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# Early prediction of invasive fungal infection risk in acute-on-chronic liver failure: a prediction model based on admission indicators

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## Abstract

**Background** Acute-on-chronic liver failure (ACLF) is a severe clinical syndrome, and the incidence of invasive fungal infection (IFI) among hospitalized patients with ACLF is steadily increasing. The aim of this study is to develop a diagnostic nomogram to assist in the identification of IFI in these patients.

**Methods** A retrospective study included 705 patients from January 1, 2019, to October 31, 2023, randomly divided into training ($n=493$) and validation ($n=212$) cohorts. The diagnosis of IFI includes proven diagnosis and probable diagnosis. Kaplan analysis was performed to analyze the survival prognosis of ACLF patients with and without IFI. A nomogram was developed based on a logistic regression model derived through least absolute shrinkage and selection operator (LASSO) regression. The discrimination, accuracy, and clinical utility of the model were assessed using receiver operating characteristic curves, Hosmer-Lemeshow tests, calibration plots, and decision curve analysis.

**Results** Kaplan–Meier survival analysis confirmed that the median survival time of ACLF patients with IFI was significantly lower (by 68 days) than that of ACLF patients without IFI, and there were significant differences in the 90-day, 180-day, and 360-day survival rates between the two groups ($P<0.05$). Based on LASSO regression, the following factors were identified as significant risk factors for predicting IFI: aminotransferase levels, prothrombin activity, hemoglobin, neutrophil-to-lymphocyte ratio, and serum total bilirubin. A nomogram was constructed incorporating these variables. The nomogram demonstrated good discriminative ability, with an area under the receiver operating characteristic curve (AUC) of 0.78 (95% confidence interval [CI]: 0.72–0.84) in the training cohort and 0.79 (95% CI: 0.70–0.87) in the validation cohort. Decision curve analysis further validated the clinical applicability of the nomogram.

**Conclusion** ACLF patients with IFI have lower survival time than those without IFI. A nomogram was developed and validated to assist clinicians in the early prediction of IFI in hospitalized patients with ACLF.

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**Clinical trial number** Not applicable.

**Keywords** Lasso, Acute-on-chronic liver failure, Invasive fungal infection, Nomogram, Risk model

## Introduction

Acute-on-chronic liver failure (ACLF) is a life-threatening syndrome characterized by acute and severe hepatic dysfunction in patients with chronic liver disease, often accompanied by multiple organ failure and a high 28-day mortality rate [1]. In patients with ACLF, the body's immune function is severely impaired, and the intestinal flora is in a state of imbalance, resulting in a significant increase in opportunistic infections including fungi. Although the application of diagnostic and treatment means such as glucocorticoids, broad-spectrum antibacterial drugs, and invasive procedures has significantly prolonged the survival period of liver failure patients, the incidence of fungal infections has shown an upward trend [2].

Invasive fungal infection (IFI) can act as a trigger of ACLF and has a signifcant impact on the disease severity in ACLF patient. According to research by Khan et al., patients with ACLF who developed IFI had a higher mortality rate compared to those with bacterial infections and experienced a shorter median survival time [3]. The mortality rate escalates successively in the presence of superficial fungal infection, invasive Candida infection, and invasive Aspergillus infection [4]. Early diagnosis of IFI is crucial for guiding appropriate treatment and improving survival rates in ACLF patients.

However, diagnosing IFI remains complex and challenging. In 2022, China released the "Expert Consensus on the Diagnosis and Treatment of Invasive Fungal Infection in Patients with Severe Liver Disease [5], suggesting that the diagnosis of severe liver disease complicated with IFI should be divided into three levels based on the evidence of fungal microbiological examination, host factors, and clinical features: proven, probable, and possible. Patients who meet the clinical diagnosis criteria (probable diagnosis) should receive preemptive treatment. Our goal is to identify the high-risk factors of IFI and develop an early predictive model, which is mainly based on the clinical characteristics of ACFL patients at admission, enabling timely intervention and improved patient outcomes.

## Methods

### Patients

A total of 753 ACLF patients between January 1, 2019 and October 30, 2023 were included in this study, based on the data collected at the Infectious and Liver Disease Center of the Second Afliated Hospital of Chongqing Medical University.

The diagnostic criteria of ACLF were based on the following criteria specified by the Asian Pacific Association for the Study of the Liver [1] and the Guideline for Diagnosis and Treatment of Liver Failure in China [6]. It can be summarized as follows: patients with chronic liver disease or cirrhosis suffer a sever liver damage manifesting as jaundice (a serum total bilirubin (TBIL) level of 5 mg/dL or higher), coagulopathy (an international normalized ratio level of 1.5 or higher, or a prothrombin activity level of less than 40%), and the development of ascites and/or hepatic encephalopathy (HE) within 4 weeks. The diagnostic criteria of IFI were based on Expert Consensus on the Diagnosis and Treatment of Invasive Fungal Infection in Patients with Severe Liver Disease [5] and The Chinese guidelines for the diagnosis and treatment of invasive fungal disease in patients with hematological disorders and cancers (the 6th revision) [7]. The diagnosis of IFI was classified into four categories: proven, probable, possible, and uncertain diagnosis. The criteria were divided into host, clinical and mycological criteria. The proven diagnosis could be defined as the culture results of the tissue obtained from the infected site were positive through the aseptic technique or blood culture was positive. The probable diagnosis required host, clinical criteria and one of mycological criteria which could be difined as galactomannan (GM) or 1,3-β-d-glucan (BDG) testing was positive in those clinical culture (serum, bronchoalveolar lavage fluid, cerebrospinal fluid). However, only those with proven or probable diagnosis were considered as case definition for IFI [8].

The Child-Turcotte-Pugh (CTP) score is calculated based on five key parameters: ascites, hepatic encephalopathy (HE), total bilirubin (TBIL), albumin (Alb), and prothrombin time (PT) extension. Each parameter is scored from 1 to 3, with the total score ranging from 5 to 15 points. HE was diagnosed according to the traditional West-Haven criteria [9], which define it as mild cognitive dysfunction, alterations in personality or behavior, psychiatric abnormalities, and, in severe cases, coma. Gastrointestinal bleeding (GIB) is characterized by hematemesis, melena, or hematochezia.

