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ORIGINAL ARTICLE

Low platelets: a new and simple prognostic marker for patients with hepatitis E virus-related acute liver failure

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Abstract

Background and aims Hepatitis E virus-related acute liver failure (HEV-ALF) rapidly worsens and has a high mortality. However, no simple and specific parameters for predicting short-term mortality are available.

Methods A derivation cohort including 97 patients with HEV-ALF and another validation cohort were enrolled. Laboratory and clinical parameters were recorded. Platelet count, model for end-stage liver disease (MELD), and King's College criteria (KCC) were separately used for predicting mortality, and the levels of cytokines associated with systemic inflammation, platelet production, and platelet activation were measured.

Results Platelet counts were significantly lower in patients with HEV-ALF, and nonsurvivors had lower platelet counts than survivors ($p < 0.001$). Platelet count was an independent risk factor for predicting 28- and 90-day mortality in patients with HEV-ALF. The AUROC of the baseline platelet count (cutoff, 131×10^9/L) for 28- and 90-day mortality was 0.786 and 0.764, respectively, which was superior to KCC score ($p < 0.05$) and comparable to MELD score. Furthermore, the platelet counts at 3 and 7 days after ALF diagnosis had similar predictive power for 28- and 90-day mortality. The value of platelet count was also confirmed in the validation cohort. Moreover, platelet-associated cytokines, including thrombopoietin, platelet factor 4, and P-selectin, were increased in patients with HEV-ALF.

Conclusions Decreased platelet count is a simple and reliable indicator for predicting 28- and 90-day mortality in patients with HEV-ALF. Overactivation of platelets is an important risk for platelet counts decrease, and treatment aiming at platelet count recovery may be considered.

Keywords Hepatitis E virus · Acute liver failure · Platelet count · Prognosis · King's College criteria · Model for end-stage liver disease · Acute hepatitis · Outcome · Mortality · Systemic inflammation

Abbreviations

ALF	Acute liver failure
HEV	Hepatitis E virus
HE	Hepatic encephalopathy
KCC	King's College criteria
MELD	Model for end-stage liver disease
HEV-ALF	Hepatitis E virus-related acute liver failure
HEV-AH	Hepatitis E virus-related acute hepatitis

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HC	Healthy control
PT	Prothrombin time
INR	International normalized ratio
WBC	White blood cell
Alb	Albumin
TBil	Total bilirubin
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
Cre	Creatine
GI	Gastrointestinal
AKI	Acute kidney injury
TPO	Thrombopoietin
PF-4	Platelet factor 4
AUROC	Area under the receiver operating characteristic curve

Introduction

Acute liver failure (ALF) is a rare and unique syndrome of severe liver impairment due to massive or submassive hepatocyte necrosis that can lead to alterations in aminotransferase levels, coagulation, and mentation in the absence of chronic liver disease [1]. Because of the high mortality rate, liver transplantation is needed in half of the patients with ALF [2]. Early diagnosis and reasonable evaluation are valuable for making accurate treatment decisions [3]. Drug-induced liver injury, viral infection, autoimmune hepatitis, and Wilson's disease are the major causes of ALF [2], and patients with virus-associated ALF are more likely to have worse outcomes [4]. Hepatitis E virus (HEV) is a major cause of acute viral hepatitis, which tends to progress into chronic viral hepatitis and ALF [2, 5]. Approximately, 6.5% of ALF cases in China had evidence of HEV infection [6]. King's College criteria (KCC) and the model for end-stage liver disease (MELD) are generally used as prognostic tools for those patients with ALF [7]. However, the lack of quantitative and specific assessment tools has made it necessary to find a more practical and simpler indicator for predicting mortality in patients with HEV-ALF.

Platelet count is a common laboratory parameter originally considered as a marker of hemostasis. Recently, the role of platelets in the formation of tumor microenvironment has gained major attention, decreased platelet count associated with systemic inflammation and high mortality in patients in the intensive care unit [8]. During viral infection, thrombocytopenia is believed to be caused by decreased platelet production, increased platelet destruction or turnover, and increased platelet consumption by the spleen [9]. Additionally, changes in portal vein pressure and an enlarged spleen can also serve as triggers for platelet activation. Once activated, platelets are recognized and cleared by circulating leukocytes or splenocytes. A low platelet count has been associated with a high risk of multi-organ system failure in patients with drug-induced ALF [10]; however, the prognostic value of platelet count in patients with HEV-ALF is unknown.

In this study, we investigated the baseline and dynamics of platelet count in patients with HEV-related acute hepatitis (HEV-AH) and HEV-ALF. Further, we separately compared the performance of platelet count with the KCC and MELD scores in derivation and validation cohorts in predicting short-term mortality. Finally, the reasons for platelet decrease were also identified.

Materials and methods

Study design

The retrospective derivation cohort of HEV-ALF was derived from the Fifth Medical Center of Chinese PLA General Hospital between June 2004 and September 2020 (Fig. 1). Patients with HEV-AH were randomly selected at a 1:2 ratio with HEV-ALF patients, according to sex, age, and time of hospitalization. In addition, a validation cohort derived from Nanning Fourth People's Hospital between June 2015 and September 2020 was also included. Clinical characteristics and outcomes were collected. Both of the derivation and validation cohort were used to develop and validate the value of platelet count for outcome prediction. Furthermore, the levels of cytokines associated with systemic inflammation, platelet production, and activation were simultaneously measured.

