



Original Article

Relationship between the Level of Serum Golgi Protein 73 and the Risk of Short-term Death in Patients with ALD-ACLF



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Abstract

Background and Aims: As a hepatocellular carcinoma biomarker, serum Golgi protein 73 (GP73) is reportedly related to inflammation. Acute-on-chronic liver failure (ACLF) is characterized by severe systemic inflammation. In this study, we aimed to explore the association between the GP73 level and short-term mortality in patients with alcohol-associated liver disease-related ACLF (ALD-ACLF). **Methods:** This retrospective cohort study involved 126 Chinese adults with ALD-ACLF. Baseline serum GP73 level was measured using enzyme-linked immunosorbent assay. Patients were followed-up for 90 d and outcomes were assessed. Data were analyzed using multivariate Cox regression and piecewise linear regression analyses. The predictive value of GP73 and classic models for the short-term prognosis of participants were evaluated and compared using receiver operating characteristic curves. **Results:** The serum GP73 level was independently associated with an increased mortality risk in patients with ALD-ACLF. Compared with the lowest tertile, the highest serum GP73 level predisposed patients with ALD-ACLF to a higher mortality risk in the fully adjusted model [at 28 days: hazard ratio (HR): 4.29 (0.99–18.54), $p=0.0511$; at 90 days: HR: 3.52 (1.15–10.79), $p=0.0276$]. Further analysis revealed a posi-

tive linear association. GP73 significantly improved the accuracy of the Child-Turcotte-Pugh score, model for end-stage liver disease score, and model for end-stage liver disease-sodium score in predicting short-time prognosis of patients with ALD-ACLF. **Conclusions:** The serum GP73 level is a significant predictor of the subsequent risk of death in patients with ALD-ACLF. GP73 improved the predictive value of classic prognostic scores.

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Introduction

Acute-on-chronic liver failure (ACLF) is a severe clinical syndrome, characterized by acute deterioration of the liver function in patients with pre-existing chronic liver disease with increased short-term (28–90 day) mortality.^{1–4} Nonetheless, some patients with ACLF may recover after receiving adequate care.^{5–8} However, in other patients, liver transplantation may be the only life-saving treatment; although, it imposes a heavy economic and psychological burden.^{5,9} Early identification of patients with a dismal prognosis helps reduce costs, facilitates more rational implementation of organ allocation, and improves the overall survival of patients with ACLF. Currently, there are various prognostic scores^{10–12} available based on biochemical data and complications to predict the short-term prognosis of ACLF patients, albeit with limited prognostic biomarkers. Pathophysiological biomarkers for ACLF may help increase our understanding of ACLF pathogenesis, improve the predictive ability of these prognostic scores, and recognize and develop strategies for the rational treatment of ACLF in the future.

Golgi protein 73 (GP73) is a 73-kDa type II Golgi transmembrane glycoprotein expressed primarily in biliary epithelial cells.¹³ Under normal conditions, GP73 is expressed at baseline levels in hepatocytes and serum, but significantly upregulated in liver diseases.¹³ GP73 was considered a serum marker for hepatocellular carcinoma (HCC).^{14–16} In

Keywords: Golgi protein 73 (GP73); Alcohol-associated liver disease; Acute-on-chronic liver failure (ACLF); Prognosis; Retrospective cohort study.

Abbreviations: AACR, APASL ACLF research consortium; ACLF, acute-on-chronic liver failure; AKI, acute kidney injury; ALB, albumin; ALD, alcohol-associated liver disease; ALD-ACLF, alcohol-associated liver disease-related ACLF; ALT, alanine aminotransferase; APASL, Asian-Pacific Association for the Study of the Liver; AST, aspartate aminotransferase; CI, confidence interval; CTP, Child-Turcotte-Pugh; ELISA, enzyme-linked immunosorbent assay; GP73, Golgi protein 73; HCC, hepatocellular carcinoma; HE, hepatic encephalopathy; HR, hazard ratio; INR, international standardization ratio; IQR, interquartile range; MELD, Model for End-Stage Liver Disease; MELD-Na, MELD-sodium; ROC, receiver operating characteristic; TBil, total bilirubin; WBC, white blood cell.