If the patients met the following criteria, they would be excluded (1) ≤ 18 years old or ≥ 85 years old, (2) hepatocellular carcinoma or other malignancies, (3) previous liver transplantation, (4) complications with other severe chronic extrahepatic diseases, (5) infection with any type of immuneodeficiency or receiving long-term immune-suppressive medication (more than one month), (6) lack of clinical information. A total of 48 patients were excluded, who had a history of

immuneodeficiency ($n = 9$), hepatocellular carcinoma or other malignancies($n = 30$), severe illnesses ($n = 6$), missing clinical data ($n = 2$). Finally, a total of 705 patients were included in the study (Fig 1). Patients were divided into the training ($n = 493$) and validation ($n = 212$) cohorts at a ratio of 7:3. Demographic data, medical histories, clinical diagnoses, laboratory data, and other relevant medical information were extracted from the hospital's electronic medical records system.

### Treatment

During hospitalization, all patients received supportive therapy, which included adequate nutritional support, monitoring of vital signs, sufficient rest, and liver protection drugs. Considering the severity of our patients' conditions, all patients had received artificial liver support system. We conducted rigorous monitoring for complications associated with ACLF. For patients with ascites, we implemented diuretic therapy or performed paracentesis drainage when indicated. In cases of HE, we provided appropriate treatment for them. For gastrointestinal bleeding, hemostatic agents, such as proton pump inhibitors (PPIs) and somatostatin analogs, were promptly used. In instances of spontaneous bacterial peritonitis (SBP), empirical antibiotic therapy was initiated. As for patients with IFI anti-fungal medications were administered according to established guidelines. Moreover, treatments of the underlying disease were also necessary.

### Statistical analysis

In our study, continuous variables that did not follow a normal distribution are presented as medians with interquartile ranges (P25–P75). Comparisons were made using the Student's t-test or the Mann–Whitney U test, as appropriate. Categorical variables are presented as numbers (percentages) and were compared using the Chi-square test or Fisher's exact test. All data were analyzed using IBM SPSS Statistics (version 26.0, Chicago, USA) and R statistical software (version 4.3.2, Vienna, Austria). A $p$-value $< 0.05$ (two-sided) was considered statistically significant. The probabilities of survival outcomes were meticulously calculated using the Kaplan–Meier methods, a well-established statistical technique for estimating the survival function from lifetime data. Lasso (Least Absolute Shrinkage and Selection Operator) is a widely used regression method in statistics. Its core principle involves shrinking the coefficients to achieve variable selection and complexity adjustment, which in turn improves the predictive accuracy and interpretability of the model. LASSO regression is particularly effective in addressing multicollinearity and high-dimensional data. The R package "glmnet" (R Foundation) was used to perform the LASSO regression analysis. The most predictive covariates were selected based on λ (λ = Lambda.1se). λ (lambda) is a regularization parameter in LASSO regression, it controls the degree of shrinkage of the regression coefficients. A smaller λ allows more variables to enter the model with relatively larger coefficients, while a larger

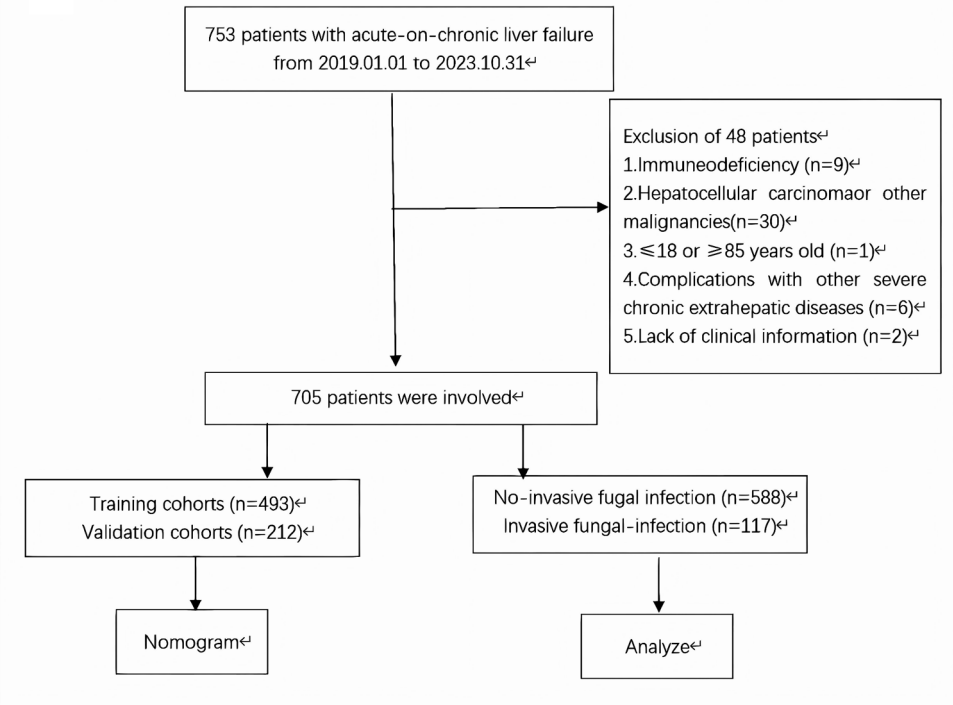


**Fig. 1** Flow chart of patient selection

λ forces more coefficients to shrink towards zero, performing variable selection and reducing model complexity. To determine the optimal value of λ, we employed cross-validation which is performed by the cv.glmnet function. In this function, the number of folds for cross-validation is 10 by default. In addition, we set the range of λ between 0.001 and 1. By doing so, we can identify the value of λ that results in the best predictive performance of the model. Subsequently, cross-validation was employed for iterative analysis, as depicted in Fig. 3B. Through this process, an optimal value of the regularization parameter λ was determined. Prognostic variables identified through LASSO regression were then analyzed using multivariable analysis, with significant risk factors ($p < 0.05$) included in the logistic regression model. Finally, the risk score is established based on nomogram and a validation cohort was used to evaluate the model's predictive ability. A nomogram is a graphical tool that combines multiple predictive factors into a single model to estimate the probability of a particular outcome. It presents a visual representation of a statistical model, allowing clinicians and researchers to quickly calculate the likelihood of an event occurring based on the values of the input variables. The diagnostic accuracy of the model is evaluated through the use of Receiver Operating Characteristic (ROC) analysis. ROC curve area under the curve (AUC) was compared using the DeLong's method. The accuracy of the model was assessed using the Hosmer-Lemeshow test and calibration curves. Additionally, decision curve analysis (DCA) was employed to evaluate the clinical utility of the model. DCA [10] is a graphical method used to evaluate the net benefit of a prediction model across a range of threshold probabilities. It compares the model's performance with two extreme strategies: one where all patients are assumed to have the disease (treat all) and the other where no patient is assumed to have the disease (treat none). By plotting the net benefit of the model against different threshold probabilities, DCA helps determine the clinical utility of the model in different decision-making scenarios.