Patients

97 patients with HEV-ALF and 194 patients with HEV-AH were included in the derivation cohort. 26 patients with HEV-ALF and 53 patients with HEV-AH were included in the validation cohort. Patients with competing causes of ALF, including co-infection with other viruses (e.g., hepatitis A/B/C virus, cytomegalovirus, Epstein–Barr virus, adenovirus, and human immunodeficiency virus), and other possible etiologies (e.g., alcohol abuse, acetaminophen or drug use, Wilson's disease, autoimmune hepatitis, pregnancy-associated liver disease, and malignant infiltration) were excluded. Furthermore, patients with age < 18 years, pregnant status, severe chronic extrahepatic diseases, carcinomas, incomplete medical records, history of liver transplantation within 90 days after enrollment, or recent platelet transfusion were further excluded. During hospitalization, all patients received integrative treatments as recommended [11, 12]. Simultaneously, 30 healthy controls (HCs) were also included in our study.

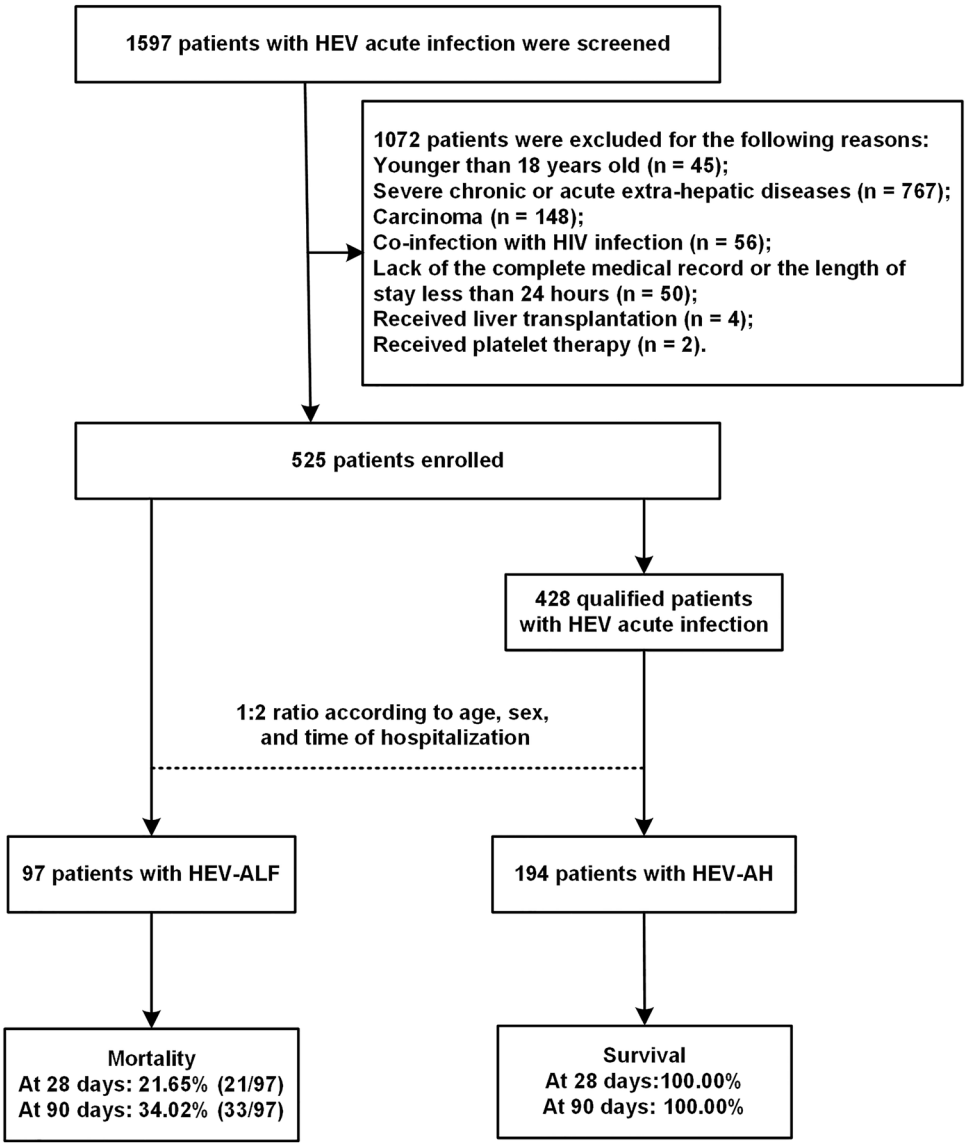


Fig. 1 Flowchart of screening and recruitment of patients with HEV-ALF and HEV-AH in the derivation cohort. *HEV* hepatitis E virus, *HEV-ALF* hepatitis E virus-related acute liver failure, *HEV-AH* hepatitis E virus-related acute hepatitis

Definitions of HEV-ALF and HEV-AH

ALF was defined according to the Japanese definition [13, 14]. In brief, patients who were hospitalized with prothrombin time (PT) values of 40% or less of the standardized value, or international normalized ratio (INR) of 1.5 or more due to severe liver damage within 8 weeks of the first symptoms were defined as having ALF. AH was defined as an acute illness with biological signs including jaundice or serum alanine aminotransferase (ALT) levels > 2.5 times the upper limit [15]. Moreover, HEV infection was diagnosed when anti-HEV IgM was detected in serum.

Data collection

We collected information on each patient from the registry databases or medical records. Laboratory parameters and complications including ascites, infections, acute kidney injury (AKI) and hepatic encephalopathy (HE) were recorded [16, 17]. The MELD and KCC scores were used to evaluate the severity of liver disease in patients with HEV-ALF. Data on laboratory parameters and systemic complications were collected for a maximum of 7 days after enrollment. Data were no longer collected in patients who underwent liver transplantation, discharged from the hospital, or died. The patients were followed up for 90 days. Death was the primary end point.

