Prognostic Nomogram for Patients with Hepatitis E Virus-related Acute Liver Failure: A Multicenter Study in China

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Abstract

Background and Aims: Timely and effective assessment scoring systems for predicting the mortality of patients with hepatitis E virus-related acute liver failure (HEV-ALF) are urgently needed. The present study aimed to establish an effective nomogram for predicting the mortality of HEV-ALF patients. Methods: The nomogram was based on a cross-sectional set of 404 HEV-ALF patients who were identified and enrolled from a cohort of 650 patients with liver failure. To compare the performance with that of the model for end-stage liver disease (MELD) scoring and CLIF- Consortium-acute-on-chronic liver failure score (CLIF-C-ACLFs) models, we assessed the predictive accuracy of the nomogram using the concordance index (C-index), and its discriminative ability using time-dependent receiver operating characteristics (td-ROC) analysis, respectively. Results: Multivariate logistic regression analysis of the development set carried out to predict mortality revealed that γ-glutamyl transpeptidase, albumin, total bilirubin, urea nitrogen, creatinine, international normalized ratio, and neutrophil-to-lymphocyte ratio were independent factors, all of which were incorporated into the new nomogram to predict the mortality of HEV-ALF patients. The area under the curve of this nomogram for mortality prediction was 0.671 (95% confidence interval: 0.602–0.740), which was higher than that of the MELD and CLIF-C-ACLFs models. Moreover, the td-ROC and decision curves analysis showed that both discriminative ability and threshold probabilities of the nomogram were superior to those of the MELD and CLIF-C-ACLFs models. A similar trend was observed in the validation set. Conclusions: The novel nomogram is an accurate and efficient mortality prediction method for HEV-ALF patients.

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Keywords: Hepatitis E; Acute liver failure; Nomogram; Mortality prediction; Scoring model.

Abbreviations: ACLF: acute-on-chronic liver failure; ALF: acute liver failure; ALT: alanine aminotransferase; AST: aspartate aminotransferase; C-index: concordance index; CHE: cholinesterase; CR: creatinine; DBIL: direct bilirubin; DCA: decision curve analysis; GGT: γ-glutamyl transpeptidase; HE: hepatic encephalopathy; HEV: hepatitis E virus; HEV-ALF: hepatitis E virus-related acute liver failure; IgM: immunoglobulin M; INR: international normalized ratio; MELD: model for end-stage liver disease; NAFLD: non-alcoholic fatty liver disease; NLR: neutrophil-to-lymphocyte ratio; OPLS-DA: orthogonal partial least squares-discriminant analysis; PT: prothrombin time; RBC: red blood count; RDW: red cell distribution width; RL: RLR: total bilirubin; td-AUC: time-dependent area under the receiver operating characteristic curve; td-ROC: time-dependent receiver operating characteristics; UREA, urea nitrogen; WBC, white blood cell.

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Introduction

Hepatitis E virus (HEV) is endemic in many developing countries because of poor sanitation. The virus is predominantly transmitted through fecal and oral routes, which is also a main cause of acute viral hepatitis.1,2 About 20.1 million HEV infection-related hepatitis cases occur worldwide, resulting in 70,000 deaths and 3,000 stillbirths in the past.3 Although hepatitis E usually causes asymptomatic and self-limiting diseases with low mortality, fulminant hepatitis that leads to acute liver failure (ALF) or acute-on-chronic liver failure (ACLF) are possible. Of all acute HEV cases, only a small fraction (0.5–4%) progresses to ALF. The rate of progression to ALF may be as high as 10–22% in pregnant women.4 Notably, the fact that HEV plays an important role in the...
development of ALF has also been frequently reported in Europe. All of these could lead to high mortality rates, ranging from 0–67%. Hence, diagnosing HEV-related ALF (HEV-ALF) patients in a timely manner is extremely important.

To date, a few scoring systems have been established for the diagnosis and prediction of prognosis in patients with different kinds of liver diseases. The model for end-stage liver disease (MELD) score,7 the integrated MELD (also known as iMELD) score,8 Child-Turcotte-Pugh score,9 and CLIF-Consortium-ACLF score (CLIF-C-ACLFs)10 have been reported for predicting prognosis in patients with liver cirrhosis. The MELD11 and the CLIF-C-ACLFs model12 have been used to assess the degree of liver damage and the prognosis of patients. Although various models have been used to predict mortality and transplant-free survival in ALF patients of both acetaminophen-induced and virus-related, a scoring model for predicting the mortality of HEV-ALF patients has not yet been reported, to the best of our knowledge.