## Results

### Baseline characteristics of patients with and without IFI

A total of 705 patients with ACLF were included and a total of 117 patients were infected by fungi. Patients with IFI showed a higher frequency of hepatic encephalopathy (HE), ascites, gastrointestinal bleeding (GIB), bacterial infection. Moreover, in laboratory data, patients with IFI had a higher level of aminotransferase (AST), serum total bilirubin (Tbil), fibrin degradation products (FDP), white blood cell (WBC), neutrophil granulocyte account (NEUT#), platelet distribution width (PDW), neutrophil to lymphocyte ratio (NLR), serum creatinine (SCr), Creactive protein (CRP) and procalcitonin (PCT). As for albumin (ALB), prothrombin activity (PTA), international normalized ratio (INR), hemoglobin (HGB), platelet account (PLT), lymphocyte account (LYMPH#), glomerular filtration rate (GFR), those indicators were much lower in patients with IFI. Howerer, in the cause of ACLF, patients without IFI had a higher rate of HBV infected. We also analized CTP score and MELD (model for End-Stage Liver Disease) scores in both groups of patients. The IFI patients got higher score in CTP ($p = 0.004$) and MELD ($p < 0.001$), which means thoses patients suffered more severe liver damage (Table 1).

According to our diagnostic criteria for IFI, we divided the patients into two groups: proven diagnosis and probable diagnosis. Detailed characteristics of patients with different diagnosis of IFI was shown in Table S1. No statistically significant differences were found between the two groups in terms of demographic characteristics, clinical features, laboratory data or antifungal treatments, except for (1,3)-beta-D-glucan (G test) levels. Based on this, we inferred that there was no substantial difference between the proven and probable diagnoses. Among patients with a proven diagnosis, invasive candidiasis was the most common fungal infection, affecting 31 patients. In contrast, only 6 patients were diagnosed with invasive aspergillosis, which was much less common than invasive candidiasis.

### Prognosis of ACLF patients with IFI

The probabilities of survival outcomes were meticulously calculated using the Kaplan–Meier methods (Fig. 2), a well-established statistical technique for estimating the survival function from lifetime data. During our one-year follow-up period, six patients required liver transplantation, so we followed the survival time of 699 patients. In the analysis of the survival data, it was found that the median survival time for patients with Invasive Fungal Infection (IFI) was significantly shorter than that of patients without IFI. Specifically, the median survival time of IFI patients was 68 days less than that of non-IFI patients. Moreover, the survival rate of non-IFI patients was greater than 50%, indicating a relatively better prognosis for this group.

Table S3 summarized the baseline clinical and laboratory parameters for predicting 90-day mortality in ACLF patients with IFI by using a univariate Cox regression analysis. The results of the univariate analysis indicated that hypertesion, GIB, Tbil, INR, PTA, FDP, HGB, WBC, PLT, neutrophils, lymphocytes, NLR, GFR, SCr and PCT were significantly associated with 90-day mortality in IFI patients, according to the results of the univariate analysis ($p < 0.05$). We calculated the variance inflation factor (VIF) to ensure no multicollinearity in these variales, and excluded neutrophils whose VIF value was 192.79 (VIF > 5). Although there is no multicollinearity between

**Table 1** Baseline characteristics of patients with and without IFI

| Variables | No Invasive funal infection($n=588$) | Invasive fungal infection($n=117$) | *P* value |
|---|---|---|---|
| Male(%) | 469(79.8) | 98(83.8) | 0.320 |
| Age(years) | 49(41–56) | 52(46–62) | 0.002 |
| Diabetes(%) | 69(11.7) | 21(17.9) | 0.066 |
| Hypertention(%) | 56(9.5) | 17(14.5) | 0.105 |
| Smoke(%) | 288(49.0) | 56(47.9) | 0.826 |
| CTP | 11(10–11) | 11(10–12) | 0.004 |
| MELD | 21.40(18.52–25.20) | 25.89(20.11–29.31) | 0.000 |
| Cause | | | 0.003 |
| HBV(%) | 508(86.4) | 89(76.1) | |
| ALD(%) | 25(4.3) | 5(4.3) | |
| AIH(%) | 18(3.1) | 8(6.8) | |
| Others(%) | 37(6.3) | 15(12.8) | |
| Crohosis(%) | 409(69.6) | 88(75.2) | 0.221 |
| Complications | | | |
| HE(%) | 98(16.7) | 31(26.5) | 0.012 |
| Ascites(%) | 449(76.4) | 104(88.9) | 0.003 |
| GI bleeding(%) | 48(8.2) | 19(16.2) | 0.007 |
| Bacterial infection | 523(88.9) | 105(89.7) | 0.801 |
| Laboratory data | | | |
| ALT(U/L) | 382(168–620) | 267(101–528) | 0.026 |
| AST(U/L) | 239(133–443) | 262(117–510) | 0.646 |
| GLP(U/L) | 139(116–176) | 136(111–179) | 0.731 |
| GGT(U/L) | 84(58–126) | 83(52–132) | 0.549 |
| ALB(g/L) | 30.9(28.4–34.0) | 30.1(27.4–32.9) | 0.044 |
| Tbil(μmol/L) | 290.4(213.1-379.3) | 403.5(309.7-484.1) | 0.000 |
| PTA(%) | 37(27–46) | 29(23–39) | 0.000 |
| INR | 2.08(1.73–2.71) | 2.47(1.88–3.18) | 0.001 |
| FDP(ug/ml) | 5.79(2.70-11.18) | 9.41(5.73–16.42) | 0.000 |
| HGB(g/L) | 123(110–136) | 107(90–122) | 0.000 |
| WBC($\times10^9$/L) | 6.21(4.82–8.42) | 7.1(5.26–10.22) | 0.006 |
| PLT(×109/L) | 90(63-123.75) | 72(41.5-107.5) | 0.000 |
| NEUT#(×109/L) | 4.51(3.24–6.45) | 5.86(3.94–8.59) | 0.000 |
| LYMPH#(×109/L) | 0.98(0.67–1.32) | 0.71(0.47–1.05) | 0.000 |
| MONO#(×109/L) | 0.53(0.38–0.76) | 0.52(0.31–0.78) | 0.339 |
| RDW(%) | 16.3(16-16.7) | 16.3(15.9–16.8) | 0.616 |
| PDW(%) | 14.85(13.6-17.08) | 17(14.45–20.75) | 0.000 |
| NLR | 4.78(3.02–7.94) | 7.90(5.90-13.28) | 0.000 |
| GFR(ml/min1.73m2) | 113.6(102.5-123.6) | 107.8(80.9-120.3) | 0.001 |
| SCr(μmol/L) | 58.2(50.3–68.4) | 63.2(50.5–79.4) | 0.032 |
| Na(mmol/L) | 135(132.3-137.1) | 134.5(132.15–137.2) | 0.595 |
| CRP(mg/L) | 10.15(6.44–18.36) | 18.82(9.16–32.89) | 0.000 |
| PCT(ng/ml) | 0.61(0.39–0.97) | 1.05(0.61–1.72) | 0.000 |