Soluble cytokine analysis

Available serum samples of patients were collected at enrollment, and the levels of different cytokines (interleukin [IL]-1β, IL-6, IL-8, thrombopoietin [TPO], platelet factor 4 [PF-4], and P-selectin) were determined through

flow cytometry using an Aimplex kit (Quantobio, Beijing, China).

Statistical analysis

All statistical analyses were performed using the following software packages: IBM SPSS (version 23.0), SAS (version 9.4), GraphPad Prism 8, and MedCalc (version 15.2.2). Continuous data were expressed as mean ± standard deviation or median (interquartile range $Q1$–$Q3$) and analyzed using Student's t test, analysis of variance, Mann–Whitney U, or Kruskal–Wallis H test, as appropriate. Categorical data were described as numbers (%) and compared using the Chi-square or Fisher's exact test. The Spearman method was used for correlation analysis. Propensity score matching was used to match the HEV-AH and HEV-ALF. Cox proportional hazards regression was performed to identify independent indicators in patients with HEV-ALF. According to the cutoff value in the derivation cohort, the cumulative survival rates at 28 and 90 days were calculated using Kaplan–Meier analysis with log-rank test. The area under the receiver operating characteristic curve (AUROC) of platelet count was compared with the KCC and MELD scores using the Z-test. The Greenhouse–Geisser method was used to analyze repeated measurement data. Statistical significance was set at $p < 0.05$.

Results

Baseline characteristics of enrolled patients

A total of 1597 patients with HEV infection were screened, and 97 patients with HEV-ALF and 428 patients with HEV-AH were selected. Of the patients with HEV-AH, 194 matched patients were enrolled and included in derivation cohort (Fig. 1).

As shown in Table 1, no significant differences in age and sex were observed between the different groups. The white blood cell (WBC) count, total bilirubin (TBil) level, aspartate aminotransferase (AST) level, creatine (Cre) level, PT, and INR were significantly higher in patients with HEV-ALF (all $p < 0.05$). In contrast, the platelet count, albumin (Alb) level, and sodium level were markedly lower in patients with HEV-ALF (all $p < 0.05$). The development of ascites (51.5%) was the most prevalent complication, followed by pathogenic microorganism infection (23.7%), HE (19.6%), AKI (18.6%), and gastrointestinal (GI) hemorrhage (3.1%). The MELD scores of patients with ALF (median 24.51, range 22.23–27.80) and AH (median 14.74, range 11.32–17.88) were calculated. All patients were followed up for 90 days, and the total 28- and 90-day mortality rates of patients with HEV-ALF were 21.65% (21/97) and 34.02% (33/97), respectively.

Development of platelet count as an important indicator for patients with HEV-ALF

According to the outcome at 28 days, patients with HEV-ALF were divided into survivors and nonsurvivors, and the baseline characteristics are shown in Table 2. Univariate and multivariate Cox regression analyses were separately performed to assess the influence of laboratory and clinical parameters on the outcome at 28 days, and Alb level, TBil level, INR, and platelet count were identified as independent prognostic predictors (all $p < 0.05$). The AUROC of platelet count (0.786; 95% confidence interval [CI] 0.691–0.863), and a cutoff of 131×10^9/L, for predicting the outcome was superior to that of Alb level, TBil level, and INR (Fig. S1).

To further identify the role of platelet count in predicting 28- and 90-day mortality in patients with HEV-ALF, we used baseline platelet count alone and combined with demographic (age, sex), laboratory (WBC count, TBil level, Cre level, INR), and clinical (HE) parameters to construct different models. Notably, as a continuous variable, baseline platelet count was negatively correlated with the risk of short-term death in patients with HEV-ALF in the crude model, model 1, and model 2 (Table S1), and adjustment for demographic variables did not weaken the associations between platelet count and mortality. In the fully multivariable model (model 3), which considered WBC count, TBil level, Cre level, INR, and HE at baseline, platelet count was still independently and negatively correlated with the 28-day (HR 0.984; 95% CI 0.975–0.993; $p = 0.001$) and 90-day (HR 0.989; 95% CI 0.981–0.997; $p = 0.009$) mortality of patients with HEV-ALF. Meanwhile, the significance of platelet count was also confirmed as a binary variable. Patients with high platelet counts ($\geq 131 \times 10^9$/L) had a lower risk of death at 28 days (HR 0.144; 95% CI 0.044–0.469) and 90 days (HR 0.319; 95% CI 0.146–0.697) (Table S1).

Evaluation of platelet count as a dynamic predictor for mortality in patients with HEV-ALF

According to the cutoff of platelet count, patients were categorized into the high platelet group ($\geq 131 \times 10^9$/L) and low platelet group ($< 131 \times 10^9$/L). The mortality rate of the high platelet group was significantly lower than the low platelet group (28-day mortality, 8.47% vs. 42.11%; 90-day mortality, 18.64% vs. 57.89%; both $p < 0.0001$; Fig. 2a, b), indicating that platelet count was an important indicator for mortality in patients with HEV-ALF. Furthermore, as shown in Fig. 2c, d, platelet counts were significantly higher at 0, 3, and 7 days in survivors than in nonsurvivors (all $p < 0.05$).