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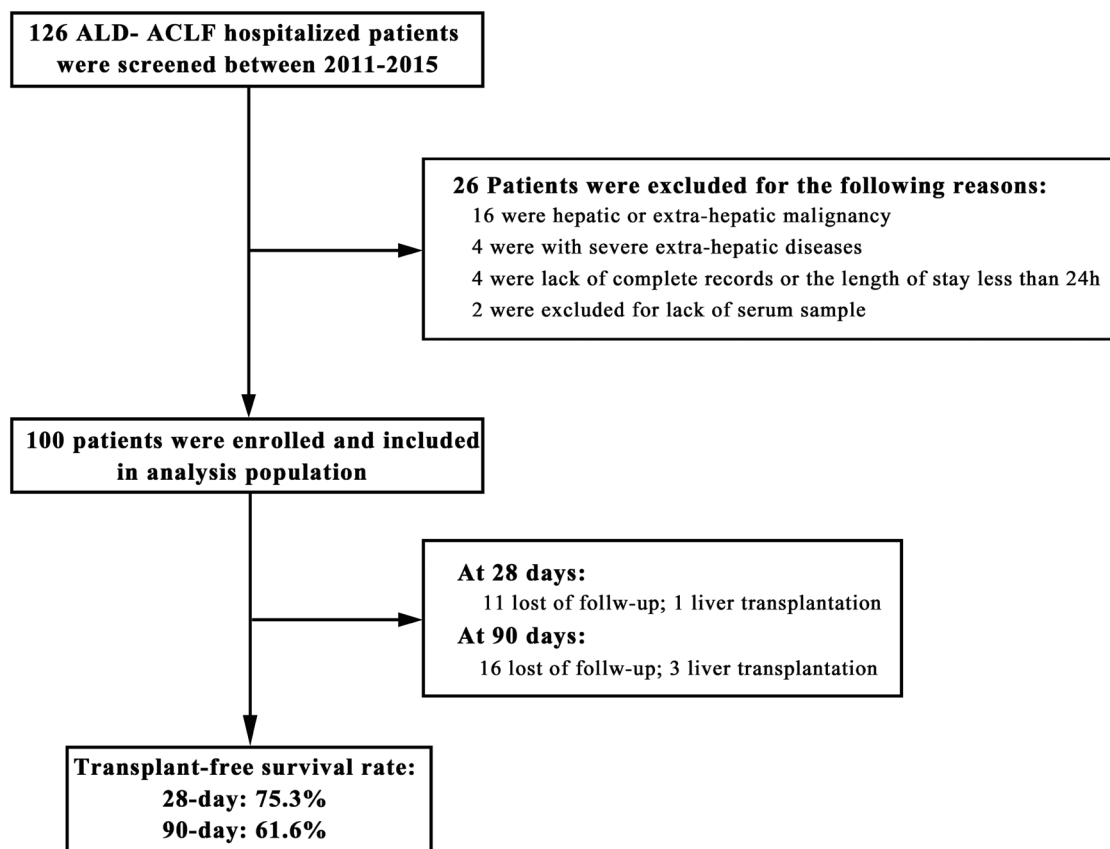


Fig. 1. Flowchart of screening and recruitment of patients with ALD-ACLF.

recent years, it has been reported to play an important role in liver inflammation and fibrosis.¹⁷⁻¹⁹ The pathologic condition of ACLF is characterized by severe liver necroinflammation in pre-existing non-cirrhotic or compensatory cirrhotic liver disease. To date, information available regarding GP73 in patients with ACLF is limited; however, its serum level is significantly higher in ACLF than in other chronic liver diseases, so that it is considered a potent serological marker for diagnosing ACLF.^{20,21}

The association between GP73 and ACLF prognosis remains to be fully clarified in the literature. Excessive alcohol consumption is one of the most frequent events precipitating ACLF. It is dominant in patients with ACLF in western countries, and the proportion of patients is also gradually increasing in eastern countries.²² In this study, we aimed to investigate the association between the serum GP73 level and short-term mortality in patients with alcohol-associated liver disease-related ACLF (ALD-ACLF) and to evaluate its predictive value.

