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$CD45^+$ erythroid progenitor cells as potential biomarkers for disease progression in hepatitis B virus-related acute-on-chronic liver failure

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Abstract

Background Hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF) is characterized by immune dysregulation and systemic inflammation, which lead to high mortality. Although immunosuppressive $CD45^+$ erythroid progenitor cells (EPCs) percentages are elevated in chronic hepatitis B (CHB) and are associated with disease progression, their role in HBV-ACLF remains unclear. This study aims to evaluate the impact of $CD45^+$ EPCs on disease progression in patients with HBV-ACLF.

Methods In this retrospective study, we analyzed the data of 102 patients with CHB and 65 patients with HBV-ACLF receiving standard drugs treatment from the Third Affiliated Hospital of Sun Yat-sen University between January 2021 and December 2023. HBV-ACLF diagnosis followed the Chinese Group on the Study of Severe Hepatitis B–Acute-on-Chronic Liver Failure criteria, with strict exclusion of comorbidities. Peripheral blood mononuclear cells (PBMCs) were isolated via density gradient centrifugation, and $CD45^+$ EPCs ($CD45^+$ $CD71^+$ $CD235a^+$) were quantified using flow cytometry. Liver tissue EPCs were assessed by immunofluorescence in biopsy/transplant specimens. Receiver operating characteristic (ROC) and multivariable logistic regression analyses identified prognostic factors associated with disease progression.

Results Our findings revealed that patients with HBV-ACLF had significantly elevated percentages of $CD45^+$ EPCs compared with those with CHB. We also observed strong correlations between $CD45^+$ EPC percentages and creatinine concentration, leukocyte count, and neutrophil-to-lymphocyte ratio (NLR). The area under the ROC curve for $CD45^+$ EPCs was 0.718, indicating a significant predictive value [95% confidence interval (CI): 0.586–0.851, $p = 0.004$]. High $CD45^+$ EPC percentage was associated with a greater incidence of hepatic encephalopathy (30.8% vs. 10.3%, $p = 0.037$) and higher rates of disease progression (73.1% vs. 35.9%, $p = 0.003$). Multivariate logistic regression

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analysis identified international normalized ratio (INR) and NLR as independent predictors of poor 28-day outcomes (INR odds ratio [OR] = 6.098, $p < 0.001$; NLR OR = 1.354, $p = 0.005$).

Conclusions The percentage of $CD45^+$ EPCs in PBMCs may be a potential biomarker for predicting 28-day disease progression in patients with HBV-ACLF. These findings highlight their possible clinical utility for risk stratification.

Keywords Acute-on-chronic liver failure, $CD45^+$ erythroid progenitor cells, Logistic regression, Biomarker, Disease progression

Introduction

Acute-on-chronic liver failure (ACLF) is a severe clinical syndrome characterized by the rapid deterioration of liver function in individuals with underlying chronic liver disease, with high mortality and significant morbidity [1]. The Asia-Pacific region demonstrates an estimated ACLF incidence of 5.7 cases per 1,000 person-years (95% confidence interval [CI]: 5.4–6.0) [2]. This contrasts sharply with European populations showing a significantly higher incidence of 20.1 cases per 1,000 person-years (95% CI: 19.5–20.6) [2]. Mortality data have highlighted profound clinical disparities among patients. Patients with ACLF experience 28-day mortality rates > 20% compared to < 5% in those with acute decompensated cirrhosis without ACLF [2]. Transplant-free mortality shows striking contrasts between ACLF and non-ACLF cohorts, with 28-day (32.8% versus 1.8%) and 90-day (51.2% versus 9.8%) mortality rates [2, 3]. Among survivors, the risk of hepatocellular carcinoma (HCC) remains elevated and comparable to that of patients with compensated cirrhosis, thus necessitating long-term surveillance [4].

Frequent complications, including renal failure, hepatic encephalopathy (HE), ascites, and infections, significantly increase mortality risk and complicate clinical management [4–6]. HE, renal failure, and sepsis occur in 30–50% of patients with ACLF and are major contributors to poorer outcomes, with infections common triggers linked to increased mortality [3, 7]. Therefore, early prognostic assessment is essential to improve patient survival and guide treatment strategies.

Recent studies have underscored the critical role of immune dysregulation in ACLF pathogenesis, particularly the involvement of systemic inflammation and immune cell dysfunction [8]. Erythroid progenitor cells (EPCs) are a heterogeneous population derived from the bone marrow, which can mature into erythrocytes [9]. EPCs are found in the spleen, peripheral blood, and bone marrow [9]. They are typically identified by the expression of CD71 (transferrin receptor) and CD235a (glycoprotein A) in humans (CD71 and Ter119 in mice), with the presence of a nucleus distinguishing immature EPCs from mature, enucleated red blood cells [10, 11]. CD45, a common leukocyte antigen, is expressed on a subset of EPCs and may inhibit erythroid differentiation to maintain the cells in an immature state and create two subpopulations ($CD45^+$ and $CD45^-$) [10, 12]. Under pathological conditions such as anemia [13], chronic infection [14], and cancer [15], impaired differentiation results in the accumulation of immature EPCs in circulation. These cells exhibit immunosuppressive properties mediated through interleukin-10 (IL-10), transforming growth factor-beta (TGF-β), and reactive oxygen species (ROS) [9]. For example, $CD45^+$ EPCs enhance ROS generation via NADPH oxidase 2 (*NOX2*) overexpression to suppress T cell function [9]. In the tumor microenvironment, EPCs work synergistically with myeloid-derived suppressor cells to deplete arginine and inhibit T cell oxidative phosphorylation, thus contributing to immune evasion [13].

Patients with chronic hepatitis B (CHB) have significantly increased $CD45^+$ EPC ($CD45^+$ $CD71^+$ $CD235a^+$) percentages in the peripheral blood and liver tissues, especially during the immune-tolerant phase [16]. These cells may also contribute to HCC progression [9, 17]. Notably, patients with hepatitis B virus-related ACLF (HBV-ACLF) exhibit more severe immune dysfunction and inflammation compared to patients with CHB [18]. Although elevated $CD45^+$ EPC percentages have been discovered in CHB, their prognostic significance in HBV-ACLF remains unclear. In this study, we investigated $CD45^+$ EPCs as potential biomarkers reflecting immune dysfunction and correlated with clinical outcomes in patients with HBV-ACLF. They may also provide a potential novel tool for early risk stratification and therapeutic targeting to improve patient management and outcomes in this high-risk population.

Methods

Study design and population

Between January 2021 and December 2023, we enrolled 167 patients (102 with CHB and 65 with HBV-ACLF) receiving standardized antiviral therapy at the Third Affiliated Hospital of Sun Yat-sen University. During admission, venous blood samples were collected from each patient, processed immediately, and cryopreserved at -80 °C until investigation of circulating EPCs. The diagnostic criteria for CHB followed the standardized Prevention and Treatment Guidelines for Chronic Hepatitis B [19]. All patients with CHB were stable outpatients without cirrhosis.

The Chinese Group on the Study of Severe Hepatitis B–Acute-on-Chronic Liver Failure criteria were used to diagnose HBV-ACLF [20]. Patients were excluded if they had drug-induced liver injury, were receiving low-dose glucocorticoids or thymosin α1, had a history of intravenous drug abuse, were infected with HIV, had alcohol-related liver disease, had concurrent hepatitis C or D infection, had malignant tumor, had autoimmune liver disease, or were pregnant. The detailed exclusion criteria are provided in the Supplementary Methods section (Additional file 1: Supplementary Materials and Methods). The inclusion and exclusion criteria for patients with HBV-ACLF are illustrated in Fig. 1. Liver tissue specimens were obtained from two distinct cohorts: six patients with CHB undergoing diagnostic biopsies and six patients with HBV-ACLF during liver transplantation. This study adhered strictly to the ethical principles of the Declaration of Helsinki (2013 revision). Additionally, the research protocol was approved by the ethics committee of Third Affiliated Hospital of Sun Yat-sen University (Approval No. RG2023-264-02). Written informed consent was obtained from all participants before enrollment.