Table 1 Baseline characteristics of healthy controls and patients with HEV-AH and HEV-ALF in the derivation cohort

Characteristics	HCs ($n=30$)	HEV-AH ($n=194$)	HEV-ALF ($n=97$)	p^a
Demographics				
Age (years)	57 (48, 67)	61 (52, 67)	58 (47, 64)	0.249
Gender (male/female)	27/3	188/6	94/3	0.165
Laboratory parameters				
White blood cells ($\times 10^9$/L)	5.37 (4.72, 6.19)	5.7 (4.56, 6.96)	7.27 (6.26, 9.22)$^{\#,\dagger}$	< 0.001
Hemoglobin (g/L)	144.5 (137.8, 150.3)	137.0 (128.0, 149.0)	140.0 (117.5, 153.0)	0.178
Platelets ($\times 10^9$/L)	236.5 (197.3, 257.8)	187.5 (150.0, 228.5)*	143.0 (102.7, 165.5)$^{\#,\dagger}$	< 0.001
Albumin (g/L)	40.65 (37.68, 43.08)	34.00 (31.00, 36.50)*	31.00 (28.25, 34.00)$^{\#,\dagger}$	< 0.001
Total bilirubin (mmol/L)	14.8 (11.3, 18.2)	159. 5 (91.2, 250.0)*	269.0 (192.9, 431.5)$^{\#,\dagger}$	< 0.001
Alanine aminotransferase (U/L)	18 (14, 23)	590 (191, 1126)*	860 (117, 1900)$^{\#}$	< 0.001
Aspartate aminotransferase (U/L)	19 (16, 22)	208 (81, 542)*	331 (101, 1138)$^{\#,\dagger}$	< 0.001
Creatinine (mmol/L)	78 (70, 93)	85 (73, 93)	90 (77, 107)$^{\#,\dagger}$	0.005
Serum sodium (mmol/L)	141 (139, 142)	138 (136, 140)*	136 (134, 138)$^{\#,\dagger}$	< 0.001
Prothrombin time (s)	11.4 (10.8, 11.8)	11.9 (11.0, 13.2)	20.5 (18.3, 26.6)$^{\#,\dagger}$	< 0.001
International normalized ratio	1.02 (0.96, 1.04)	1.04 (0.97, 1.13)	1.78 (1.575, 2.27)$^{\#,\dagger}$	< 0.001
Complications, *n* (%)				
Ascites	–	21 (10.8)	50 (51.5)†	–
Infections	–	8 (4.1)	23 (23.7)†	–
Acute kidney injury	–	4 (2.1)	18 (18.6)†	–
Hepatic encephalopathy	–	0 (0)	19 (19.6)†	–
Gastrointestinal bleeding	–	0 (0)	3 (3.1)†	–
Prognosis scores				
MELD	–	14.74 (11.32, 17.88)	24.51 (22.23, 27.80)†	–
Mortality, % (*n*/*N*)	–			–
28-day	–	0 (0)	21.65 (21/97)†	–
90-day	–	0 (0)	34.02 (33/97)†	–

HCs healthy controls, *HEV-AH* hepatitis E virus-related acute hepatitis, *HEV-ALF* hepatitis E virus-related acute liver failure

[a]*p* value(< 0.05) for comparisons between HCs, HEV-AH, and HEV-ALF; **p* value (< 0.05) for comparisons between HCs and HEV-AH; [#]*p* value (< 0.05) for comparisons between HCs and HEV-ALF; [†]*p* value (< 0.05) for comparisons between HEV-AH and HEV-ALF

Moreover, the platelet count continuously decreased in non-survivors regardless of death within 28 or 90 days ($p<0.05$). Therefore, the sequential decrease in platelet count was associated with mortality.

Identification of the predictive power of platelet count for mortality in patients with HEV-ALF

Our study compared the predictive power of platelet count with that of existing classical prognostic scores (KCC and MELD) in a sequential manner, at the time of ALF diagnosis, 3 days and 7 days after the diagnosis (Fig. 2e–j). The baseline platelet count showed a significantly higher predictive power for death in patients with HEV-ALF than the KCC (AUROC = 0.555, $p<0.05$ for 28-day mortality; AUROC = 0.598, $p<0.05$ for 90-day mortality), and showed a comparable predictive power to the MELD (AUROC = 0.791 for 28-day mortality, AUROC = 0.771 for 90-day mortality, both $p>0.05$). Importantly, the platelet count at 3 and 7 days both had similar power to the MELD score for predicting 28- and 90-day mortality (all $p>0.05$), but had superior predictive ability to the KCC score (all $p<0.05$). In addition, both platelet count and MELD score showed improved predictive performance when sequentially assessed at 3 and 7 days after the HEV-ALF diagnosis, especially for predicting the 90-day mortality (all $p<0.05$) (Table S2).