CTP, Child-Turcotte-Pugh score; MELD, model for End-Stage Liver Disease; HBV, Hepatitis B virus infection; ALD, alcoholic liver disease; AIH, autoimmune hepatitis; HE, hepatic encephalopathy; GI bleeding, gastrointestinal bleeding; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GLP, gamma-glutamyltransferase; GGT, gamma-glutamyltransferase; ALB, Albumin; Tbil, total bilirubin; PTA, prothrombin activity; INR, international normalized ratio; FDP, fibrinogen degradation products; HGB, hemoglobin; WBC, white blood cells; PLT, platelet; NEUT#, neutrophils; LYMPH#, lymphocytes; MONO#, monocytes; RDW, red cell distribution width; PDW, platelet distribution width; NLR, neutrophil-to-lymphocyte ratio; GFR, glomerular filtration rate; SCr, serum creatinine; Na, sodium; CRP, C-reactive protein; PCT, procalcitonin

PTA and INR, they are generally regarded as representing the same type of clinical indicator. Therefore, we chose INR, which has a wider confidence interval. There were 13 factors were incorporated into the multivariate Cox proportional hazards model and only 6 factors showed statistical significance: hypertension, INR, HGB, WBC, NLR and PCT. However, hypertension and PCT were identified as protective factors for 90-day mortality in IFI patients. This results might be due to the limited number of IFI patients in our study or the fact that it is a single center study. I believed that with more research and data accumulation, clearer conclusions and stronger evidence would emerge to validate these findings.

When examining the mortality rates at different time points, it was observed that there was a significant difference in the 90-day mortality rate between patients with and without IFI. The bar chart in Fig. 2 clearly shows that the percentages of IFI patients who died within 90 days, 180 days and 360 days were much higher compared to non-IFI patients (90days: 57.4% vs. 33.5%, 180days: 61.5% vs. 37.1%, 360days: 64.1% vs. 38%) with the p-value being less than 0.001, indicating a statistically significant difference. However, when looking at the 28-day mortality rate, there was no significant difference between the two groups (19.7% vs. 19.4%), as suggested by the p-value of 0.952.

We also conducted corresponding research on antifungal treatment. In our study, each patient was evaluated on the basis of imaging findings, laboratory indicators as criteria to assess the effectiveness of antifungal medication. In the 90-day survival group, 42 individuals had effective antifungal treatment (79.2%), while in the death group, only 14 individuals (24.6%) (HR 0.169, 95%CI 0.092–0.312, $p<0.05$). As for antifungal medication duration, the median duration of antifungal medication in the survival group was 15 days, while in the death group, it was only 6 days (HR 0.919, 95%CI 0.886–0.953, $p<0.05$). Due to some patients discontinuing treatment or dying during the course of treatment, the antifungal medication duration for some patients was inadequate, which might introduce some bias in our results. However, it was undeniable that prolonging the antifungal medication duration could increase the survival rate of IFI patients.

### Prediction model based on lasso regression

The patients were divided into two cohorts: the training cohort and the validation cohort. The splitting ratio was set at 7:3, resulting in 493 patients in the training cohort and 212 patients in the validation cohort. Statistical analysis demonstrated that there was no significant difference between these two cohorts, as shown in Table S2. The training cohort was primarily utilized for two important purposes: feature selection and model development. In order to identify the key parameters, LASSO (Least

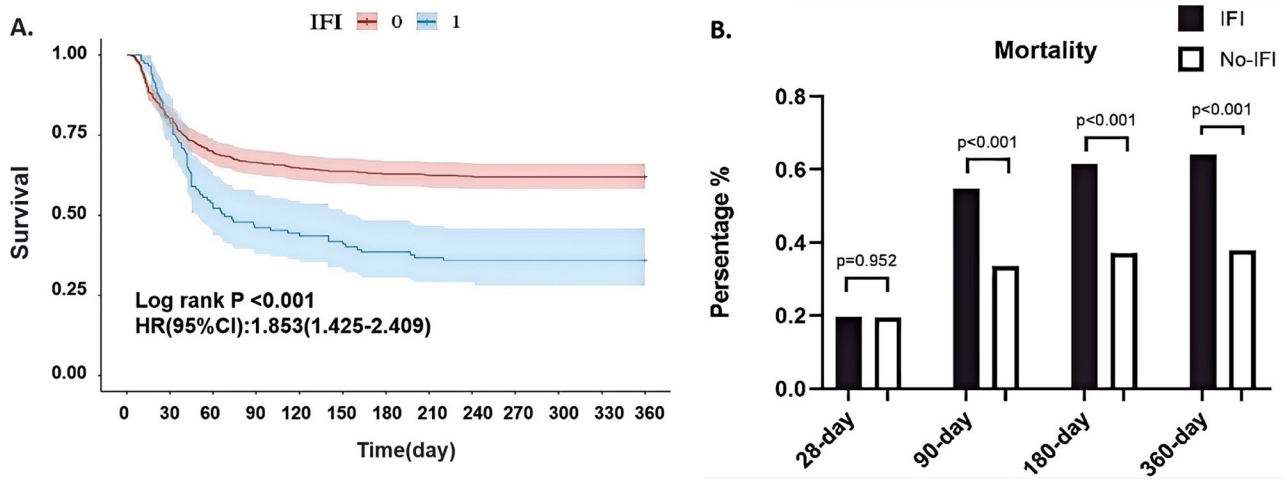


**Fig. 2** Prognosis of ACLF with and without IFI. (**A**) Kaplan- Meier survival curves comparing patients with invasive fungal infection (IFI, red line) and those without IFI (blue line). (**B**) Bar chart showing the mortality percentage of patients with IFI (black bars) and without IFI (white bars) at different time points (28-day, 90-day, 180-day, and 360-day)