Flow cytometry for EPCs expression

Peripheral blood samples (10 mL) were collected from each participant using ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes. Peripheral blood mononuclear cells (PBMCs) were isolated via Lymphoprep™ density gradient centrifugation (Stemcell, Vancouver, Canada) according to the manufacturer's protocol and then cryopreserved in liquid nitrogen until flow-cytometry analysis. CytoFLEXLX (Beckman Coulter Life Sciences) was used to assess the cell phenotypes, and data were analyzed using CytExpert and FlowJo V10.0.7 (FlowJo, OR, USA). Data were obtained from a minimum of 50,000 events as the percentage of tagged cells within a live-cell gate. Flow cytometric sorting was performed using a BD FACSAria III cell sorter (BD Bioscience). Fluorescently labeled antibodies (listed in Additional file 2: Table S1) were titrated to optimal concentrations based on preliminary checkerboard experiments. Additional methodology can be found in the supplemental material (Additional file 1: Supplementary Materials and Methods), including a list of the reagents and antibodies used (Additional file 2: Table S1).

Baseline, complications, and outcomes

A comprehensive dataset, including demographic characteristics, biochemical parameters, and clinical outcomes, was systematically collected in a dedicated HBV-ACLF registry database. At admission, standardized data collection included baseline characteristics (age, sex), hepatic function markers (aspartate aminotransferase [AST], gamma-glutamyl transpeptidase [GGT], alanine aminotransferase [ALT], albumin [ALB], total, direct, and

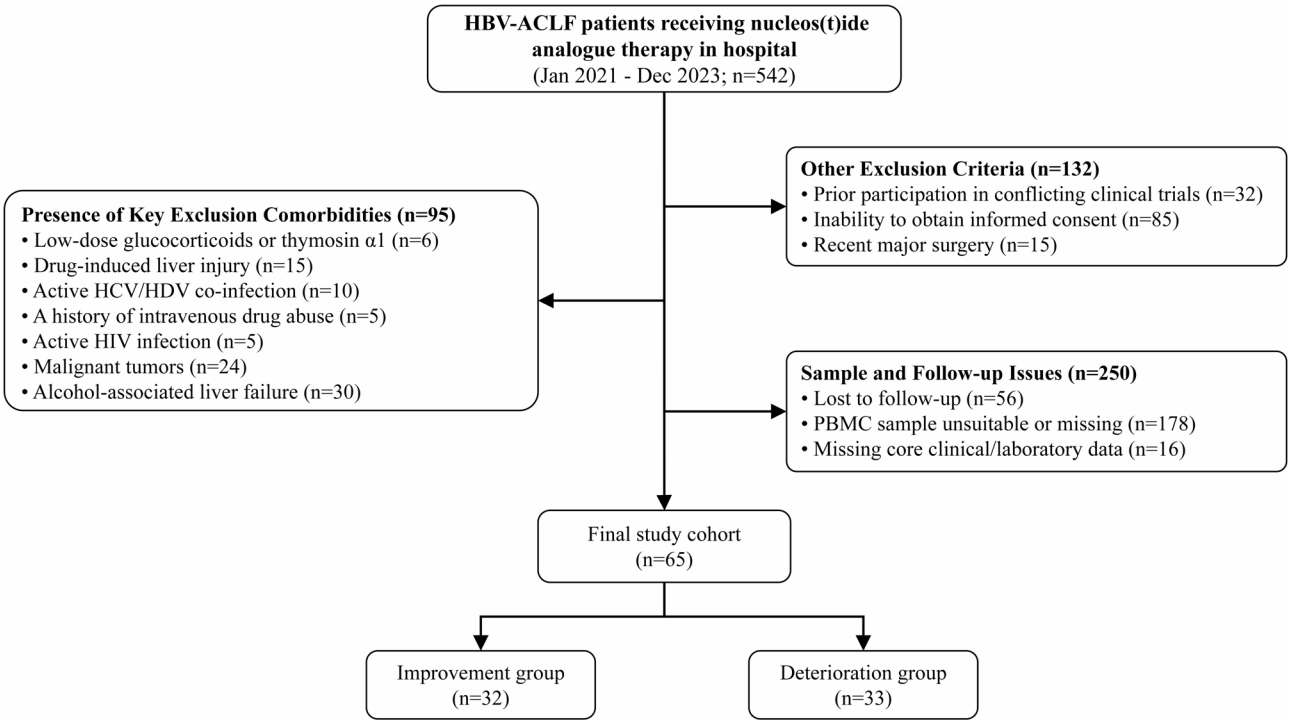

Fig. 1 Flowchart of patients with HBV-ACLF. HBV-ACLF, hepatitis B virus-related acute-on-chronic liver failure; HCV, hepatitis C virus; HDV, hepatitis D virus; IV, intravenous; HIV, human immunodeficiency virus; PBMC, peripheral blood mononuclear cell; Jan, January; Dec, December

indirect bilirubin), renal/metabolic indicators (creatinine, sodium), coagulation profiles (prothrombin activity [PTA], international normalized ratio [INR]), hematologic parameters (hemoglobin [Hb], hematocrit [Hct], white blood cell [WBC] count, neutrophil-lymphocyte ratio [NLR], platelet [PLT]) count, inflammatory biomarkers (procalcitonin [PCT]), prognostic scores (Model for End-Stage Liver Disease [MELD]), and quantitative measures of erythroid progenitors (EPCs in PBMC, $CD45^+$ EPCs in PBMC), along with documentation of complications including HE, infection, and organ failures. The severity and prognosis of liver disease were assessed using the MELD score.

Patients with ACLF were prospectively stratified into improvement ($n=32$) and deterioration ($n=33$) groups based on their 28-day clinical trajectories following treatment initiation. The improvement group was characterized by the resolution of clinical symptoms (fatigue, anorexia, ascites, bleeding, HE), normalized liver function (total bilirubin [TB] < 5×upper limit of normal [ULN], PTA > 40%, INR < 1.5), and reduced MELD scores post-treatment. The deterioration group exhibited progressive worsening of symptoms (fatigue, anorexia, ascites, bleeding), deteriorating liver function, new/persisting complications (e.g., organ failure), and elevated MELD scores post-treatment. Outcomes at 28 days were assessed using medical records or direct phone calls with patients or their family members.

Statistical analysis

Data are presented as means ± standard deviation (SD), medians (interquartile range, IQR), or counts (%). Continuous variables with a normal distribution were compared using Student's t-test, while non-normally distributed variables were analyzed using the Mann–Whitney U test. Categorical variables are expressed as frequencies (%) and compared using Fisher's exact or chi-squared (χ^2) tests, as appropriate. Correlation analyses were performed using Pearson's correlation coefficients for normally distributed continuous variables and Spearman's rank coefficients for non-normally distributed variables. Receiver operating characteristic (ROC) curve analysis was employed to assess the predictive value of $CD45^+$ EPCs in PBMCs for 28-day clinical deterioration. The area under the curve (AUC) and 95% CIs were also computed. The Youden index was used to determine the optimal cutoff value. A binary logistic regression model was applied to generate odds ratios (ORs) with 95% CIs for $CD45^+$ EPC percentage cutoff. Statistical significance was defined as a two-tailed $p<0.05$. Bidirectional stepwise logistic regression analysis was performed using the MASS package (version 7.3.61) in R (version 4.4.1). All remaining statistical analyses were performed with IBM SPSS Statistics for Windows, version 26.0 (Armonk, NY, USA).