A nomogram is a graphical representation, which can be used to diagnose or predict disease occurrence or progression with multiple indicators. Moreover, nomograms can provide a user-friendly interface, which has a demonstrated advantage over the traditional staging systems used to predict patient outcomes for many critical diseases.14,15 As a result, nomogram has been proposed as an alternative method, or even as the new standard. Hence, this study aimed to develop a nomogram for predicting the mortality of HEV-ALF patients, and to compare the performance of this nomogram with that of the CLIF-C-ACLFs and MELD models.

Methods

Patients

A total of 404 eligible HEV-ALF patients were recruited from among 650 patients with liver failure from five hospitals in different regions of China. The patient enrollment flow chart is shown in Supplementary Figure 1. All diagnosed HEV-ALF patients, who were referred to The First Affiliated Hospital (Zhejiang University School of Medicine), The Fifth People’s Hospital of Wuxi, The First People’s Hospital of Yancheng City, The People’s Hospital of Dafeng City, and The Linyi Traditional Hospital of Wuxi, China according to manufacturer’s instructions. Samples were collected for anti-HEV IgM, IgG antibodies and HEV RNA quantification.

Data collection and scoring model calculation

We collected all enrolled patients’ clinical, demographic information and laboratory variables, including age, sex, coagulation parameters, hepatic encephalopathy (HE), arterial blood ammonia, laboratory parameters, length of hospitalization and intensive care unit stay, and prognosis. The diagnosis of HE met the West Haven criteria. The MELD and CLIF-C-ACLFs scoring model calculations are described in the supplemental material.

HEV RNA detection

HEV RNA was tested by means of internally controlled quan-
Comparing nomogram-predicted vs. observed Kaplan-Meier evaluated by calibration and discrimination, and assessed by tingen, Germany). The performance of the nomogram was of HEV-ALF patients using SIMCA software (Sartorius, Gottingen, Germany). The performance of the nomogram was evaluated by calibration and discrimination, and assessed by comparing nomogram-predicted vs. observed Kaplan-Meier estimates of survival probability. The rcorr.cens package in Hmisc in R software was used to compare the time-dependent area under the receiver operating characteristic curve (td-AUC). The decision curve analysis (DCA) was also used to assess the net benefits of the nomogram.

**Results**

**Patient characteristics and follow-up**

The majority of the HEV-ALF patients were males (71.5%), with 115 (28.5%) patients being female. The mean patient age was 57.25 years (range: 43.33–69.17 years). Nine of the four hundred and four total eligible HEV-ALF patients were pregnant women. In addition to the liver, the most frequent failure organ was the kidney (14.4%), followed by cerebral (7.9%), coagulation (5.9%) and lung failure (4.0%). Among all the HEV-ALF patients, 83.9% exhibited just failure in liver, followed by 9.2% with failure in two organs, and 6.9% with failure in three or more. The mean follow-up times were 5.7 months (range: 3.2 to 9.6 months) and 5.5 months (range: 3.1 to 9.2 months) for the development and validation sets, respectively. The 7-day, 28-day and 90-day overall survival rates of the HEV-ALF patients were 201 (49.8%), 157 (38.9%) and 155 (38.4%), respectively. The characteristics of all recruited patients are summarized in Table 1, and show that there was no significant difference among all variables between the development and validation sets.

**Prognostic factors for HEV-ALF patients’ 7-day, 28-day and 90-day mortality**

A univariate Cox analysis was firstly performed to observe the influences of clinical and laboratory parameters on HEV-ALF patients’ 7-day, 28-day and 90-day mortality, which indicated that HE, bacterial infection, alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), albumin (ALB), urea nitrogen (UREA), creatinine (CR), total bilirubin (TBIL), direct bilirubin (DBIL), the INR, prothrombin time (PT), cholinesterase (CHE), triglyceride (TG), total cholesterol, glucose, total triiodothyronine), neutrophil-to-lymphocyte ratio (NLR), RDW to lymphocyte ratio (RLR), platelet (PLT) count, and organ failure were all prognostic factors for HEV-ALF patients’ survival. Subsequently, multivariable analyses continued to demonstrate that GGT, ALB, TBIL, UREA, CR, INR, and NLR levels were independent risk factors for HEV-ALF patients’ survival (Table 1).