Absolute Shrinkage and Selection Operator) regression was applied. A comprehensive set of 35 baseline variables was incorporated into the LASSO regression analysis. In Fig 3, the coefficients of these 35 baseline variables are illustrated. This graphical representation provides an overview of the relative importance of each variable in the context of the regression analysis.

When λ = min (0.01041921), seven variables were selected as feature variables: AST (Aspartate Aminotransferase), PTA (Prothrombin Time Activity), HGB (Hemoglobin), NLR (Neutrophil-to-Lymphocyte Ratio), PCT (Procalcitonin), TBIL (Total Bilirubin), and PLT (Platelet). Multivariable logistic regression analysis confirmed that AST, PTA, HGB, NLR, Tbil were independent risk factors ($p < 0.05$) which were subsequently incorporated into the nomogram (Table 2). The nomogram serves as a predictive tool for estimating the probability of IFI in patients with ACLF, as depicted in Fig. 4.

Figure 4 illustrates an example of using the nomogram to predict IFI in hospitalized ACLF patients. The total score is calculated based on the individual scores derived from the nomogram. The following conditions are associated with an increased risk of IFI: elevated AST levels, impaired coagulation (PTA), reduced hemoglobin (HGB), a higher neutrophil-to-lymphocyte ratio (NLR), and more severe jaundice (TBIL). The longer the line for each variable, the greater its influence on the likelihood of IFI. By summing the individual scores, a total score can be calculated, which is then used to estimate the risk of IFI in hospitalized ACLF patients.Therefore, in order to make our nomogram more intuitive, we provided an example: a patient with AST = 1000 U/L, PTA = 20%, HGB = 80 g/L, NLR = 10, and TBIL = 400 μmol/L, the scores for each parameter are as follows: AST = 24, PTA = 27, HGB = 51, NLR = 11, and TBIL = 35. The total score is 148, indicating an approximate 70% probability of a fungal infection.

### Validation of predictive model

To verify the prediction ability of this model, we formulated a risk score equation: 0.1309583 + 0.001225×AST-0.0282114×PTA-0.0295534×HGB + 0.0574862×NLR + 0.0045628×Tbil. Figure 5 presents a comprehensive evaluation of the nomogram's performance in predicting invasive fungal infection (IFI) in patients with acute-on-chronic liver failure (ACLF), encompassing calibration curves, decision curve analysis (DCA), and receiver operating characteristic (ROC) curves for both the training and validation cohorts.

In the training cohort (Fig. 5A), the DCA curve demonstrates that the nomogram offers net benefits across a broad spectrum of threshold probabilities. This implies that integrating the nomogram into clinical decision-making processes can enhance patient management strategies by optimizing the balance between true positives and false positives, ultimately leading to improved patient outcomes. Similarly, in the validation cohort (Fig. 5B), the DCA curve exhibits net benefits, and its resemblance in shape and position to that of the training cohort attests to the model's robustness and generalizability. This consistency indicates that the nomogram can be effectively applied in diverse clinical settings. The calibration curves in both cohorts (Fig. 5C for the training cohort and Fig. 5D for the validation cohort) exhibit a remarkable concordance between the predicted probabilities of IFI by the nomogram and the actual observed incidences. This alignment validates the model's ability to accurately estimate the likelihood of IFI occurrence, providing clinicians with reliable prognostic information.