Table 1 Clinical characteristics and $CD45^+$ EPC expression in CHB and ACLF patients

Clinical Feature	CHB ($n=102$)	ACLF ($n=65$)	*p* value
Age (years)	39.67 ± 9.73	47.05 ± 13.47	<0.001
Gender (Male%)	87.3	89.2	0.701
ALT (U/L)	63.10 ± 106.72	116.84 ± 175.25	0.031
AST (U/L)	40.06 ± 40.26	123.16 ± 123.09	<0.001
Hb (g/L)	139.85 ± 21.83	100.73 ± 23.72	<0.001
WBC count (10^9/L)	5.81 ± 2.16	7.70 ± 4.15	0.001
PLT count (10^9/L)	205.79 ± 67.51	99.69 ± 62.28	<0.001
EPC in PBMC (%)	0.84 ± 0.78	9.02 ± 13.98	<0.001
$CD45^+$EPC in PBMC (%)	0.18 ± 0.15	1.48 ± 1.79	<0.001

Data are presented as mean ± standard deviation (SD); ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHB, chronic hepatitis B; $CD45^+$EPC, $CD45^+$ erythroid progenitor cells; EPC, erythroid progenitor cells; Hb, hemoglobin; PLT, platelet; WBC, white blood cell

Results

Clinical characteristics of patients with CHB and HBV-ACLF

This study included 65 patients with HBV-ACLF and 102 patients with CHB. Compared with patients with CHB, patients with HBV-ACLF exhibited significantly elevated markers of liver injury, including ALT (116.84 ± 175.25 vs. 63.10 ± 106.72 U/L; $p=0.031$) and AST (123.16 ± 123.09 vs. 40.06 ± 40.26 U/L; $p<0.001$). Hematologic analysis showed reduced Hb concentration (100.73 ± 23.72 vs. 139.85 ± 21.83 g/L; $p<0.001$) and PLT count (99.69 ± 62.28 vs. 205.79 ± 67.51 × 10^9/L; $p<0.001$), alongside elevated WBC count (7.70 ± 4.15 vs. 5.81 ± 2.16 × 10^9/L; $p=0.001$). Flow cytometry analysis showed a significantly higher percentage of EPCs in patients with HBV-ACLF (9.02% ± 13.98% vs. 0.84% ± 0.78%; $p<0.001$), with a corresponding increase in the percentage of $CD45^+$ EPCs in PBMCs (1.48% ± 1.79% vs. 0.18% ± 0.15%; $p<0.001$). No significant differences were observed in sex distribution between the two groups ($p>0.05$; Table 1). Although the age between CHB and ACLF groups exhibited a significant difference ($p<0.001$), we stratified the CHB cohort into two age subgroups and compared the older subgroup with ACLF group to eliminate age-related confounding factors. Notably, further analysis revealed that despite comparable age distributions between the groups ($p=0.891$), the percentages of EPCs and $CD45^+$EPCs in PBMCs remained significantly elevated in the ACLF group (Additional file 2: Table S2).

$CD45^+$EPC percentage in PBMCs and counts in liver tissues from patients with CHB and HBV-ACLF

We analyzed the percentages of circulating EPCs and $CD45^+$ EPCs in PBMCs from 102 patients with CHB and 65 patients with HBV-ACLF using flow cytometry. The gating strategy for detecting $CD45^+$EPCs in circulating PBMCs is illustrated in Fig. 2a. The results revealed

significantly elevated percentages of EPCs and $CD45^+$ EPCs in patients with HBV-ACLF (both $p < 0.0001$, Fig. 2b). Additionally, we evaluated intrahepatic $CD45^+$ EPCs counts in liver tissue samples from six patients with CHB and six with HBV-ACLF. As shown in Fig. 2c, immunofluorescence staining demonstrated higher counts of $CD45^+$ EPCs in liver tissues of patients with HBV-ACLF than in those of patients with CHB, suggesting a potential role for these cells in hepatic injury and ACLF progression.

Correlation of $CD45^+$EPCs percentages with clinical indicators and complications

We analyzed the correlations between the percentages of $CD45^+$ EPCs and clinical indicators in patients with ACLF using Pearson's correlation analysis. The associations between the percentages of $CD45^+$ EPCs and clinical indicators in patients with ACLF showed significant positive relations between the percentage of $CD45^+$ EPCs in PBMCs and WBC count ($r = 0.306$, $p = 0.013$; Fig. 3a), NLR ($r = 0.344$, $p = 0.006$; Fig. 3b), and serum Cr concentration ($r = 0.511$, $p < 0.001$; Fig. 3c). No significant associations were observed with AST, ALT, TB, sodium, ALB, PTA, INR, Hb concentration, or PLT count (Fig. 3 and Additional file 3: Figure S1). Spearman's correlation analysis revealed that the percentage of $CD45^+$ EPCs in PBMCs was positively correlated with 28-day disease progression ($r = 0.348$, $p = 0.005$; Fig. 4a) and HE occurrence ($r = 0.247$, $p = 0.047$; Fig. 4b). Death after the 28-day follow-up ($p = 0.291$), gastrointestinal bleeding ($p = 0.265$), acute renal failure ($p = 0.805$), and infection ($p = 0.849$) showed no significant associations. These results suggest that the percentage of $CD45^+$ EPCs in PBMCs may serve as a potential biomarker for HE and short-term prognosis in patients with ACLF, although further mechanistic studies are required.

Clinical characteristics of patients with HBV-ACLF stratified by clinical outcomes

Patients with HBV-ACLF were further divided into improvement ($n = 32$) and deterioration groups ($n = 33$) based on 28-day clinical outcomes. Table 2 presents the clinical parameters and EPC data for both groups. The results demonstrated a significantly higher percentage of $CD45^+$EPCs in PBMCs in the deterioration group compared to the improvement group (3.16% ± 5.30% vs. 0.95% ± 1.12%, $p = 0.025$). The improvement group showed significantly higher ALT (165.00 ± 231.40 U/L vs. 67.13 ± 55.81 U/L, $p = 0.026$) and GGT (74.35 ± 59.94 U/L vs. 40.23 ± 19.99 U/L, $p = 0.005$) concentrations compared to the deterioration group. In contrast, the deterioration group exhibited higher TB (436.87 ± 135.26 μmol/L vs. 252.52 ± 116.18 μmol/L, $p < 0.001$), direct bilirubin (236.87 ± 79.25 μmol/L vs. 154.83 ± 63.38 μmol/L, $p < 0.001$), and indirect bilirubin (200.00 ± 74.03 μmol/L vs. 103.63 ± 49.50 μmol/L, $p < 0.001$) concentrations. The deterioration group also had significantly lower Hb concentration (94.32 ± 26.19 g/L vs. 107.34 ± 19.08 g/L, $p = 0.026$), lower PTA (25.26% ± 9.20% vs. 37.93% ± 9.58%, $p < 0.001$), and higher INR (3.32 ± 1.22 vs. 2.02 ± 0.64, $p < 0.001$) compared with the improvement group.

ROC curve analysis

ROC curve analysis was used to evaluate the prognostic value of $CD45^+$ EPCs% in PBMCs for 28-day outcomes in patients with ACLF. These results demonstrated a significant predictive capacity, with an AUC of 0.718 (95% CI: 0.586–0.851; $p = 0.004$) (Fig. 5). Multiple laboratory variables were assessed for their diagnostic efficacy in predicting 28-day disease deterioration in patients with ACLF. Among these, $CD45^+$ EPC percentage showed a moderate diagnostic value (AUC: 0.718). In comparison, both the MELD score (AUC: 0.830, 95% CI: 0.727–0.933; $p < 0.001$) and INR (AUC: 0.815, 95% CI: 0.705–0.925; $p < 0.001$) exhibited a high accuracy, indicating a strong diagnostic performance. The NLR had a weaker discriminatory power (AUC: 0.698, 95% CI: 0.555–0.840; $p = 0.009$) but remained statistically significant. These results suggested that the MELD score and INR are the most robust predictors of 28-day outcomes in ACLF, whereas $CD45^+$ EPCs may serve as a supplementary biomarker for prognosis assessment. Using the optimal cutoff of 1.016% (determined by Youden index, sensitivity = 0.636, specificity = 0.781), patients were stratified into the low ($n = 39$, $CD45^+$ EPCs% <1.016) and high ($n = 26$, $CD45^+$ EPCs% ≥1.016) $CD45^+$ EPCs groups.