To further evaluate and rank the ability of the parameters to predict the mortality of HEV-ALF patients, OPLS-DA was next used. Nonsurvivors could be unambiguously distinguished from survivors using OPLS-DA (Fig. 1A, B). The top seven predictors were In (UREA), In (NLR), In (GGT), In (TBIL), In (INR), In (ALB), and In (CR) (Fig. 1C,D). UREA, NLR, GGT, TBIL, INR, ALB, and CR were finally identified as the seven best prognostic indicators, since they influenced the mortality of HEV-ALF patients independent from other parameters (identified by Cox regression) and were the top seven indicators with highest predictive capability (identified by OPLS-DA).

**Prognostic nomogram for HEV-ALF patients**

A prognostic nomogram was created to predict HEV-ALF patients’ 7-day, 28-day and 90-day survival using the significant independent risk factors for HEV-ALF patients’ survival (Fig. 2). The prognostic nomogram allows the user to predict the mortality of HEV-ALF patients, corresponding to a patient’s particular combination of covariates. For example, we can locate the patient’s GGT level and draw a line straight upward to the ‘points’ axis to determine the score associated with that GGT level. The same process was applied for ALB, TBIL, UREA, CR, INR, and NLR levels, and then we summed the scores achieved for each covariate, and located this sum on the ‘total points’ axis. Then, we drew a line straight down to determine the probability of mortality at each time point.

**Comparison of predictive accuracy for HEV-ALF patients’ 7-day, 28-day and 90-day mortality between the nomogram, MELD score, and CLIF-C-ACLFs in the development set**

Calibration tests were used to compare the predictive accuracy for the mortality of HEV-ALF patients between the nomogram, MELD score, and CLIF-C-ACLFs scores. The C-index for predicting HEV-ALF patients’ survival using the nomogram was 0.671 (95% CI: 0.602–0.740), which was statistically significantly greater than that of the MELD score at 0.540 (95% CI: 0.540–0.540) (all p<0.05). The calibration curve had an optimal agreement between the prognostic nomogram and the actual observation (Fig. 3A–C; Supplementary Table 1).

To estimate the prognostic efficiency of the nomogram, we compared the td-AUC between the nomogram, MELD and CLIF-C-ACLFs scores. Figure 4A–C shows the time-dependent receiver operating characteristics (td-ROC) curves of the nomogram, MELD score, and CLIF-C-ACLFs for predicting HEV-ALF patients’ mortality. The td-AUC for predicting 7-day mortality using the nomogram was 0.921 (0.872–0.970), and was statistically significantly greater than that obtained using the MELD score (0.474 [0.363–0.586]), and the CLIF-C-ACLFs (0.489 [0.376–0.603]) (both p<0.05). The td-
Table 1. Characteristics of the enrolled patients