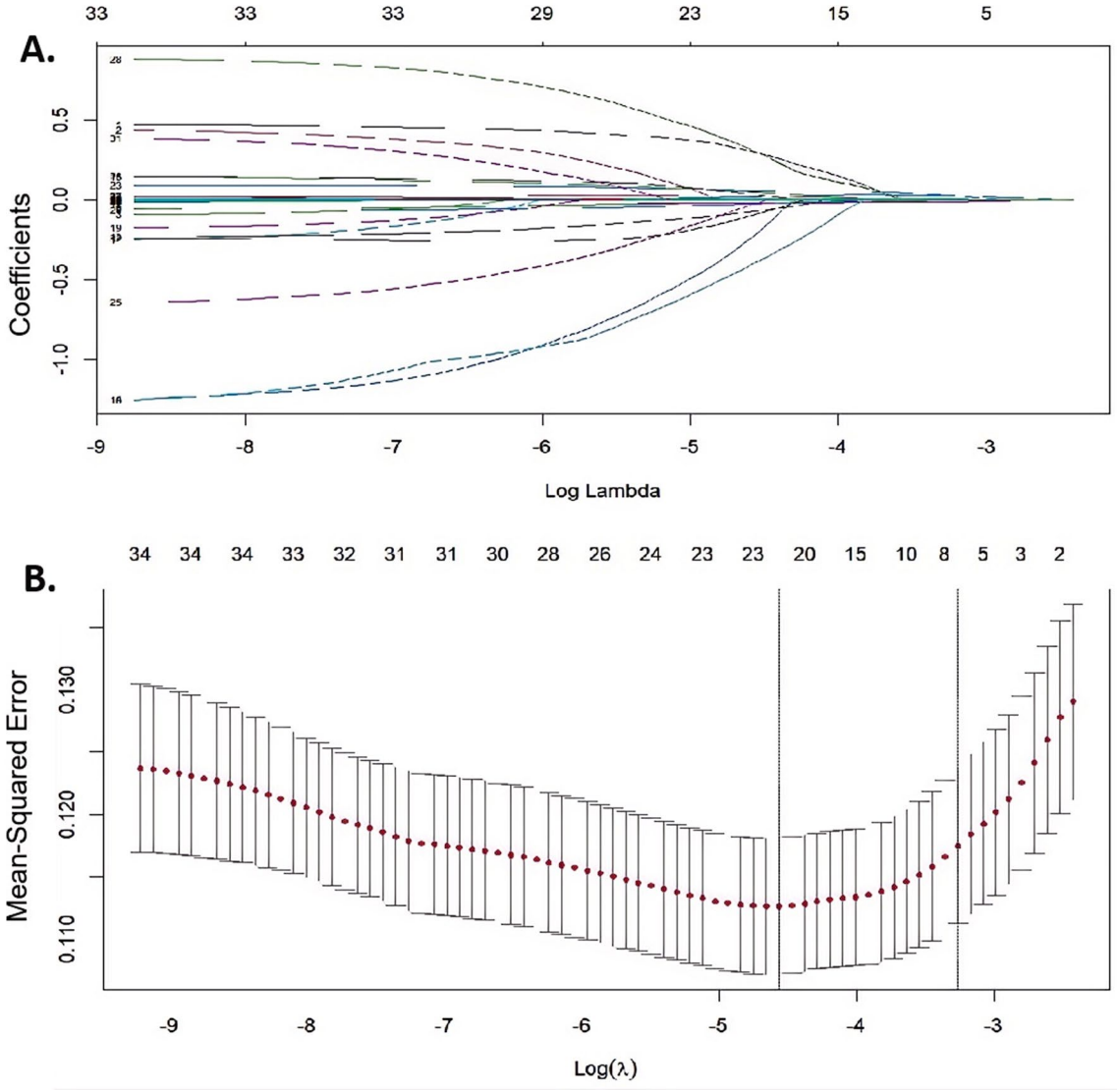


**Fig. 3** Screening of variables based on Lasso regression. (**A**) The variation characteristics of the coefficient of variables; (**B**) the selection process of the optimum value of the parameter λ in the Lasso regression model by cross-validation method

**Table 2** Multivariate logistic regression analysis for the risk of IFI in ACLF

| Variables | OR | 95%CI | *p*-value |
|---|---|---|---|
| AST | 1.000 | 1.000-1.002 | 0.007 |
| PTA | 0.952 | 0.952–0.998 | 0.034 |
| HGB | 0.962 | 0.962–0.987 | 0.000 |
| NLR | 1.015 | 1.015–1.091 | 0.006 |
| PCT | 0.949 | 0.949–1.330 | 0.177 |
| Tbil | 1.002 | 1.002–1.007 | 0.000 |
| PLT | 0.989 | 0.989–1.001 | 0.111 |

The ROC curves for both cohorts (Fig. 5E for the training cohort and Fig. 5F for the validation cohort) reveal excellent discriminative capabilities. In the training cohort, the area under the curve (AUC) is 0.78 (95% confidence interval [CI]: 0.72–0.84), while in the validation cohort, it is 0.79 (95% CI: 0.70–0.87).

## Discussion

Infection plays a crucial role in the context of ACLF. It not only acts as a common trigger that initiates the development of ACLF in patients with underlying liver cirrhosis but also complicates the clinical course. The link between ACLF and increased infection-related mortality has been well-documented in multiple studies [11]. When ACLF is due to infection, the patient's prognosis is often poorer compared to those with ACLF caused by other non-infectious etiologies [12]. The immunodeficient state that accompanies end-stage liver disease weakens the patient's immune defenses, making them more vulnerable to various pathogens, especially

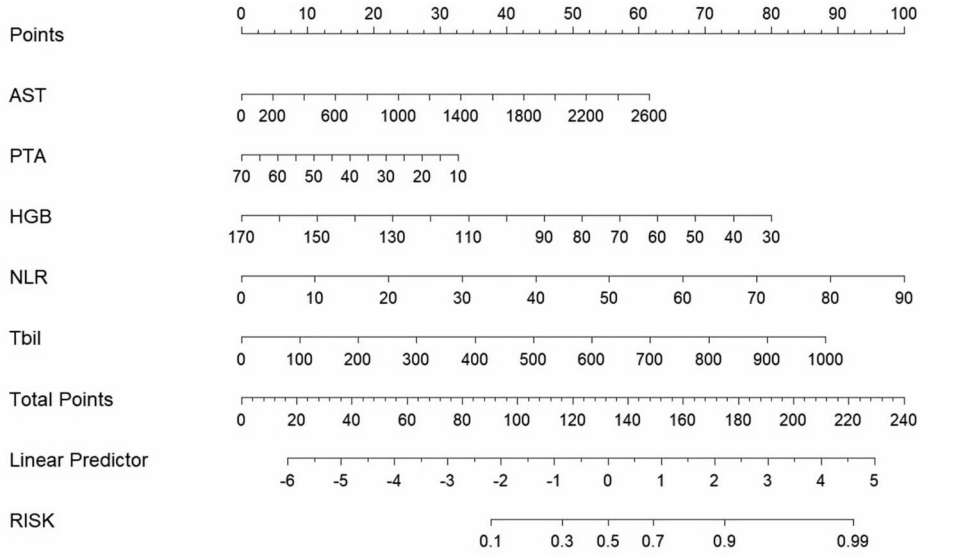


**Fig. 4** Nomogram used to predict IFI in hospitalized ACLF patients. The total score is calculated based on the individual scores derived from the nomogram. AST aminotransferase; PTA, prothrombin activity; HGB, hemoglobin; NLR, neutrophil to lymphocyte ratio; Tbil, serum total bilirubin

