treat hepatitis B-related liver cirrhosis and have little effect on the normal metabolism of cell deoxynucleosides and mitochondrial structure [40, 41]. Li et al. [42] reported that the combination of lamivudine and adefovir dipivoxil could effectively treat hepatitis B-related liver cirrhosis. Wang et al. [43] reported that the combined use of adefovir dipivoxil and anluo huaxian in the treatment of hepatitis B-related liver cirrhosis may significantly reduce the inflammatory stress response and improve the fibrosis index and immune function. Therefore, controlling the inflammatory response in patients with hepatitis B-related liver cirrhosis and restoring the stability of immune cells may help to control or even reverse the progression of cirrhosis.

The present study reports that in group B, compared to before treatment, the HBV-DNA concentration, serum IL-18 and IL-22 levels, and $CD8^+$ concentration were lower after treatment. The concentrations of $CD3^+$ and $CD4^+$ cells and the $CD4^+/CD8^+$ ratio in the peripheral blood increased after treatment. However, the concentration of $CD8^+$ cells significantly decreased after treatment. Moreover, the inflammatory response was inhibited after treatment, which improved the immune function. This indicates that delaying the progression of hepatitis B-related liver cirrhosis may be a primary approach to inhibiting HBV replication. Therefore, IL-18, IL-22, and T lymphocyte subset levels in the peripheral blood can be considered as assessment indices for the treatment of patients with hepatitis B-related liver cirrhosis.

HRS is one of the most common and serious complications of liver cirrhosis, occurring in 50%-70% of patients during decompensation. The survival rate of patients with HRS is extremely low as it is a difficult condition to treat [44]. Yap et al. [45] reported a poor prognosis in patients with HRS. They also reported that the serum IL-18 concentration was significantly higher in patients with HRS than in those without. Higher or lower IL-22 levels and the protein/IL-22 ratio are associated with the occurrence and mortality of acute hepatic failure [37, 46]. However, the role of IL-18, IL-22, and T lymphocyte subsets in the diagnosis of HRS remains unclear. In the present study, the concentrations of $CD3^+$ and $CD4^+$ cells and the $CD4^+/CD8^+$ ratio in the peripheral blood were significantly lower in patients with HRS than in those without. The serum levels of IL-18, IL-22, and $CD8^+$ were significantly higher in patients with HRS than in those without. The ROC curve revealed that the determination of IL-18, IL-22, and T lymphocyte subsets in the peripheral blood before treatment could be effective in the diagnosis of HRS in patients with hepatitis B-related liver cirrhosis. The IL-18 levels in the peripheral blood were reported to be significantly higher in patients with hepatitis B-related liver cirrhosis than in the normal population. Furthermore, the IL-18 level is positively correlated with the ALT, AST, and TBIL levels, model for end-stage liver disease scores, flow velocity in the portal and superior mesenteric veins, velocity and diameter of the splenic vein, and the splenic volume [47]. Zhang et al. [48] reported that increased Th17 cells (>5.9%) at admission indicated a poor prognosis in patients with HBV-induced acute chronic liver failure. Thus, the possibility of HRS development in patients with hepatitis B-related liver cirrhosis could be assessed based on the optimal cutoff of IL-18, IL-22, and T lymphocyte subsets before treatment. This result was confirmed in our study, which revealed that the IL-18, IL-22, and T lymphocyte subset levels in the peripheral blood were able to predict HRS development in patients with hepatitis B-related liver cirrhosis.

We used logistic regression analysis to identify independent risk factors for HRS among the variables assessed. The results indicated that the IL-18 and IL-22 levels and the $CD4^+/CD8^+$ ratio were independent risk factors for HRS in patients with hepatitis B-related liver cirrhosis. This finding suggests that monitoring and targeting of these factors may be useful in the management of the progression of hepatitis B-related liver cirrhosis and prevention of HRS. Future studies are required to identify the mechanisms by which these factors contribute to HRS and to explore potential therapeutic interventions to mitigate their impact on disease progression.

Although this study confirmed the important role of IL-18, IL-22, and T lymphocyte subsets in hepatitis B-related liver cirrhosis, it has some limitations. First, the specific mechanism of

action of IL-18, IL-22, and T lymphocyte subsets in patients with hepatitis B-related liver cirrhosis and the risk factors for HRS were not analyzed. Second, the role of IL-18, IL-22, and T lymphocyte subsets in the patients who died was not thoroughly investigated owing to the lack of long-term follow-up. Urinary neutrophil gelatinase-associated lipocalin and kidney injury molecule 1 levels can serve as biomarkers for predicting HRS in advanced cases of cirrhosis [45]. Thus, a more comprehensive combination of indicators is warranted in future studies. These design limitations need to be addressed in the future, and the results of this study require further external validation.

In conclusion, this study revealed that IL-18, IL-22, and T lymphocyte subsets may play an important role in the diagnosis of patients with hepatitis B-related liver cirrhosis. The estimation of these markers may facilitate the treatment, evaluation, and prediction of HRS in patients with chronic HBV infections.

Disclosure of conflict of interest

None.

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