| Variable                                      | Total (n=404) | Development set (n=249) | Validation set (n=155) | p | Univariate analysis | Multivariate analysis |
|------------------------------------------------|---------------|-------------------------|------------------------|---|---------------------|-----------------------|
|                                                |               |                         |                        |   | OR (95% CI)         | OR (95% CI)           |
|                                                |               |                         |                        |   | p                   | p                     |
| Clinical characteristics                       |               |                         |                        |   |                     |                       |
| Age, years                                    | 57.25±12.92   | 56.11±12.07             | 57.54±12.88            | 0.177 | 1.02 (0.97–1.12)     | 0.892                 |
| Sex, F/M                                      | 115/289       | 73/176                  | 42/113                 | 0.631 | 1.39 (0.42–5.06)     | 0.601                 |
| BMI                                           | 23.74±2.98    | 23.55±2.37              | 23.91±2.99             | 0.599 | 1.07 (0.92–1.08)     | 0.794                 |
| PH                                            | 7.42 (7.32–7.48) | 7.41 (7.33–7.47)     | 7.42 (7.31–7.49)       | 0.554 | 2.19 (0.94–4.99)     | 0.077                 |
| MAP, mm Hg                                    | 88.56±12.39   | 90.59±13.39             | 85.86±10.77            | 0.592 | 1.07 (0.91–1.04)     | 0.293                 |
| HE mild                                       | 30            | 19                      | 11                     | 0.842 | 2.84 (1.09–7.41)     | 0.033                 |
| HE severe                                     | 68            | 40                      | 28                     | 0.601 | 2.28 (1.14–4.55)     | 0.019                 |
| Muscle and/or joint pain (mild/serve)         | 81/34         | 48/22                   | 33/12                  | 0.585 | 1.09 (0.79–1.09)     | 0.322                 |
| Abdominal pain and/or vomiting(mild/serve)    | 106/64        | 62/39                   | 44/25                  | 0.753 | 1.12 (0.89–1.51)     | 0.554                 |
| Ascites, mild/serve                           | 94/51         | 58/31                   | 36/20                  | 0.914 | 1.05 (0.81–1.17)     | 0.488                 |
| Bacterial infection                           | 62(15.3%)     | 39(15.7%)               | 23(14.8%)              | 0.823 | 1.51 (1.12–2.19)     | 0.021                 |
| Laboratory parameters                         |               |                         |                        |   |                     |                       |
| WBC, 10^9/L                                    | 6.29 (5.46–8.91) | 6.21 (5.44–8.90)     | 6.44 (5.48–8.95)       | 0.687 | 2.25 (0.97–5.65)     | 0.897                 |
| RBC, 10^12/L                                   | 4.10±0.67     | 4.12±0.69               | 4.09±0.61              | 0.655 | 1.19 (0.87–1.38)     | 0.503                 |
| ALT, U/L                                       | 401.00 (133.00–1,134.00) | 418.00 (145.00–1,240.00) | 396.00 (124.00–1,042.00) | 0.599 | 1.00 (1.00–1.00)     | 0.043                 |
| AST, U/L                                       | 219.50 (84.50–617.00) | 223.00 (92.00–636.00) | 217.00 (82.00–610.00) | 0.673 | 1.00 (1.00–1.00)     | 0.477                 |
| GGT, U/L                                       | 98.00 (56.00–176.00) | 99.00 (57.00–177.00) | 97.00 (55.00–172.00) | 0.988 | 0.99 (0.99–1.00)     | 0.003                 |
| TP, g/L                                        | 57.21±8.81    | 57.87±8.51              | 56.63±9.12             | 0.164 | 0.99 (0.95–1.05)     | 0.331                 |
| ALB, g/L                                       | 31.72±5.77    | 32.12±5.71              | 31.42±5.66             | 0.227 | 0.95 (0.91–1.00)     | 0.039                 |
| TBIL, mol/L                                    | 299.54±165.81 | 297.76±156.64           | 305.12±176.41          | 0.660 | 1.00 (1.00–1.00)     | 0.016                 |
| DBIL, mol/L                                    | 217.60±116.82 | 213.87±110.12           | 223.87±124.32          | 0.396 | 1.00 (1.00–1.00)     | 0.035                 |
| UREA, mmol/L                                   | 4.58 (3.62–6.79) | 4.49 (3.51–6.61)     | 4.67 (3.81–7.30)       | 0.551 | 2.48 (1.33–4.64)     | 0.004                 |
| CR, mol/L                                      | 76.91 (66.00–94.00) | 77.20 (66.50–95.00) | 76.50 (64.00–94.50) | 0.956 | 1.00 (1.00–1.00)     | 0.016                 |
| PT, s                                          | 17.45 (15.50–23.55) | 17.35 (14.50–24.20) | 17.65 (14.50–23.90) | 0.912 | 1.05 (1.02–1.07)     | <0.001                 |