Clinical characteristics and outcomes of patients with HBV-ACLF stratified by the percentage of $CD45^+$ EPCs

Comparative analysis revealed distinct clinical profiles between the two groups (Table 3). The low-$CD45^+$ EPCs group had significantly higher GGT concentration (67.54 ± 55.85 vs. 39.91 ± 19.37 U/L; $p = 0.007$), whereas the high-$CD45^+$ EPCs group exhibited elevated bilirubin concentrations, including TB (422.35 ± 107.50 vs. 298.90 ± 162.70 μmol/L; $p = 0.001$), direct bilirubin (232.09 ± 55.87 vs. 174.48 ± 88.34 μmol/L; $p = 0.007$), and indirect bilirubin (191.11 ± 70.17 vs. 130.89 ± 76.55 μmol/L; $p = 0.004$).

Inflammatory profiling further highlighted marked differences between the groups. The high-$CD45^+$ EPCs group displayed higher neutrophil count (fraction of total leukocytes) (0.72 ± 0.10 vs. 0.60 ± 0.13; $p < 0.001$), reduced lymphocyte count (fraction of total leukocytes) (0.15 ± 0.08 vs. 0.24 ± 0.10; $p = 0.001$), and elevated NLR (6.53 ± 4.28 vs. 3.70 ± 3.50; $p = 0.005$). Clinically, the high-$CD45^+$EPCs group also had significantly

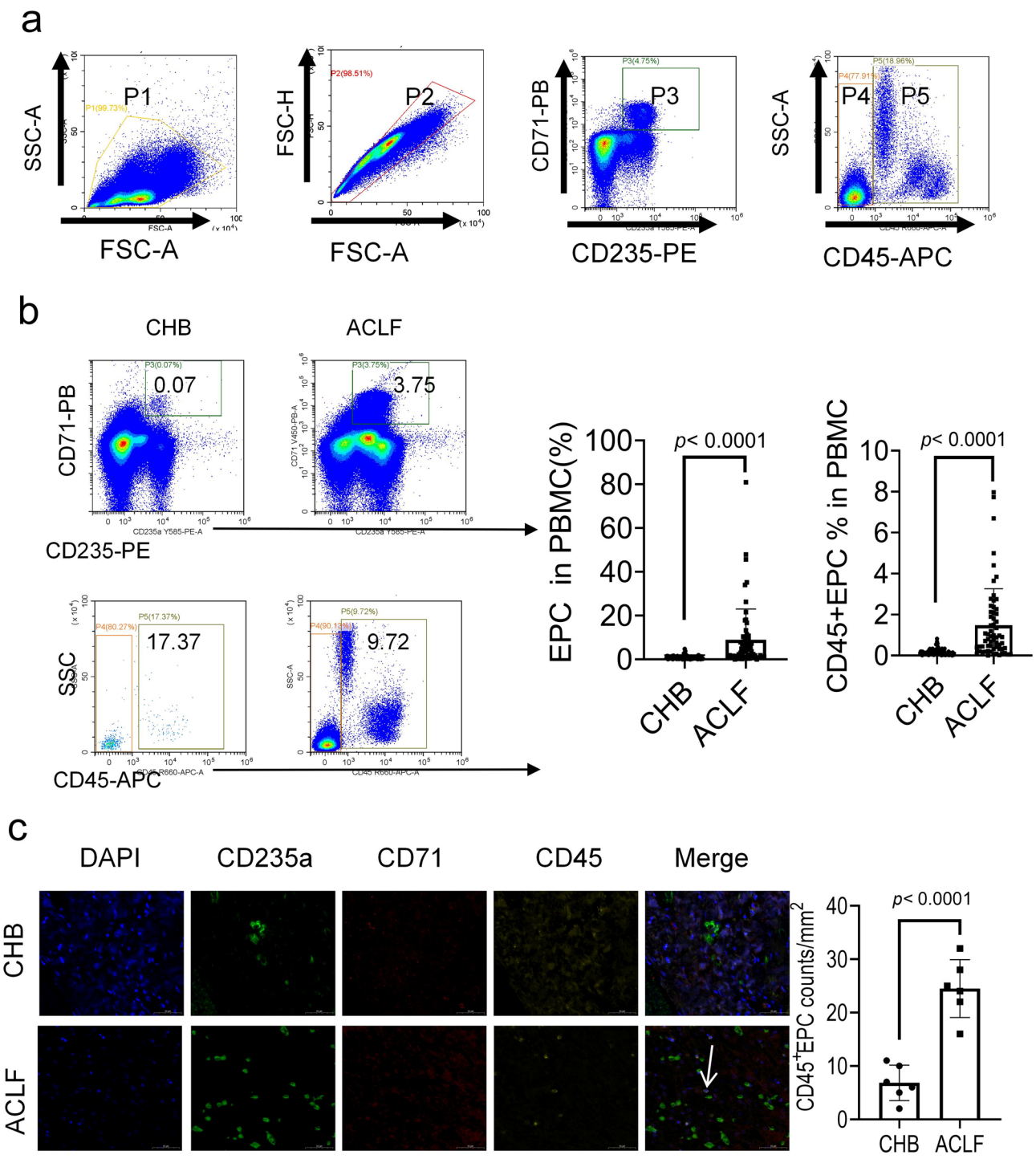

Fig. 2 $CD45^+$ EPCs percentages in PBMCs and counts in liver tissue of patients with ACLF and CHB. (**a**) Gating strategy for detecting $CD45^+$EPCs in circulating PBMCs using flow cytometry. (**b**) Representative flow cytometry plots showing the percentages of $CD45^+$ EPCs in PBMCs from patients with CHB ($n = 102$) and ACLF ($n = 65$). Data are presented as mean ± SD. $CD45^+$EPCs ($CD45^+CD71^+CD235a^+$) were quantified using flow cytometry. (**c**) Immunofluorescence staining of formalin-fixed liver tissue from patients with CHB and HBV-ACLF. $CD45^+$ $CD71^+$ $CD235a^+$ erythroid cells visualized using the following antibodies: anti-CD71 with goat anti-rabbit Alexa Fluor 594 (red), anti-CD235a with goat anti-rat Alexa Fluor 488 (green), anti-CD45 with goat anti-mouse Alexa Fluor 647 (yellow) and DAPI (blue) for nuclei. White arrows indicate $CD45^+$EPCs. Scale bar = 50 μm. CD45, cluster of differentiation 45; EPC, erythroid progenitor cell; PBMC, peripheral blood mononuclear cell; ACLF, acute-on-chronic liver failure; CHB, chronic hepatitis B; CD71, transferrin receptor; CD235a, glycophorin A; HBV, hepatitis B virus; DAPI, 4′,6-diamidino-2-phenylindole; SD, standard deviation