Association of in vivo inflammation with platelet decrease

To examine the causes of thrombocytopenia, we first assessed the length of the spleen, which may be an indicator for the destruction of platelets. No correlation was found between platelet count and the length of the spleen ($p>0.05$, Fig. S2a). In addition, there was also no significant difference in platelet counts between patients with and

Table 2 Characteristics of the HEV-ALF patients in the derivation cohort on admission according to the outcome of 28 days

Characteristics	Nonsurvivors	Survivors	*p*	Univariate Cox regression		Multivariate Cox regression	
				HR (95% CI)	*p*	HR (95% CI)	*p*
Demographics							
Age (years)	62.91 ± 13.99	53.92 ± 13.33	0.014	1.044 (1.008, 1.081)	0.015		
Gender (male/female)	21/0	3/73	0.097	21.185 (0.001, 718,128.25)	0.566		
Laboratory parameters							
White blood cells ($\times 10^9$/L)	9.24 (7.17, 13.49)	7.06 (6.21, 8.40)	0.004	1.228 (1.097, 1.374)	< 0.001		
Hemoglobin (g/L)	117 (92, 128)	145 (132, 155)	< 0.001	0.975 (0.962, 0.989)	< 0.001		
Platelets ($\times 10^9$/L)	96 (65, 128)	149 (124, 170)	< 0.001	0.982 (0.972, 0.991)	< 0.001	0.986 (0.975, 0.998)	0.019
Albumin (g/L)	28.05 ± 5.38	31.74 ± 4.23	0.001	0.873 (0.802, 0.949)	0.001	0.846 (0.772, 0.928)	< 0.001
Total bilirubin (mmol/L)	452.6 (259.1, 534.6)	245.3 (186.0, 361. 5)	0.003	1.004 (1.001, 1.007)	0.002	1.005 (1.001, 1.008)	0.008
Alanine aminotransferase (U/L)	91 (64, 1210)	1158 (315, 2047)	0.003	0.999 (0.999, 1)	0.023		
Aspartate aminotransferase (U/L)	270.5 (73.5, 697.8)	361.5 (118.3, 11,154.3)	0.041	1 (0.999, 1)	0.437		
Creatinine (mmol/L)	116.0 (89.0, 159.5)	87.5 (75.3, 98.5)	0.002	1.006 (1.003, 1.009)	< 0.001		
Serum sodium (mmol/L)	133.91 ± 3.92	136.01 ± 3.18	0.014	0.867 (0.770, 0.976)	0.018		
Prothrombin time (s)	21.3 (18.2, 33.2)	20.4 (18.2, 25.8)	0.372	0.970 (0.931, 1.010)	0.137		
International normalized ratio	1.91 (1.53, 3.09)	1.77 (1.59, 2.2)	0.545	1.659 (1.054, 2.610)	0.029	2.606 (1.558, 4.360)	< 0.001
Complications, *n* (%)							
Ascites	11 (52.4)	39 (51.3)	0.931	1.0250 (0.435, 2.415)	0.954		
Infection	12 (57.1)	11 (14.5)	< 0.001	5.332 (2.241, 12.689)	< 0.001		
Acute kidney injury	11 (52.4)	7 (9.2)	< 0.001	6.560 (2.774, 15.510)	< 0.001		
Hepatic encephalopathy	7 (33.3)	12 (15.8)	0.116	2.467 (0.995, 6.120)	0.051		
Gastrointestinal bleeding	2 (9.5)	1 (1.3)	0.117	4.416 (1.021, 19.104)	0.047		

HEV-ALF hepatitis E virus-related acute liver failure

without AKI ($p > 0.05$, Fig. S2b), which indicated that a low platelet count was not associated with spleen and kidney dysfunction.

Second, we found that HEV-ALF patients with infection had significantly lower platelet counts than those without infection ($p < 0.05$), and platelet count was negatively associated with procalcitonin levels ($r = -0.299$, $p < 0.05$) (Fig. 3a, b). In ROC curve analysis, the presence of bacterial infections did not confound the value of platelet count in predicting mortality in patients with HEV-ALF. Additionally, the AUROC of platelet count in predicting the outcome of patients with HEV-ALF was not different from the MELD score, whereas the AUROC of both platelet count and MELD score were better than the KCC in the absence of infection (Fig. 3c).

In addition, serum levels of systemic inflammatory cytokines (IL-1β, IL-6, and IL-8) and cytokines associated with platelet destruction (PF-4 and P-selectin) and platelet production (TPO) were evaluated in 20 patients with HEV-ALF, 40 patients with HEV-AH, and 30 HCs. We found that the levels of all cytokines were higher in patients with HEV-ALF and HEV-AH than in HCs (all $p < 0.05$, Fig. S3a–f).

Validation of the performance of platelet count in a new cohort

We used a validation cohort to verify the predictive power of platelet count for mortality in patients with HEV-ALF. The baseline demographics, laboratory parameters, and clinical parameters of the validation cohort were similar to those of the derivation cohort (Table S3). In the validation cohort, the 28-day (34.62%) and 90-day (50.00%) mortality rates of patients with HEV-ALF were not significantly different from the derivation cohort (all $p > 0.05$). The platelet counts of patients with HEV-ALF