(continued)
Table 1. (continued)

| Variable | Total (n=404) | Development set (n=249) | Validation set (n=155) | \( p \) | Univariate analysis OR (95% CI) | \( p \) | Multivariate analysis OR (95% CI) | \( p \) |
|----------|---------------|-------------------------|------------------------|------|-------------------------------|------|-------------------------------|------|
| INR      | 1.50 (1.30–2.12) | 1.50 (1.32–2.15) | 1.51 (1.30–2.07) | 0.881 | 7.02 (2.59–19.01) | <0.001 | 7.72 (2.35–25.16) | 0.001 |
| Ammonia, \( \mu \)mol/L | 150.25 (87.56–185.55) | 143.50 (78.22–178.25) | 152.55 (80.20–182.50) | 0.674 | 1.01 (1.00–1.02) | 0.008 | 1.01 (1.00–1.02) | 0.186 |
| CRP, mg/L | 9.92 (7.15–14.87) | 9.77 (7.04–13.98) | 10.21 (7.19–14.97) | 0.697 | 0.98 (0.95–1.09) | 0.912 | 0.98 (0.95–1.09) | 0.912 |
| TG, mmol/L | 1.02 (0.82–1.54) | 1.04 (0.87–1.55) | 0.99 (0.80–1.32) | 0.812 | 0.42 (0.25–0.68) | <0.001 | 0.59 (0.21–1.65) | 0.310 |
| TCH, mmol/L | 2.26±0.78 | 2.30±0.67 | 2.21±0.79 | 0.218 | 0.71 (0.52–0.90) | 0.005 | 0.80 (0.41–1.54) | 0.496 |
| GLU, mmol/L | 3.67 (2.96–5.98) | 3.64 (2.99–5.98) | 3.78 (2.97–5.46) | 0.512 | 0.89 (0.82–0.97) | 0.008 | 0.92 (0.84–1.01) | 0.087 |
| Potassium, mmol/L | 4.55±0.73 | 4.59±0.77 | 4.49±0.69 | 0.184 | 1.31 (0.96–1.72) | 0.156 | 0.94 (0.69–1.41) | 0.542 |
| Sodium, mmol/L | 38.98±65.09 | 139.76±65.18 | 137.12±64.81 | 0.928 | 1.09 (0.92–1.21) | 0.542 | 0.92 (0.84–1.01) | 0.012 |
| Total T3, nmol/L | 103.97 (57.07–136.79) | 102.50 (58.25–123.22) | 105.50 (60.05–139.50) | 0.662 | 1.01 (1.00–1.01) | 0.004 | 1.00 (1.00–1.01) | 0.226 |
| Total T4, nmol/L | 0.94 (0.69–1.41) | 0.92 (0.75–1.32) | 0.96 (0.74–1.45) | 0.892 | 0.98 (0.71–1.31) | 0.552 | 0.80 (0.41–1.54) | 0.496 |
| TSH, mIU/L | 1.73 (1.05–3.12) | 1.67 (0.97–2.41) | 1.81 (1.05–3.09) | 0.446 | 0.92 (0.79–1.32) | 0.152 | 0.92 (0.79–1.32) | 0.152 |
| RDW | 14.88 (13.30–17.90) | 14.85 (13.20–16.00) | 14.95 (13.35–18.25) | 0.875 | 1.07 (0.98–1.17) | 0.132 | 1.07 (0.98–1.17) | 0.132 |
| RLR | 0.77 (0.59–1.42) | 0.77 (0.59–1.29) | 0.78 (0.59–1.41) | 0.596 | 2.46 (1.21–4.57) | 0.012 | 1.34 (0.71–2.52) | 0.367 |
| AFP, ng/mL | 38.37 (6.62–119.26) | 37.55 (6.00–112.00) | 39.90 (6.40–125.20) | 0.905 | 0.99 (0.79–1.22) | 0.812 | 0.99 (0.79–1.22) | 0.812 |
| CHE, U/L | 2,693.24 (2,412.50–3,312.09) | 2,656.50 (2,363.60–3,155.12) | 2,681.20 (2,450.30–3,486.80) | 0.472 | 1.00 (1.00–1.00) | <0.001 | 1.00 (1.00–1.00) | 0.228 |
| FER, ng/mL | 2,912.54 (1,395.43–4,957.72) | 2,907.79 (1,391.20–4,922.12) | 2,932.60 (1,399.20–4,997.54) | 0.976 | 1.00 (1.00–1.00) | 0.497 | 1.00 (1.00–1.00) | 0.497 |
| Other organ failure except for liver and cerebral | | | | | | | | |
| Kidney, \( n \) (%) | 58 (14.4%) | 35 (14.1%) | 23 (14.8%) | 0.827 | 4.08 (2.79–7.93) | <0.001 | 1.81 (0.62–5.30) | 0.279 |
| Coagulation, \( n \) (%) | 24 (5.9%) | 15 (6.0%) | 9 (5.8%) | 0.928 | 5.69 (4.02–7.97) | <0.001 | 2.39 (0.27–21.22) | 0.433 |
| Lung, \( n \) (%) | 16 (4.0%) | 10 (4.0%) | 6 (3.9%) | 0.942 | 1.60 (1.09–2.29) | <0.001 | 3.18 (0.18–55.80) | 0.428 |
| 2 organs failure (%) | 37 (9.2%) | 23 (9.2%) | 14 (9.0%) | 0.945 | 3.23 (2.79–4.29) | <0.001 | 3.23 (2.79–4.29) | <0.001 |
| ≥3 organs failure (%) | 28 (6.9%) | 17 (6.8%) | 11 (7.1%) | 0.917 | 3.48 (1.24–9.77) | 0.018 | 3.48 (1.24–9.77) | 0.018 |