Methods

Patients

In total, 126 hospitalized patients with ALD-ACLF were retrospectively and consecutively screened at the Fifth Medical Center of Chinese PLA General Hospital from January 2011 to December 2015. The enrolment criteria for the patients with ALD were as follows:²³ 1) regular alcohol consumption of >20 g/day in females and >30 g/day in males, together

with the presence of clinical and/or biological abnormalities suggestive of liver injury; and 2) absence of evidence of viral hepatitis, autoimmune liver disease, drug-induced liver disease, and metabolic liver disease. The diagnostic criteria of ACLF followed the Asian-Pacific Association for the Study of the Liver (APASL)-ACLF consensus. The exclusion criteria for patients were as follows: 1) age less than 18 years; 2) severe extra-hepatic diseases; 3) suspected or confirmed hepatic or extra-hepatic malignancy; 4) pregnancy; 5) hospitalization for less than 24 h; or 6) no remaining serum sample after laboratory analysis. Consequently, 100 patients were ultimately enrolled in the study.

The screening and enrollment processes are summarized in Figure 1. Pre-existing chronic liver disease included non-cirrhotic (hepatitis) and cirrhosis. Cirrhosis was diagnosed on the basis of history, liver biopsy, laboratory analysis, endoscopic findings (esophageal and gastric varices), and ultrasonographic and radiological imaging of portal hypertension and/or liver nodularity.²⁴

This study was approved by the Ethics Committee of the Fifth Medical Center of Chinese PLA General Hospital (No. 2019017D). The ethics committee waived the requirement of patient consent because data were analyzed anonymously and the retrospective nature of the study did not compromise the health, safety, and privacy of the patients. The study was conducted in accordance with the tenets of the 2013 Helsinki Declaration.

Data collection, follow-up, and end-point

The clinical data obtained at enrollment were demographic

characteristics, pre-existing liver disease, and laboratory indicators [e.g., white blood cell (WBC) count, hemoglobin level, blood platelet count, albumin (ALB), total bilirubin (TBil), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, γ -glutamyl transferase, creatinine, total cholesterol, sodium, alpha-fetoprotein and PTA (prothrombin activity) levels, and the international standardization ratio (INR)]. Complications including ascites, infection, acute kidney injury (AKI), hepatic encephalopathy (HE), and gastrointestinal hemorrhage were evaluated. Furthermore, the Child-Turcotte-Pugh (CTP) score, (10) Model for End-Stage Liver Disease (MELD) score, (11) and MELD-sodium (MELD-Na) score (12) were determined.

Patients were followed-up for 90 days. Patient death was the primary endpoint. Prognostic data of patients discharged from the hospital were verified through the assessment of medical records, telephone contacts, or visits.

In total, 126 patients with ALD-ACLF were screened; among them, 26 were excluded and 100 were included in the final study population. During the 90-day follow-up, three patients underwent transplantation and sixteen were lost to follow-up (Fig. 1). Patients who received a liver transplant were considered lost to follow-up.

Measurement of serum GP73 level

Blood samples, to obtain serum, were collected at enrollment and stored at -80°C until analysis. Serum GP73 was quantified in accordance with the manufacturer's protocol for a commercially available double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit (Hotgen Biotech Inc., Beijing, China).

Clinical data and serum GP73 level were assessed in a blinded manner.