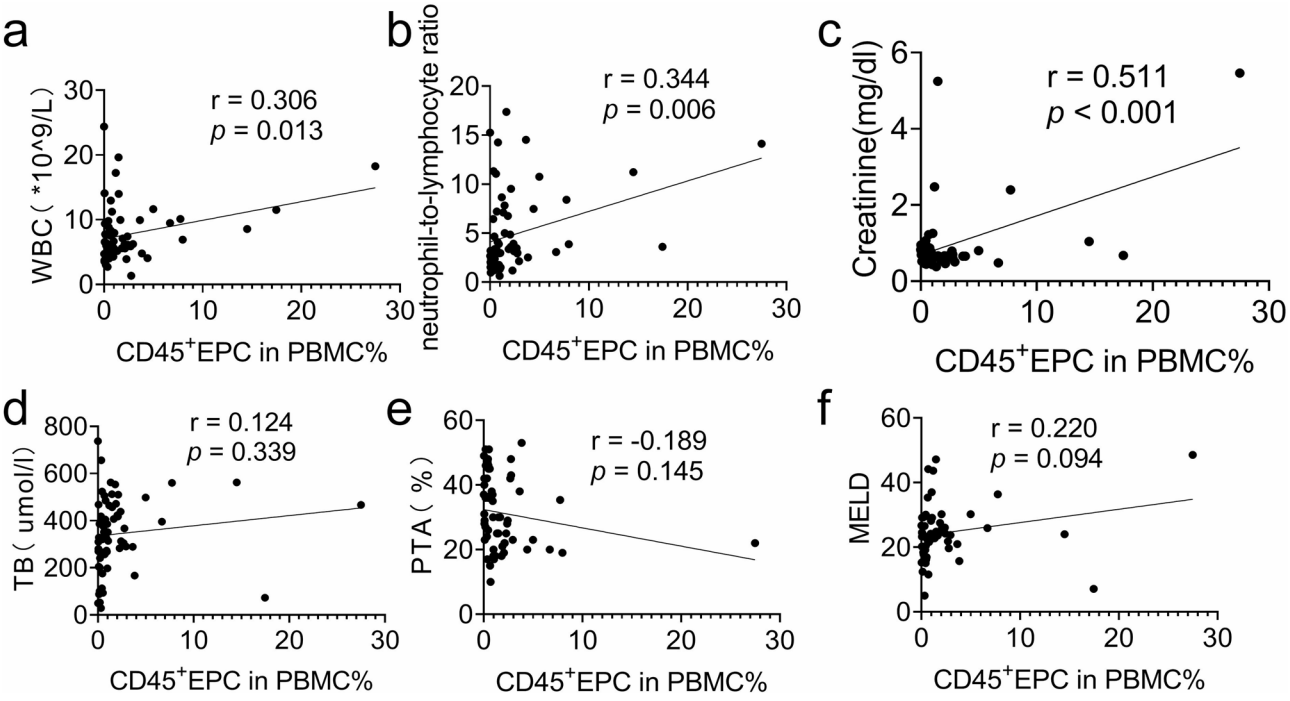

Fig. 3 Pearson correlation analysis of the associations between the percentages of $CD45^+$ EPCs in PBMCs and clinical parameters in HBV-ACLF ($n = 65$). HBV-ACLF, hepatitis B virus-related acute-on-chronic liver failure; CD45⁺EPC, CD45-positive erythroid progenitor cell; PBMCs, peripheral blood mononuclear cells; TB, total bilirubin; PTA, prothrombin time activity; MELD, Model for End-Stage Liver Disease

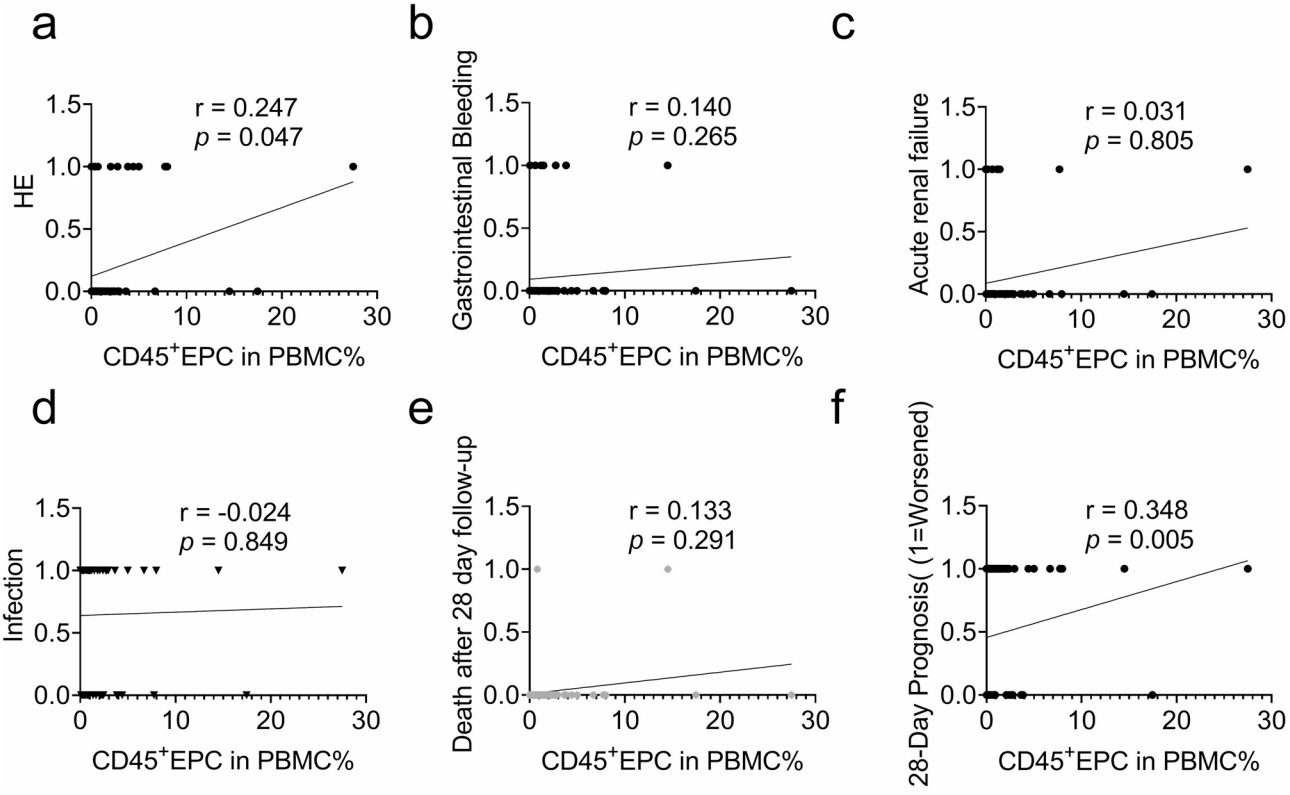

Fig. 4 Spearman correlation analysis showing the percentage of $CD45^+$EPC in PBMCs associated with complications and outcomes in HBV-ACLF ($n = 65$). HBV-ACLF, hepatitis B virus-related acute-on-chronic liver failure; CD45⁺EPCs, CD45-positive erythroid progenitor cells; PBMCs, peripheral blood mononuclear cells; HE, hepatic encephalopathy

Table 2 Comparison of clinical characteristics and CD45$^+$ EPC percentages between the improvement and deterioration groups with ACLF based on their clinical status at 28 days post treatment