Compare the difference between the development set and validation set by \( p \) or compare the difference between different prognosis in the development set by \( p \) under univariate analysis. BMI, body mass index; PH, degree of acid or alkali; MAP, mean arterial pressure; FER, Ferritin; WBC, white blood cell; RBC, red blood count; T3, triiodothyronine; T4, tetraiodothyronine; TSH, thyroid-stimulating hormone; GLU, glucose.
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**Fig. 2.** The nomogram for HEV-ALF patients' 7-day, 28-day and 90-day mortality, including UREA, NLR, GGT, TBIL, INR, ALB, and CR levels. The nomogram allows the user to obtain a probability of 7-day, 28-day and 90-day mortality corresponding to a patient's particular combination of covariates. To use the nomogram, locate the patient's value and draw a line straight upward to determine the score received for the variable. The sum of these scores is obtained for each covariate, which is then located on the 'Total Points' axis. A line is drawn downward to determine the likelihood of 7-day, 28-day and 90-day mortality on the survival axis.

**Fig. 1.** OPLS-DA was used to evaluate and rank the ability of the parameters to predict the mortality of HEV-ALF patients. (A) ROC of OPLS-DA. (B) In the three-dimensional scatter plot of all samples in the OPLS-DA model, the predictive component was used to distinguish survivors and nonsurvivors. (C) Loading plot showing the relation of each parameter to the predictive component (x) and the first orthogonal component (y); parameters that deviated from zero on the x-axis were considered potentially predictive. (D) The higher predictive VIP (VIP pred) value.
AUC within 28-day using the nomogram was 0.809 (0.710–0.907), and was statistically significantly greater than that using the MELD score, which was 0.683 (0.559–0.807), and the CLIF-C-ACLFs, which was 0.632 (0.498–0.766) (both \( p < 0.05 \)). A similar trend was seen with 90-day predictions. Comparisons of the td-AUC of all models for predicting HEV-ALF patients’ mortality are shown in Supplementary Table 2.

Moreover, DCA was used to further assess the net benefits of nomogram, MELD score, and CLIF-C-ACLFs assisted decisions at different threshold probabilities. Supplementary Figure 2A–C shows that the nomogram gave a better performance than the MELD score and CLIF-C-ACLFs over the entire range of threshold probabilities.

Validation of the predictive accuracy of the nomogram in the validation set

The clinical characteristics and laboratory parameters of the validation set are shown in Table 1. A good agreement was shown using the nomogram and the calibration curve between the prediction and actual observation of the probability of HEV-ALF patients’ 7-day, 28-day and 90-day survival (Fig. 3D–F). The C-index for the established nomogram was 0.671 (95% CI: 0.608–0.735), which was significantly greater than that of the MELD score, 0.578 (95% CI: 0.504–0.651), and the CLIF-C-ACLFs, 0.604 (95% CI: 0.530–0.675) (Supplementary Table 2). Notably, the performance of the established nomogram was also superior to that of MELD score and CLIF-C-ACLFs, which was confirmed by td-AUC (Fig. 4D–F; Supplementary Table 2) and DCA (Supplementary Fig. 2D–F).