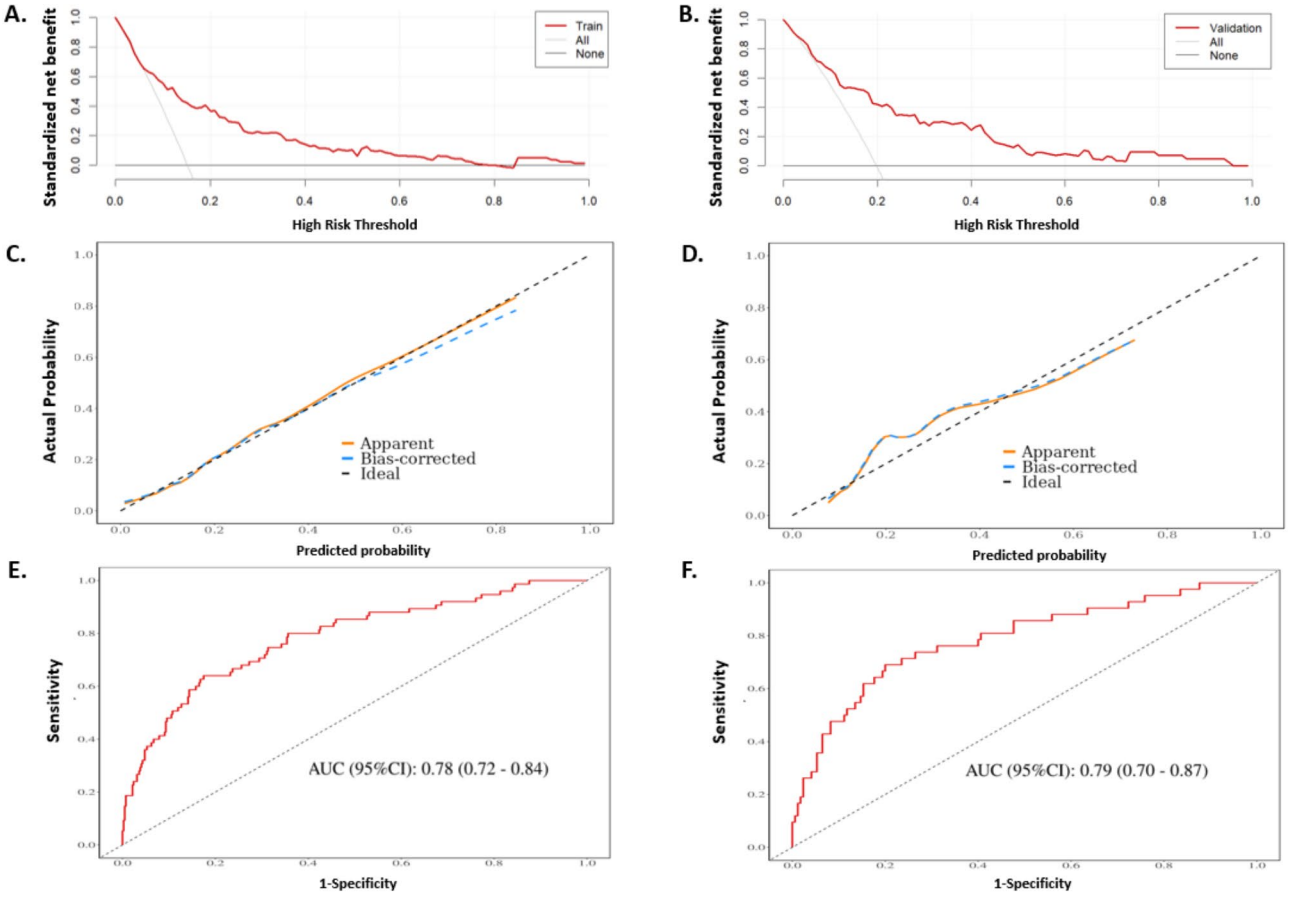


**Fig. 5** The calibration curves, the decision curve analysis and the receiver operating characteristic curves of the nomogram in the training cohort and validation cohort. (**A**)Decision curve analysis in the training cohort. (**B**)Decision curve analysis in the validation cohort. (**C**)The calibration curve in the training cohort. (**D**)The calibration curve in the validation cohort. (**E**)The receiver operating characteristic curves in the training cohort. (**F**)The receiver operating characteristic curves in the validation cohort

opportunistic ones such as fungi. Invasive fungal infections, which have not been fully appreciated in the past, are now being recognized as a significant contributor to the high mortality rate in ACLF cases [13, 14]. The recent research findings have shed light on the fact that fungal infections have a more profound impact on the progression of ACLF. They are associated with the occurrence of severe forms of ACLF, a greater likelihood of patients requiring admission to the intensive care unit for closer monitoring and more intensive treatment, and ultimately, a higher risk of death when compared to bacterial infections [15, 16]. The same result was also confirmed in our study. We found that the median survival time of patients with invasive fungal infection (IFI) is 68 days less than that of patients without IFI, and the survival rate of non-IFI patients exceeds 50%, which further verifies that IFI has an impact on the prognosis of ACLF. When it comes to mortality rates at different time points, there is no significant difference in the 28-day mortality between ACLF patients with and without IFI (with a p-value of 0.952), while significant differences exist in the 90-day, 180-day, and 360-day mortality rates (with p-values less than 0.001). We hypothesized that the observed difference in the 90-day mortality rate, but not in the 28 day mortality rate, could be attributed to the effectiveness of the treatment provided. It is possible that the treatment strategies implemented during the initial 28 days were able to stabilize both groups of patients to some extent, preventing a significant divergence in mortality at this early stage. However, as time progressed to 90 days, the cumulative effects of IFI, perhaps due to its more chronic and invasive nature, led to a significant increase in mortality among IFI patients, despite the treatment efforts. This implies that while the initial treatment may have been adequate in the short-term, additional or more targeted interventions may be required to address the long-term survival challenges posed by IFI. This emphasizes the need for a better understanding of the unique characteristics and consequences of fungal infections in the setting of ACLF and the development of more effective preventive and therapeutic strategies.

With the aim of facilitating the early identification of patients at high risk of invasive fungal infection (IFI) by clinicians during admission, LASSO regression was employed in this study to analyze 35 baseline features at the time of admission. AST, PTA, HGB, NLR and TBIL were finally confirmed independent risk factors ($p < 0.05$) by multivariate logistic regression analysis, which were subsequently incorporated into the nomogram. The strength of this methodology lies in its adeptness at handling multicollinearity and high-dimensional data, culminating in a more parsimonious and readily interpretable model.