Statistical analysis

Serum GP73 level was divided into three tertiles (T1, T2, and T3) in an ascending order. The serum GP73 level was simultaneously analyzed as a continuous variable in the three tertile groups. Continuous variables are expressed as mean \pm standard deviation (normal distribution) or median (interquartile range [IQR]) (skewed distribution). Categorical variables are expressed as count and percentage in each group and among the groups. The differences among the tertiles of GP73 were assessed using the one-way ANOVA (normal distribution) or Kruskal-Wallis rank sum test (skewed distribution) for continuous variables and the chi-squared test for categorical variables. Patients lost to follow-up or those receiving a liver transplant contributed to censored data. Cox proportional hazard models were used for the multivariate analysis to assess hazard ratio (HR) and the 95% confidence interval (CI) for the risk of death at 28 and 90 days. Both non-adjusted and multivariate-adjusted models were used. We compared the changes in regression coefficients by adding covariates to the basic model or eliminating the covariates individually in the complete model of Cox regression. Covariates with a change in regression coefficient of $>10\%$ were selected as adjustment variables for the final model (Model 3). The linear trend test was performed using the median value of each tertile of GP73 as a continuous variable in the models. Crude cumulative incidence across GP73 tertiles was examined using Kaplan-Meier curves. The relationship between the GP73 level and log-transformed values of the relative risk of short-time death was assessed using the smoothing function. We conducted a log-likelihood ratio test to compare the one-line linear model with two-piecewise linear regression models. Fur-

thermore, the receiver operating characteristic (ROC) curve was used to evaluate the predictive value of various models for the short-term prognosis of patients with ALD-ACLF.

All statistical tests were performed using SPSS version 24.0 (IBM Corp., Armonk, NY, USA), GraphPad Prism version 7.00 (GraphPad Software Inc., La Jolla, CA, USA), statistical package R version 3.4.3 (The R Foundation, Vienna, Austria), and EmpowerStats (X&Y Solutions, Inc., Boston, MA, USA). Results with two-sided p -values of <0.05 were considered statistically significant.

Results

Baseline characteristics of patients with ALD-ACLF and the serum GP73 level

In total, 100 patients with ALD-ACLF were included in the study (Fig. 1). Table 1 summarizes the demographic and clinical parameters of the study participants, classified in accordance with the serum GP73 tertiles (T) as follows: T1: 135.72–212.30 ng/mL; T2: 213.04–268.83 ng/mL; and T3: 271.68–414.88 ng/mL. The mean age of participants was 43.88 ± 7.86 years and most participants (94 subjects, 94.00%) were male. Furthermore, 94% of patients with ALD-ACLF had the basis of compensatory liver cirrhosis; a similar proportion was observed in each group ($p > 0.05$). The serum GP73 level was directly proportional to the TBil, AST, and creatinine levels and inversely proportional to the ALB level ($p < 0.05$). The CTP, MELD and MELD-Na scores and the proportion of infection and AKI at baseline were significantly higher among participants with increased GP73 level ($p < 0.05$). The baseline characteristics are well-balanced between patients in the study and those lost to follow up at 90 days. The statistical analyses are described in Supplementary Table 1.

Association between the serum GP73 level and short-term mortality in patients with ALD-ACLF

During the follow-up period, 23 and 34 deaths occurred within 28 days and 90 days, respectively. The cumulative survival rate of each group was 90.6% (T1), 73.0% (T2), and 61.6% (T3) at 28 days and 83.6% (T1), 49.7% (T2), and 50.1% (T3) at 90 days. Kaplan-Meier curves showed that the cumulative survival rate was the highest in patients within the lowest tertile of GP73 (T1), followed by those within tertiles T2 and T3 ($p = 0.007$ at 28 days and $P = 0.005$ at 90 days) (Fig. 2).

As a continuous variable, the baseline GP73 level independently and significantly positively correlated with the risk for short-term death at both 28 days (HR: 1.01 [1.00–1.02], $p = 0.0097$) and 90 days (HR: 1.01 [1.00–1.02], $p = 0.0074$) after adjusting for the potential confounders of age, sex, pre-existing liver disease, WBC and PLT counts, ALB, TBil, and PTA levels, and ascites, infection, AKI, and HE presence (Model 3). The same trend was observed in the crude models (Model 1 and Model 2). In Model 3, the HR of GP73 for the mortality risk within 28 days in groups T2 and T3 was 2.93 (0.68–12.69) and 4.29 (0.99–18.54), respectively, compared with that in group T1 ($p_{\text{trend}} = 0.0505$). Furthermore, the HR for the mortality risk at 90 days was 3.82 (1.24–11.76) and 3.52 (1.15–10.79), respectively ($p_{\text{trend}} = 0.0351$). Details are provided in Table 2.