Performance of the nomogram in stratifying risk among HEV-ALF patients

We determined the cut-off value by grouping the patients in the development set into two groups, on average after sorting according to the total score (low risk: 0–200, and high risk: \( \geq 201 \)); each group showed a different mortality (\( p<0.0001; \) Supplementary Fig. 3A). Similar results were obtained in the validation set. The nomogram performed well, allowing a remarkable distinction between the Kaplan-Meier curves for survival outcomes when stratifying into two risk subgroups (\( p<0.0001; \) Supplementary Fig. 3B).

Discussion

In the current study, a multicenter and multisample design was used with HEV-ALF patients. A new nomogram model was established and compared with traditional liver disease models to prognosticate the mortality of HEV-ALF patients. The nomogram integrated UREA, NLR, GGT, TBIL, INR, ALB, and CR levels, which are all significant independent risk factors for HEV-ALF patient survival. Notably, the nomogram
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had better predictive accuracy than the current conventional prognostic prediction scoring systems for liver failure.

The nomogram generated from the development set had a C-index that was superior to that of MELD score and the CLIF-C-ACLFs models. The calibration curves for the probability of 7-day, 28-day and 90-day overall survival showed optimal agreement between the nomogram prediction and actual observation values. Moreover, the td-ROC and DCA also showed that the nomogram was superior to the MELD and CLIF-C-ACLFs models. In addition, stratification into two risk subgroups (low-risk and high-risk) allowed remarkable distinction between Kaplan-Meier curves for survival outcomes. Similar results were also confirmed in the validation set.

Both multivariate logistic regression and OPLS-DA revealed that UREA, NLR, GGT, TBIL, INR, ALB, and CR levels are all independent risk factors for HEV-ALF patients’ survival. Both UREA and CR are important indicators for evaluating renal function. Consistent with previous studies, HEV infection and the associated renal injury is likely to be a causal factor. Cases of membranoproliferative glomerulonephritis with and without cryoglobulinemia, and membranous glomerulonephritis in HEV patients have been reported. A case of renal impairment during acute HEV infection in a solid organ transplant recipient has also been reported. INR is an important index to evaluate the coagulation function of patients. HEV infection is associated with certain hematological diseases. Severe thrombocytopenia has been reported in patients with acute HEV infection. All these symptoms are further aggravated with the development of HEV, especially for HEV-ALF patients. NLR, which was combined with neutrophils and lymphocytes, two inflammation indicators, has been reported to predict the prognosis of patients with stable cirrhosis, NAFLD and hepatitis B virus-related decompensated cirrhosis. Several other extra-hepatic disorders, such as myocarditis, thyroiditis, and myasthenia gravis, have been described with HEV infection.

Jiang et al. revealed that hypoalbuminemia was associated with an increased risk of ALF in patients with acute hepatitis A and B. In addition, Manka et al. reported that ALB levels were inversely correlated with the MELD score, INR, and bilirubin. Our study also confirmed ALB was an independent risk factor for HEV-ALF patient survival. Compared with the majority of ALF-cohorts in the worldwide literature, the mean age of our cohort was 57.25±12.92 years, being significantly older. We consider that this is related to the high incidence of hepatitis E failure in the elderly, the mechanism of which remains to be further studied. The 7-day, 28-day and 90-day overall survival rates of the HEV-ALF patients were significantly better than patients of other etiologies. All of these are consistent with the report by Shalimar et al.

This was a retrospective study, which inherently limits the generalization of its findings. First, all HEV-ALF patients were enrolled from five hospitals located in different regions of China. Therefore, the study was easily subject to selection bias and there was considerable heterogeneity likely between units. Second, the nomogram may not be useful for pregnant females, as this cohort only include nine pregnant females. Third, the role of nomogram in HEV-related ACLF patients has not been discussed in this study and requires further focused investigation.

Conclusions

In summary, the noninvasive nomogram may serve as an important method of HEV-ALF mortality evaluation for clini-
cians, and also enhance patient stratification in clinical trials.

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Conflict of interest
The authors have no conflict of interests related to this publication.

Author contributions
Conception and design (JW, CS, HC, LL), conduct of the literature search and writing of the manuscript (JW, CS), collection of patients’ samples and medical information (JX, XS, GL, XZ), data analysis and generation of the tables and figures (XS, YY), statistical analysis (JY); obtained funding and critically revised the manuscript (HC, LL).

Data sharing statement
All data are available upon request.

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