Variable	Improvement Group ($n=32$)	Deterioration Group ($n=33$)	p value
Age (years)	45.53 ± 11.60	48.52 ± 15.10	0.255
Gender (Male%)	26 (87.5%)	30 (90.9%)	0.658
ALT (U/L)	165.00 ± 231.40	67.13 ± 55.81	0.026
AST (U/L)	127.69 ± 135.39	118.48 ± 111.01	0.769
GGT (U/L)	74.35 ± 59.94	40.23 ± 19.99	0.005
Total Bilirubin (umol/L)	252.52 ± 116.18	436.87 ± 135.26	<0.001
DB (umol/L)	154.83 ± 63.38	236.87 ± 79.25	<0.001
IB (umol/L)	103.63 ± 49.50	200.00 ± 74.03	<0.001
Creatinine (mg/dL)	0.72 ± 0.16	1.14 ± 1.27	0.088
Sodium (mmol/L)	137.34 ± 3.52	137.29 ± 5.50	0.964
ALB	33.57 ± 4.53	34.25 ± 3.80	0.527
PTA (Prothrombin Activity, %)	37.93 ± 9.58	25.26 ± 9.20	<0.001
INR	2.02 ± 0.64	3.32 ± 1.22	<0.001
WBC count (10^9/L)	6.68 ± 2.51	8.69 ± 5.13	0.049
Hb (g/L)	107.34 ± 19.08	94.32 ± 26.19	0.026
Hct	0.29 ± 0.05	0.27 ± 0.05	0.106
PLT count (10^9/L)	113.13 ± 81.39	86.67 ± 31.38	0.093
Neutrophils (fraction of total leukocytes)	0.61 ± 0.09	0.69 ± 0.15	0.018
Lymphocytes (fraction of total leukocytes)	0.24 ± 0.08	0.17 ± 0.11	0.004
Neutrophil to Lymphocyte Ratio	3.11 ± 2.37	6.50 ± 4.64	0.001
PCT (ng/ml)	0.56 ± 0.39	0.93 ± 0.71	0.044
MELD	19.97 ± 6.07	29.09 ± 8.29	<0.001
EPC in PBMC (%)	7.12 ± 11.40	11.56 ± 15.86	0.201
CD45$^+$EPC in PBMC (%)	0.95 ± 1.12	3.16 ± 5.30	0.025
CD45$^+$EPC in PBMC (%)			0.004
≤ 0.80	21 (65.6%)	10 (30.3%)	
>0.80	11 (34.3%)	23 (69.7%)	
Complication and Outcome			
HE	3 (9.4%)	9 (27.3%)	0.063
Infection	18 (56.3%)	24 (72.7%)	0.165
Gastrointestinal Bleeding	3 (9.4%)	4 (12.1%)	0.721
Hepatorenal Syndrome	0 (0)	5 (15.2%)	0.022
Respiratory Failure	0 (0)	6 (18.2%)	0.011
Circulatory Failure	0 (0)	4 (12.1%)	0.042
Death	0 (0)	2 (6.1%)	0.157

Data are presented as mean ± standard deviation (SD). ACLF, acute-on-chronic liver failure; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CD45$^+$EPC, CD45-positive erythroid progenitor cells; DB, direct bilirubin; PBMC, peripheral blood mononuclear cells; GGT, gamma-glutamyl transpeptidase; HE, hepatic encephalopathy; Hb, hemoglobin; Hct, hematocrit; IB, indirect bilirubin; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; PCT, procalcitonin; PLT, platelet; PTA, prothrombin activity; WBC, white blood cell

higher deterioration rates (73.1% vs. 35.9%; $p=0.003$) and increased HE incidence (30.8% vs. 10.3%; $p=0.037$) (Table 3).

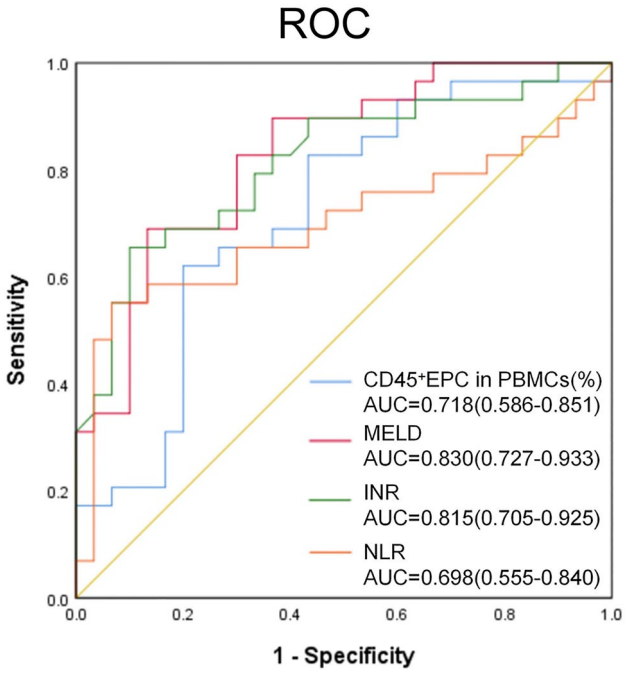

Fig. 5 ROC curve analysis of percentages of CD45$^+$ EPCs in PBMCs to predict 28-day prognosis in ACLF. ACLF, acute-on-chronic liver failure; AUC, area under the curve; CD45$^+$EPCs, CD45-positive erythroid progenitor cells; INR, international normalized ratio; PBMC, peripheral blood mononuclear cell; NLR, neutrophil-to-lymphocyte ratio; MELD, Model for End-stage Liver Disease; ROC, receiver operating characteristic

Multivariate logistic regression analysis for disease progression

In accordance with the Events Per Variable criterion, we retained five variables to mitigate overfitting and parameter estimation bias. And we performed stepwise multivariate logistic regression analysis incorporating CD45$^+$EPC in PBMCs (%), Hb (g/L), INR, ALT (U/L), and NLR. The final model identified INR (adjusted OR 6.098, 95% CI 2.189–16.984, $p<0.001$) and NLR (adjusted OR 1.354, 95% CI 1.098–1.669, $p=0.005$) as independent predictors of 28-day clinical deterioration in patients with HBV-ACLF (Table 4). Given the limited sample size, these results require cautious interpretation prior to confirmation in large-scale prospective cohort studies.

Discussion

HBV-ACLF is a severe clinical condition marked by the rapid deterioration of liver function in patients with underlying chronic liver disease [5, 21]. This condition presents significant health risks and is associated with high morbidity and mortality rates [22]. The pathophysiology of HBV-ACLF involves a complex interaction between chronic liver injury, acute exacerbations, and multiorgan failure, leading to a poor patient prognosis if not addressed promptly [6]. Currently, ACLF outcomes are assessed using scoring systems such as MELD, which

Table 3 Comparisons of clinical characteristics and outcomes between dichotomized CD45$^+$EPCs in PBMCs (%) in patients with ACLF

Variable	Low CD45$^+$EPC ($n=39$)	High CD45$^+$EPC ($n=26$)	*p* value
Age (years)	45.62 ± 13.04	49.19 ± 14.08	0.288
Gender (Male%)	33 (84.6%)	25 (96.2%)	0.658
ALT (U/L)	138.44 ± 213.92	81.75 ± 71.45	0.134
AST (U/L)	128.49 ± 146.42	114.50 ± 72.74	0.665
GGT (U/L)	67.54 ± 55.85	39.91 ± 19.37	0.007
Total Bilirubin (umol/L)	298.90 ± 162.70	422.35 ± 107.50	0.001
DB (umol/L)	174.48 ± 88.34	232.09 ± 55.87	0.007
IB (umol/L)	130.89 ± 76.55	191.11 ± 70.17	0.004
Creatinine (mg/dL)	0.75 ± 0.20	1.20 ± 1.41	0.144
Sodium (mmol/L)	137.45 ± 3.61	137.12 ± 5.81	0.783
ALB	33.58 ± 3.63	34.44 ± 4.95	0.446
PTA (Prothrombin Activity, %)	33.61 ± 11.75	27.94 ± 9.86	0.053
INR	2.51 ± 1.33	2.94 ± 0.84	0.152
WBC count (10^9/L)	7.27 ± 3.86	8.35 ± 4.55	0.306
Hb (g/L)	101.27 ± 25.93	99.92 ± 20.42	0.824
Hct	0.28 ± 0.05	0.28 ± 0.05	0.571
PLT count (10^9/L)	109.88 ± 73.90	84.42 ± 34.99	0.107
Neutrophils (fraction of total leukocytes)	0.60 ± 0.13	0.72 ± 0.10	<0.001
Lymphocytes (fraction of total leukocytes)	0.24 ± 0.10	0.15 ± 0.08	0.001
Neutrophil to Lymphocyte Ratio	3.70 ± 3.50	6.53 ± 4.28	0.005
PCT (ng/ml)	0.60 ± 0.49	0.92 ± 0.67	0.086
MELD	22.41 ± 7.90	27.90 ± 8.61	0.015
Complication and Outcome			
HE	4 (10.3%)	8 (30.8%)	0.037
Infection	24 (61.5%)	18 (69.2%)	0.525
Gastrointestinal Bleeding	2 (5.1%)	5 (19.2%)	0.072
Hepatorenal Syndrome	2 (5.1%)	3 (11.5%)	0.342
Respiratory Failure	4 (10.3%)	2 (7.7%)	0.726
Circulatory Failure	2 (5.1%)	2 (7.7%)	0.673
Deterioration	14 (35.9%)	19 (73.1%)	0.003
Death	1 (2.6%)	1 (3.8%)	0.769

High CD45$^+$EPC percentages correspond to CD45$^+$EPC in PBMCs (%) ≥ 1.016. Data are presented as mean ± standard deviation (SD). ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; ALB, albumin; AST, aspartate aminotransferase; EPC in PBMC (%), the percentage of erythroid progenitor cells in peripheral blood mononuclear cells; GGT, gamma-glutamyl transpeptidase; DB, direct bilirubin; Hct, hematocrit; Hb, hemoglobin; HE, hepatic encephalopathy; IB, indirect bilirubin; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; PLT, platelet; PCT, procalcitonin; PTA, prothrombin activity; WBC, white blood cell

primarily focus on liver function and do not fully capture immune dysfunction for prognosis [23].