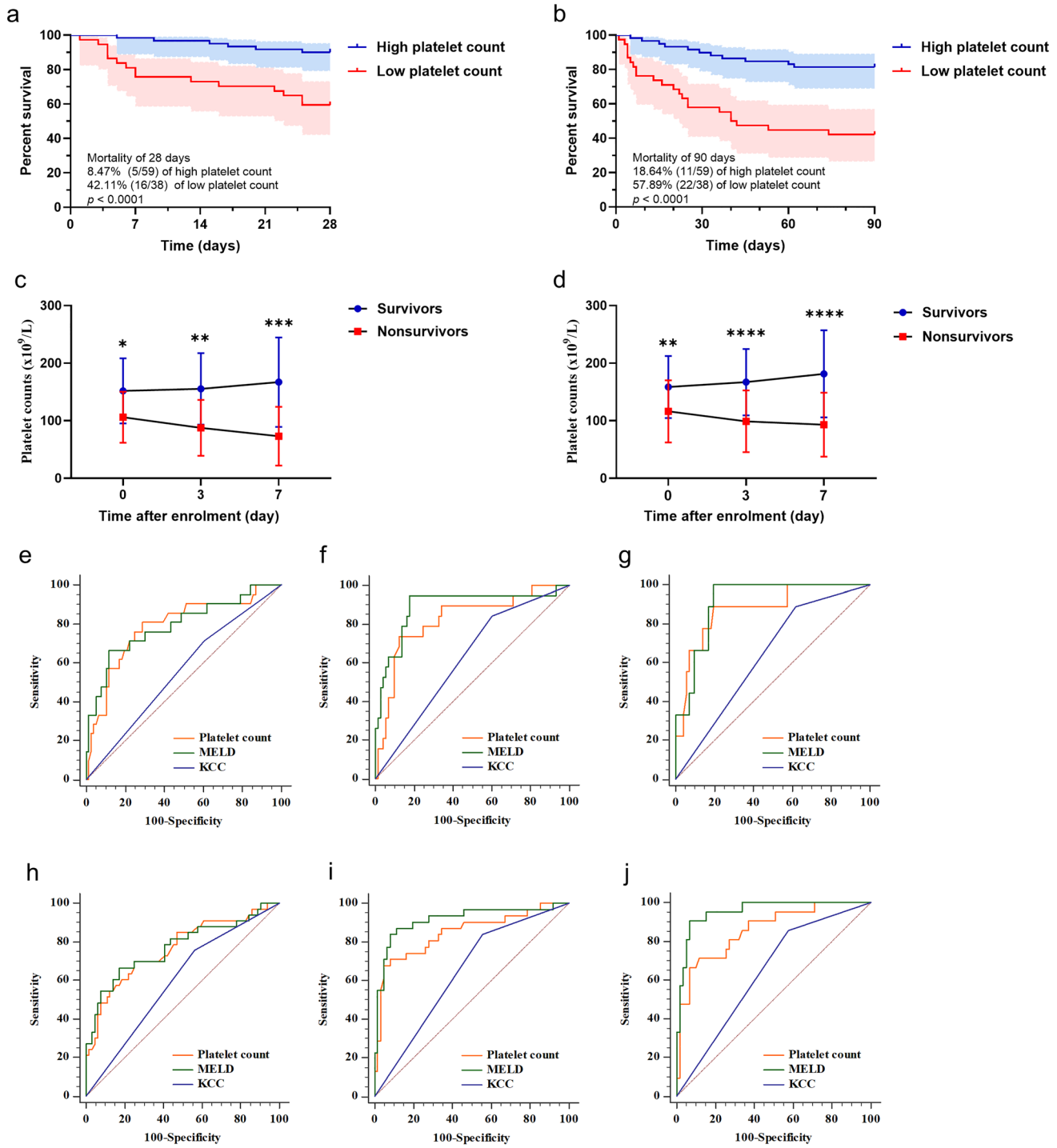
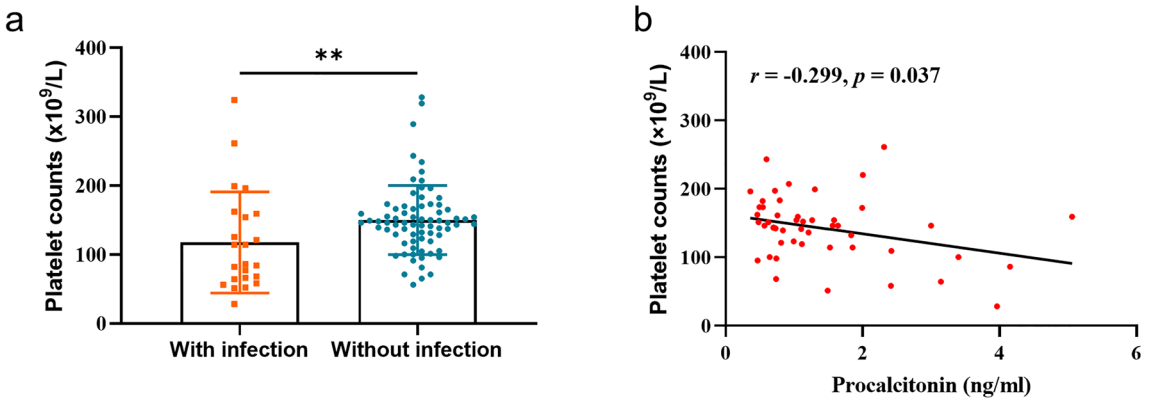


Fig. 2 Low platelet count predicts poor outcomes in patients with HEV-ALF in the derivation cohort. **a**, **b** Kaplan–Meier survival curves of patients with HEV-ALF categorized according to platelet counts for predicting 28-day mortality (**a**) and 90-day mortality (**b**). **c**, **d** Longitudinal changes in platelet counts within 7 days according to 28-day (**c**) and 90-day (**d**) outcomes. HEV-ALF, hepatitis E virus-related acute liver failure. **e**–**j** Predictive value of platelet count, MELD score, and KCC score at enrollment, 3 days, and 7 days for 28-day mortality (**e**–**g**) and 90-day mortality (**h**–**j**). *ROC* receiver operating characteristic curve, *MELD* model for end-stage liver disease, *KCC* King's College criteria, *HEV-ALF* hepatitis E virus-related acute liver failure. Data were analyzed using Kaplan–Meier analysis with log-rank test and repeated-measures analysis of variance. Data were analyzed by comparing ROC curves with the *Z*-test (Delong's method) results. *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$