AST, PTA, and Tbil serve as pivotal biomarkers indicative of liver injury and play a decisive role in prognosticating ACLF [17]. The progression of liver disease to an advanced stage is usually accompanied by a compromised immune system, thereby augmenting the susceptibility to opportunistic fungal infections [18]. The nexus between these markers and IFI risk is multifaceted. AST, ubiquitously present in tissues, is crucial for liver mitochondrial gluconeogenesis [19]. In acute liver injury, elevated AST indicates potential mitochondrial damage [20]. Despite ALT's liver specificity, AST better predicts liver-related mortality [21]. In ACLF, the AST-IFI risk link may stem from mitochondrial damage disrupting hepatic and immune function, favoring fungal invasion, supported by correlations with other markers. Impaired coagulation function is also a known susceptibility factor for fungal infections [22], it mainly due to the the severity of the liver disease [23]. INR-PTA multicollinearity complicates analysis; thus, INR was excluded for model accuracy. Future research should disentangle coagulation-IFI interplay for refined risk stratification. HGB, essential for oxygen transport, has a complex role in fungal-host interaction. HGB degradation yields antimicrobials [24] but also provides fungal iron, depleting reserves and reducing Hb, exacerbated by decompensated liver failure inflammation [25]. In ACLF, lower HGB relates to increased IFI risk via immune and iron availability mechanisms. NLR, a valuable biomarker, reflects immune balance and is linked to various conditions [2627]. Elevated NLR (> 3.0) indicates pathology. In our research, a suite of clinical infectious indicators was incorporated, namely PCT, CRP, WBC, NEUT#, LYMPH#, MONO#, and NLR. Notably, among these, only NLR manifested statistical significance, whereas the remaining five did not. One plausible explanation lies in the fact that the majority of liver failure patients typically reside in a state of systemic inflammatory response. This renders the differentiation between infectious and non-infectious etiologies arduous [28]. Additionally, fungi can express specific virulence factors, thereby eluding the antimicrobial actions of neutrophil extracellular traps (NETs) [29, 30].

These identified risk factors proffer a comprehensive elucidation of the pathophysiological underpinnings of IFI in ACLF. Comprehending how variations in these variables coalesce to augment susceptibility to fungal infection provides invaluable insights for the formulation of targeted preventive and therapeutic regimens. The devised nomogram in this study stands as a potent and trailblazing tool. With its remarkable predictive prowess, evidenced by Areas Under the Curve (AUC) of 0.79 (95% CI: 0.70–0.87) in the validation cohort and 0.78 (95% CI: 0.72–0.84) in the training cohort, it streamlines the early and accurate identification of patients at elevated risk of IFI. Clinicians can readily harness this nomogram to

make judicious decisions with alacrity. Merely by inputting patient data related to the identified risk factors, they can obtain a meticulous assessment of IFI risk.

A crucial and distinguishing aspect of our study lies in the concurrent inclusion of both proven IFI and clinically diagnosed IFI, which marks a substantial leap forward. In accordance with the 2022 Expert Consensus on the Diagnosis and Treatment of Severe Liver Disease Complicated with Invasive Fungal Infection, this approach broadens the vista of patient identification. By encompassing not only those patients with conclusive evidence of IFI but also those who meet the clinical diagnostic benchmarks, our study enhances its clinical salience. This comprehensive strategy more faithfully reflects the actual clinical panorama of ACLF patients with IFI. It endows clinicians with the capacity to detect a more extensive array of cases, thus expediting earlier and more precise diagnosis. For instance, patients presenting with specific clinical symptoms and risk factors, yet lacking definitive microbiological verification, can now be incorporated. This effectively mitigates the potential for treatment initiation delays.

This study inevitably has several limitations that warrant consideration. Firstly, being a retrospective clinical data analysis carried out at a single center, it is susceptible to potential biases. The data might not be fully representative due to the specific patient population and clinical practices of that particular center. Secondly, the absence of external validation with independent datasets restricts the generalizability of our results. Without testing the model on diverse and external data, we cannot be certain of its performance in different settings. Thirdly, only proven and probable diagnoses of IFI were accounted for in the study, while possible diagnoses were excluded. This exclusion could have led to an undercounting of IFI cases and an elevated risk of false negatives. It is possible that some patients with early or mild forms of IFI, who would have been classified as possible cases, were missed. Finally, the sample size of our study is relatively limited. Larger-scale studies in the future will be essential to confirm and expand upon our current findings, ensuring greater statistical power and more reliable conclusions.

## Conclusion

Our study identified several risk factors associated with IFI in ACLF patients, including AST, PTA, HGB, NLR, and TBIL. Additionally, we developed a nomogram that demonstrated strong predictive capabilities for identifying IFI in these patients. This model enables the early identification of individuals at high risk for IFI, facilitating timely and targeted treatment interventions.

### Abbreviations

| | |
|---|---|
| ACLF | Acute-on-Chronic Liver Failure |
| AIH | Autoimmune Hepatitis |
| ALB | Albumin |
| ALD | Alcoholic Liver Disease |
| ALT | Alanine Aminotransferase |
| AST | Aspartate Aminotransferase |
| CRP | C-Reactive Protein |
| CTP | Child-Turcotte-Pugh score |
| FDP | Fibrinogen Degradation Products |
| GGT | Gamma-Glutamyltransferase |
| GI bleeding | Gastrointestinal Bleeding |
| GLP | Gamma-Glutamyltransferase; BDG:1,3-β-d-glucan |
| GFR | Glomerular Filtration Rate |
| GM | Galactomannan |
| HBV | Hepatitis B Virus infection |
| HE | Hepatic Encephalopathy |
| HGB | Hemoglobin |
| IFI | Invasive Fungal Infection |
| INR | International Normalized Ratio |
| LYMPH# | Lymphocytes |
| MELD | Model for End-Stage Liver Disease |
| MONO# | Monocytes |
| Na | Sodium |
| NEUT# | Neutrophils |
| NLR | Neutrophil-to-Lymphocyte Ratio |
| PCT | Procalcitonin |
| PDW | Platelet Distribution Width |
| PLT | Platelet |
| PTA | Prothrombin Activity |
| RDW | Red Cell Distribution Width |
| SCr | Serum Creatinine |
| Tbil | Total Bilirubin |
| WBC | White Blood Cells |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03819-6.

Supplementary Material 1

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### Author contributions

Shan Zhong contributed to the conception and design, and critical revision of important intellectual content. Jie Li, Xuelian Dan, Yanli Yang, and Li Zhang collected the data. Xiaohao Wang and Xu Yang performed the analyses, wrote and revised the manuscript. Hu Li, Zhi Zhou and Dachuan Cai interpreted the data and critically revised important intellectual content. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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### Data availability

We declared that data described in the manuscript would be freely available to any scientist wishing to use them for non commercial purposes.

## Declarations

### Ethical approval

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University and conducted in accordance

with the ethical guidelines of the Declaration of Helsinki (approval number:2024IIT322). As a retrospective cohort study, the requirement for informed consent was waived.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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