The results of the present study identified CD45$^+$ EPCs as a potential novel biomarker for HBV-ACLF, highlighting their role in disease progression. We observed that patients with HBV-ACLF had significantly elevated circulating and intrahepatic CD45$^+$ EPC percentages or

Table 4 Logistic regression of factors influencing poor outcome

Variable	Adjusted OR	95%CI	Adjusted *p* value
INR	6.098	2.189, 16.984	<0.001
NLR	1.354	1.098, 1.669	0.005
ALT(U/L)	0.993	0.984, 1.002	0.133

Variables included in the model: CD45$^+$EPC in PBMC (%), Hb (g/L), INR, ALT (U/L), and NLR. ALT, alanine aminotransferase; INR, international normalized ratio; NLR, neutrophil-to-lymphocyte ratio; CD45$^+$ EPC in PBMC (%), the percentage of CD45$^+$ erythroid progenitor cells in peripheral blood mononuclear cells; Hb, hemoglobin

counts compared with patients with CHB. This increase suggests the systemic and localized involvement of CD45$^+$ EPC in ACLF pathogenesis. One potential explanation for this increase is compensatory myelopoiesis, a phenomenon observed in conditions such as anemia [24] and malignancy [17], in which bone marrow stress triggers extramedullary hematopoiesis. In ACLF, anemia and systemic inflammation may similarly drive the expansion of CD45$^+$ EPCs as part of a dysregulated hematopoietic response. However, the absence of a control group with compensated cirrhosis limits our ability to determine whether the elevated EPC percentage reflected acute decompensation or was related to chronic background cirrhosis. Future studies should include such controls to help clarify this distinction.

High percentages of CD45$^+$ EPCs were positively correlated with clinical parameters, such as creatinine concentration, WBC count, and NLR, indicating their strong association with multiorgan dysfunction and systemic inflammation. In this cohort, although CD45$^+$ EPC percentage showed only a weak correlation with MELD scores ($r=0.22$, $p=0.094$), patients with high CD45$^+$ EPC percentages had worse 28-day outcomes even within similar MELD score ranges. This finding suggests that CD45$^+$ EPCs may improve clinical risk stratification. Previous studies have reported that CD45$^+$ EPCs in HCC promote coagulation during portal vein tumor thrombus by upregulating key coagulation factors [17]. However, our study revealed no significant association between the percentages of CD45$^+$ EPCs and coagulation markers. The baseline coagulation profiles in patients with ACLF are systemically disrupted, potentially obscuring detectable correlations between CD45$^+$ EPCs and conventional coagulation parameters. Considering the small sample size, further validation in studies with larger samples is warranted.

Notably, patients with high percentages of CD45$^+$ EPCs had a significantly higher rate of 28-day disease deterioration and a greater incidence of HE. This highlights the potential of CD45$^+$ EPCs as complementary biomarkers to MELD scores to predict complications such as HE and short-term deterioration. However, we did not track CD45$^+$ EPC percentage over time, which could provide valuable insights for monitoring disease progression. The

absence of longitudinal tracking of CD45⁺ EPCs is a key limitation, as dynamic changes in EPC percentage during disease progression could provide critical insights into their temporal relationship with organ failure. Moreover, this retrospective analysis could not fully account for potential confounding factors, such as variations in infection characteristics (such as pathogen type and severity) and heterogeneity in treatment regimens, which may have biased the outcomes.

Patients with HBV-ACLF who experienced clinical deterioration within 28 days showed elevated percentages of CD45⁺ EPCs, along with reduced Hb concentrations and PTA, suggesting a potential link between ineffective erythropoiesis and coagulopathy. Mechanistically, anemia in ACLF may stimulate erythropoietin production [25], which paradoxically enhances EPC mobilization while failing to support their differentiation into mature erythrocytes. Future studies are needed to elucidate the pathways responsible for this impaired differentiation of EPCs in the context of ACLF.

The results of the ROC curve analysis confirmed the predictive value of CD45⁺ EPC for disease progression (AUC = 0.718), with percentages ≥ 1.016% predicting 28-day deterioration. Patients in the high-CD45⁺EPCs group exhibited marked hyperbilirubinemia, elevated NLR, and increased HE incidence. Notably, the elevated ALT levels in the improvement group contrasted with higher bilirubin levels in the deterioration group, suggesting distinct immune states. This dichotomy supports the "immune paralysis" hypothesis in ACLF [26], in which CD45⁺ EPCs may promote immunosuppressive myeloid cell expansion, potentially increasing infection risks and organ failure. Multivariate regression identified INR and NLR as independent predictors of a poor 28-day prognosis. These findings align with previous reports showing significant associations between total bilirubin, neutrophils, INR and age-adjusted Charlson comorbidity index and 28-day transplant-free mortality in HBV-ACLF [27]. Our results corroborate established knowledge about ACLF's core pathological features, including systemic inflammation and immune suppression [8, 28–30]. Additionally, the findings also address limitations of traditional MELD scores by providing dynamic immune status assessment and improved short-term prognosis prediction [31, 32].

We hypothesized that the association between CD45⁺ EPCs and short-term prognosis in ACLF may be mediated by their immunomodulatory roles in disease pathogenesis. One key immune regulatory mechanism of CD45⁺ EPCs is the suppression of T-cell function through metabolic interference. These cells highly express arginase-2 (Arg2), which depletes L-arginine in the microenvironment, thereby disrupting the T cell receptor signaling pathway and impairing cytokine secretion [33–35]. Additionally, CD45⁺ EPCs generate ROS, which damages T cell mitochondrial function and induces apoptosis [13, 36]. This combined metabolic stress may weaken the antiviral immune response, rendering patients with ACLF more vulnerable to secondary infections.

Furthermore, CD45⁺ EPCs may regulate immune responses in ACLF by secreting TGF-β, which promotes CD45⁺ EPC differentiation into regulatory T cells (Tregs). Through this pathway, CD45⁺ EPCs indirectly suppress T-cell activity [37]. Shahbaz et al. demonstrated continuous and high expression of TGF-β by CD71⁺ EPCs to support Treg development and function. In ACLF, excessive Treg activation may worsen immune paralysis, hinder virus clearance, and increase infection risk [37]. Moreover, the immunosuppressive properties of EPCs may diminish as they mature. Late-stage CD45⁻ EPCs, with reduced IL-4, IL-6, IL-10, and TGF-β expression, lose the ability to suppress T cell proliferation and inhibit dendritic cell activation [24]. Therefore, promoting EPC differentiation into mature erythrocytes may help alleviate immune suppression, thus providing a promising therapeutic strategy for ACLF.