c

Severity score	AUROC (95% CI)	Cut-off	Sensitivity	Specificity	Youden	*p*	*p* vs. platelet count
With infection							
28-day							
Platelet count	0.678 (0.453-0.855)	66	50.00	90.91	0.4091	0.1360	-
MELD	0.659 (0.434-0.841)	25.83	66.67	72.73	0.3939	0.1868	0.9008
KCC	0.64 (0.416-0.827)	0	91.67	36.36	0.2803	0.1061	0.7987
90-day							
Platelet count	0.730 (0.507-0.892)	86	64.71	83.33	0.4804	0.0398	-
MELD	0.873 (0.668-0.974)	24.02	88.24	83.33	0.7157	< 0.0001	0.3351
KCC	0.691 (0.466-0.865)	0	88.24	50.00	0.3824	0.1077	0.8064
Without infection							
28-day							
Platelet count	0.811 (0.703-0.893)	131	77.78	75.38	0.5316	< 0.0001	-
MELD	0.862 (0.762-0.931)	28.29	77.78	92.31	0.7009	< 0.0001	0.6417
KCC	0.578 (0.457-0.692)	0	36.36	60.00	0.1556	0.4031	0.0334
90-day							
Platelet count	0.764 (0.651-0.855)	149	93.75	53.45	0.4720	< 0.0001	-
MELD	0.730 (0.614-0.826)	26.72	62.50	84.48	0.4698	0.0070	0.7376
KCC	0.528 (0.408-0.645)	0	62.50	43.10	0.0560	0.6914	0.0299

Fig. 3 Analysis of causes related to platelet count decrease. **a** Difference in platelet counts between patients with HEV-ALF with and without infection. **b** Correlation between the baseline platelet count and procalcitonin levels. **c** Accuracy of platelet count in predicting mortality in patients with HEV-ALF with and without infection, as shown by the AUROC values. *AUROC* area under the receiver operating characteristic curve, *MELD* model for end-stage liver disease, *KCC* King's College criteria, *HEV-ALF* hepatitis E virus-related acute liver failure. Data were analyzed using the Mann–Whitney *U* test, Spearman correlation test, and *Z*-test (Delong's method). ** $p < 0.01$

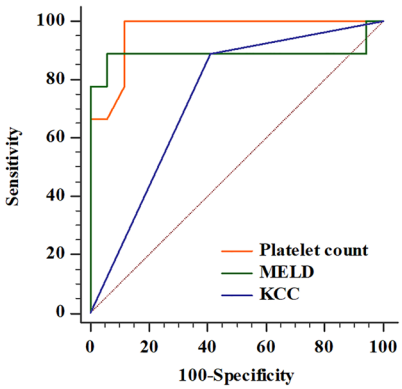
were consistently lower than patients with HEV-AH and showed reasonable sensitivity and specificity in predicting mortality. The AUROC of platelet count for 28-day mortality was 0.964, which was significantly higher than the KCC score (0.739, $p = 0.019$) and comparable to the MELD score (0.889, $p = 0.5071$) (Fig. 4a). In addition, the value of platelet count was also identified in patients who died within 90 days (Fig. 4b, c).

Discussion

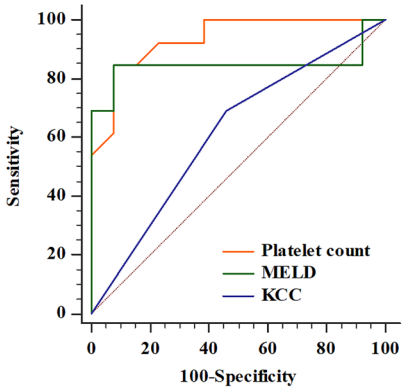
HEV-ALF is a serious syndrome of severe liver injury that rapidly worsens and has a high mortality rate [3]. Thus, it is necessary to find a simple and accurate prognostic indicator for evaluating outcomes. Platelet count is a common clinical parameter with an increasingly important influence on liver homeostasis and viral infection [8]. In our study, a derivation cohort including 97 patients and a validation cohort including 26 patients with HEV-ALF were used to verify the predictive value of platelet count for predicting mortality in patients with HEV-ALF. Our results showed that platelet count was an independent risk factor for 28- and 90-day mortality. Platelet count was superior to the KCC score and comparable to the MELD score in predicting mortality in patients with HEV-ALF. Therefore, platelet count may be a new simple prognostic marker for predicting short-term mortality.

Platelets are considered to play an active role in liver inflammation by promoting leukocyte recruitment [8], modifying the hepatic cellular and cytokine milieu, and driving both hepatoprotective and hepatotoxic processes. In our study, we found that platelet count decreased after liver failure and was an independent risk indicator for short-term

a



b



c

Severity score	AUROC (95% CI)	Cut-off	Sensitivity	Specificity	Youden	*p*	*p* vs. platelet count
28-day							
Platelet count	0.964 (0.807-0.999)	115	77.78	92.86	0.706	0.0113	-
MELD	0.889 (0.704-0.977)	26.53	88.89	92.86	0.818	0.0006	0.5071
KCC	0.739 (0.530-0.890)	0	88.89	42.86	0.318	0.0722	0.0190
90-day							
Platelet count	0.935 (0.765-0.994)	121	69.23	100.00	0.692	0.0001	-
MELD	0.846 (0.651-0.956)	22.78	84.62	90.00	0.746	0.0015	0.4292
KCC	0.615 (0.406-0.798)	0	76.92	40.00	0.169	0.4059	0.0028

Fig. 4 ROC curve analysis of platelet count in the validation cohort. **a**, **b** Predictive value of platelet count, MELD score, and KCC score in the validation cohort for mortality at 28 days (**a**) and 90 days (**b**). **c** Details of the analysis results. *ROC* receiver operating characteristic curve, *MELD* model for end-stage liver disease, *KCC* King's College criteria. Data were analyzed by comparing ROC curves with the *Z*-test (Delong's method) results

mortality in patients with HEV-ALF. Furthermore, platelet count was found to be negatively correlated with the risk of 28- and 90-day mortality, regardless of whether it was treated as a continuous or segmented variable. Notably, during the course of HEV-induced ALF, the platelet count was stable when it was sequentially assessed at enrollment, after 3 days, and after 7 days, suggesting that sequential measurements of platelet count may be used to predict the outcome of patients with HEV-ALF.

In patients with ALF, the American Gastroenterological Association suggested using MELD and KCC as prognostic scoring systems [7]. Encouragingly, our data showed that platelet count seemed superior to the KCC score and comparable to the MELD score in both the derivation and validation cohorts. Although the MELD score is considered as the gold standard for predicting the outcome of chronic liver disease and ALF [18], the inferiority of the KCC score in predicting mortality in patients with HEV-ALF may be associated with its sensitivity and applicability in drug-induced liver failure [19]. In our patients, the platelet count and MELD score both at baseline and after the following 3 days showed equal predictive powers for mortality. In addition, both the platelet count and MELD score at 7 days were even better than the baseline values in predicting 28- and 90-day mortality, highlighting the link between platelet count and disease progression. In addition, the combination of platelet count and MELD scores did not improve prognostication (data not shown), which indicated that only one laboratory parameter, platelet count, was a powerful predictor of the outcome of HEV-ALF.

The reasons for low platelet count in patients with chronic liver disease include infection, hypersplenism, increased destruction of platelets, decreased platelet production, antiplatelet antibodies, and translocated toxins [20, 21]. Various hepatotropic viruses are known to cause severe thrombocytopenia [22]. Lower platelet counts at baseline were highly associated with liver transplantation or liver-related death in patients with drug-induced liver injury [3].

The reasons for decreased platelets during HEV infection were carefully checked. Firstly, no correlation was found between platelets and spleen length. Thus, the relationship between splenomegaly and decreased platelet count was ruled out. Secondly, lower platelet counts were negatively associated with procalcitonin levels, indicating that pathogenic infection may be an important inducer. Platelet count showed good performance in predicting mortality in patients with and without pathogenic infection and had no statistical significance in nonsurvivors with and without infection (data not shown). Therefore, the relationship between infection and platelet decrease may be weak and other causes may be involved.

Furthermore, we analyzed the factors associated with platelet production. On the one hand, we assessed the major regulator of platelet production, TPO, which is primarily produced by the liver and kidney [23] and supports the survival, proliferation, and differentiation of megakaryocytes, which was the precursors of platelets [24]. We found that the occurrence of AKI did not influence the platelet count. Moreover, as some HEV-infected patients with thrombocytopenia had normal function of bone marrow [25], ineffective thrombopoiesis may not existed. On the other hand, the levels of TPO and other cytokines (PF-4 and P-selectin) were all increased in patients with HEV-AH and HEV-ALF. Although there is no direct evidence of platelet destruction by HEV infection to date, the lower performance of platelet count in predicting the outcome of patients with drug-induced ALF (unpublished data) emphasizes the direct importance of HEV infection on platelets. Furthermore, the increase of IL-6, IL-8, and IL-1β levels may support the relationship between inflammation and platelet destruction, which was previously identified during hepatic dysfunction and inflammation [26]. Therefore, the direct destructive effects of HEV and systemic inflammation may work together to influence the platelet count; however, the associated mechanisms are still unclear.

This study had some limitations. The association between platelet count and the prognosis of patients with HEV-ALF was observed only in a retrospective cohort. Confirmatory studies involving multicenter prospective cohorts are needed. Furthermore, we did not conduct more in-depth investigations of the reasons for platelet reduction.

In conclusion, our study highlighted the value of platelet count, either at baseline or at sequential follow-up time points, in predicting the short-term outcome of patients with HEV-ALF. The findings of this study confirmed that platelet count is at least comparable to the MELD score and superior to the KCC score in predicting mortality in patients with HEV-ALF. Furthermore, overactivation of platelets mediated by HEV infection and inflammation may be the major causes of platelet decrease.

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Author contributions XM, JZ, JC, JT, LM, PN, HL, ZF, TY, KL, WC, MJZ, CZ, JYZ, YMJ, JWS, XF, MS, and JH participated in the data acquisition and soluble cytokines analysis; XM, JZ, RX, and FSW designed the study; XM and JZ performed analyses and interpretation of data; XM and JZ wrote the first draft of the manuscript and incorporated revisions; XM, JZ, RX, and FSW prepared the final version; All authors approved the final manuscript to be published.

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Declarations

Conflict of interest Xiuying Mu, Jun Zou, Jing Chen, Jingjing Tong, Lian Ma, Peng Ning, Huajie Li, Zhiqian Feng, Tao Yang, Kai Liu, Wen-Jing Cao, Ming-Ju Zhou, Chao Zhang, Ji-Yuan Zhang, Yan-Mei Jiao,Jin-Wen Song, Xing Fan, Ming Shi, Jinhua Hu, Ruonan Xu and-Fu-Sheng Wang declare that they have no conflict of interest.

Ethical approval Ethical approvals were obtained from the Fifth Medical Center of Chinese PLA General Hospital, and the study was performed in accordance with the 1975 Declaration of Helsinki.

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