CD45⁺ EPCs can also enhance immune suppression through the abnormal expression of immune checkpoint molecules. Recently, Long et al. showed that tumors induce a subset of CD45⁺ EPCs to differentiate into a cellular "erythroid-myeloid hybrid" population, known as erythroid-differentiated myeloid cells (EDMCs) [13]. EDMCs derived from CD45⁺ EPCs express high levels of programmed death-ligand 1 (PD-L1) [13]. This ligand binds to the PD-1 receptor on T cells activates Src homology 2 domain-containing phosphatase 2 (SHP2), and inhibits the phosphoinositide 3-kinase/protein kinase b/mechanistic target of rapamycin pathway (PI3K/AKT/mTOR) pathway [13]. This leads to metabolic reprogramming of T cells, inhibiting their activation and proliferation [33, 38]. This pathway not only weakens the antiviral capacity of T cells but may also explain the failed virus clearance and accelerated hepatocyte necrosis in HBV-ACLF by creating a dense immunosuppressive network. Taken together, this immune checkpoint-based inhibitory mechanism suggests that CD45⁺ EPCs likely play a complex immune regulatory role in ACLF.

In the liver environment of HBV-ACLF, the immunosuppressive effect of CD45⁺ EPCs may primarily occur through two pathways: metabolic interference and TGF-β-mediated expansion of Tregs. Because patients with ACLF often experience anemia and extramedullary hematopoiesis [39–41], the depletion of L-arginine caused by high Arg2 expression may be a key change in the hepatic microenvironment. Additionally, TGF-β-induced increase in Tregs may further exacerbate immune suppression, potentially driving disease

progression. The synergistic effects of these pathways may create a strong immunosuppressive state locally within the liver.

In summary, our findings suggest that an accumulation of immature CD45$^+$ EPCs may exacerbate immune dysfunction in HBV-ACLF. However, the mechanistic speculation is not experimentally validated in our study cohort. Future studies elucidating the mechanisms by which CD45$^+$ EPCs contribute to ACLF pathogenesis are critically needed. Besides, CD45$^+$ EPCs are a promising candidate biomarker that warrants further validation. Patients with elevated percentage of CD45$^+$ EPCs should be monitored closely, with regular assessments of disease progression.

This study has several strengths. It systematically reveals the association of CD45$^+$ EPCs with clinical indicators, complications, and 28-day disease progression in ACLF, offering both a tool for real-time monitoring of immunosuppressive status and a promising therapeutic target to reverse immune tolerance in ACLF management. The significantly elevated expression of CD45$^+$ EPCs not only establishes this cell subset as a novel prognostic biomarker but also suggests its pivotal role in modulating the hepatic immune microenvironment during disease progression [13, 42, 43]. If validated, high percentages of CD45$^+$ EPCs could become an integral component of precision medicine in ACLF management.

Despite its strengths, this study has some limitations that should be considered when interpreting the results. First, the generalizability of our findings is limited by the small sample size and single-center retrospective design. Additionally, the CHB group may differ in baseline characteristics such as liver fibrosis, reducing comparability. Therefore, future studies should include both patients with compensated and decompensated cirrhosis (without ACLF) as controls to better investigate changes in CD45$^+$ EPCs during acute decompensation episodes. Specifically, our results must be confirmed in future larger-scale, multicenter studies that incorporate independent validation cohorts. In addition, further validation through expanded liver tissue sampling is warranted to confirm these mechanistic associations. Second, the short 28-day follow-up period is insufficient to assess the long-term prognostic significance of CD45$^+$ EPCs. Future research is also needed to extend the follow-up durations to 90 days, incorporate liver transplantation outcomes and HCC risk, and explore targeted intervention strategies [13, 14, 44]. Third, we rigorously controlled for potential confounding effects of pharmacological interventions, including nucleos(t)ide analogues and immunomodulatory agents. Nevertheless, we acknowledge that some variations in supportive treatments (e.g., albumin administration and artificial liver-support systems) and the inherent risk of unmeasured confounding may have occurred and could not be fully adjusted for. Fourth, as CD45$^+$ EPC percentages were measured only at admission, how these percentage change over time with treatment or disease progression remains unclear. Longitudinal tracking of CD45$^+$ EPC percentage could provide valuable insights into disease trajectory. Moreover, current limitations in flow cytometry for detecting CD45$^+$ EPCs emphasize the need for simpler, more accessible methods such as enzyme-linked immunosorbent assay (ELISA) for broader clinical applications. Fifth, the absence of single-cell RNA sequencing spatial transcriptomics data limits the ability to resolve the heterogeneity of CD45$^+$ EPC subpopulations and their associated surface marker signatures. Ultimately, comprehensive studies incorporating both in vivo (animal models) and in vitro (cell-based) approaches are essential to elucidate the precise mechanistic role of CD45$^+$ EPCs in ACLF pathogenesis.

Conclusions

The results of this study demonstrated the significant elevation of CD45$^+$ EPC percentages in patients with HBV-ACLF. Moreover, this increase was associated with 28-day clinical deterioration and adverse complications. Immunofluorescence analysis further confirmed the marked accumulation of CD45$^+$ EPCs in the liver tissues of patients with ACLF. These findings suggest that CD45$^+$ EPCs may be a promising prognostic biomarker for disease progression and a potential therapeutic target for early intervention and precision treatment in ACLF.

Abbreviations

ACLF	Acute-on-chronic liver failure
ALB	Albumin
ALT	Alanine aminotransferase
Arg2	Arginase-2
AUC	Area under the curve
AST	Aspartate aminotransferase
CD235a	Glycoprotein A
CD45	Cluster of differentiation 45
CD71	Transferrin receptor
CHB	Chronic hepatitis B
CI	Confidence interval
Cr	Creatinine
DAPI	4′,6-diamidino-2-phenylindole
DB	Direct bilirubin
EDMCs	Erythroid-differentiated myeloid cells
EDTA	Ethylenediaminetetraacetic acid
EPC	Erythroid progenitor cell
ELISA	Enzyme-linked immunosorbent assay
GGT	Gamma-glutamyl transpeptidase
Hb	Hemoglobin
HBV-ACLF	Hepatitis B virus-related acute-on-chronic liver failure
HCC	Hepatocellular carcinoma
Hct	Hematocrit
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HE	Hepatic encephalopathy
HIV	Human immunodeficiency virus
IB	Indirect bilirubin
IL-10	Interleukin-10
INR	International normalized ratio

IQR	Interquartile range
IV	Intravenous
MELD	Model for End-stage Liver Disease
NLR	Neutrophil-to-lymphocyte ratio
NOX2	NADPH oxidase 2
OR	Odds ratio
PD-L1	Programmed death-ligand 1
PBMCs	Peripheral blood mononuclear cells
PCT	Procalcitonin
PI3K/AKT/mTOR	Phosphoinositide 3-kinase/protein kinase b/mechanistic target of rapamycin
PLT	Platelet
PTA	Prothrombin activity
ROC	Receiver operating characteristic
ROS	Reactive oxygen species
SD	Standard deviation
SHP2	Src homology 2 domain-containing phosphatase 2
TB	Total bilirubin
TGF-β	Transforming growth factor-beta
Tregs	Regulatory T cells
ULN	Upper limit of normal
WBC	White blood cell

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions
Nan Zhang, Hai-Shi Wu, and Xiu-Qing Pang: Investigation, Methodology, Software, Visualization, Writing- Original draft, and Writing- Reviewing and Editing. Cheng-You Yu: Methodology, and Writing- Reviewing and Editing. Xing Li: Project administration, Supervision, Writing- Original draft, and Writing- Reviewing and Editing. Zhi-Liang Gao: Funding acquisition, Project administration, Supervision, Writing- Original draft, and Writing- Reviewing and Editing.

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Data availability
The datasets used and analyzed in this investigation will be made available by the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Every procedure used in this study complied with the principles of the 1964 Declaration of Helsinki and its subsequent amendments, as well as institutional and national research committee's ethical standards, or similar ethical standards. The scientific and ethical committees of Sun Yat-sen University's Third Affiliated Hospital approved this study (Approval No. RG2023-264-02). Every participant included in this study provided their informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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