Threshold effect analysis of GP73 on the risk of short-term death in patients with ALD-ACLF

Smoothing function analysis was performed to investigate

Table 1. Baseline characteristics of the study participants in accordance with serum GP73 level

| Variables | Total | GP73 tertiles | | | p-value |
|---------------------------------------|------------------------|------------------------|------------------------|------------------------|---------|
| | | T1 (135.72-212.30) | T2 (213.04-268.83) | T3 (271.68-414.88) | |
| Participants, n | 100 | 33 | 33 | 34 | |
| Age in years | 43.88 (7.86) | 44.39 (8.71) | 43.27 (7.43) | 43.97 (7.60) | 0.845 |
| Males, n (%) | 94 (94.00) | 31 (93.9) | 31 (93.94) | 32 (94.12) | 0.999 |
| Pre-existing liver disease, n (%) | | | | | 0.869 |
| Hepatitis | 6 (6.00) | 2 (6.06) | 1 (3.03) | 3 (8.82) | |
| Cirrhosis | 94 (94.00) | 31 (93.94) | 32 (96.97) | 31 (91.18) | |
| WBCs as $\times 10^9/L$ | 9.50 (6.02-16.73) | 7.47 (4.31-13.40) | 10.91 (6.10-19.40) | 11.88 (8.11-17.30) | 0.015 |
| Hemoglobin in g/L | 93.50 (69.00-110.25) | 85.00 (69.00-114.00) | 98.00 (73.00-107.00) | 94.50 (66.50-112.75) | 0.992 |
| Platelets as $\times 10^9/L$ | 79.00 (47.00-114.50) | 77.00 (49.00-119.00) | 67.00 (47.00-99.00) | 81.50 (45.25-121.75) | 0.605 |
| ALB in g/L | 27.00 (24.00-30.25) | 28.00 (26.00-31.00) | 28.00 (24.00-31.00) | 26.00 (21.50-27.75) | 0.018 |
| TBil in $\mu\text{mol/L}$ | 259.30 (196.02-376.80) | 204.90 (180.40-307.30) | 288.90 (208.10-401.70) | 316.65 (221.50-402.17) | 0.038 |
| ALT in U/L | 36.00 (22.00-61.75) | 36.00 (22.00-65.00) | 27.00 (20.00-50.00) | 46.00 (27.75-72.00) | 0.095 |
| AST in U/L | 87.00 (52.00-144.25) | 71.00 (50.00-150.00) | 71.00 (50.00-100.00) | 114.50 (79.50-163.75) | 0.009 |
| Alkaline phosphatase in U/L | 138.00 (109.25-189.25) | 158.00 (113.00-212.00) | 139.00 (107.00-169.00) | 131.00 (109.75-159.00) | 0.259 |
| γ -glutamyl transferase in U/L | 79.00 (41.00-162.25) | 77.00 (32.00-131.00) | 72.00 (43.00-144.00) | 105.00 (60.25-182.50) | 0.159 |
| Creatinine in $\mu\text{mol/L}$ | 89.00 (74.75-132.25) | 80.00 (69.00-101.00) | 88.00 (77.00-113.00) | 122.00 (86.25-187.50) | 0.004 |
| Total cholesterol in mmol/L | 1.76 (1.10-2.51) | 2.05 (1.10-2.44) | 1.98 (1.35-2.93) | 1.33 (0.73-2.18) | 0.076 |
| Na in mmol/L | 134.00 (130.00-137.25) | 136.00 (132.00-138.00) | 133.00 (129.00-137.00) | 133.00 (130.00-135.75) | 0.105 |
| PTA as % | 37.35 (30.87-42.62) | 38.30 (33.20-44.00) | 36.00 (30.90-40.40) | 37.45 (26.62-43.15) | 0.244 |
| Ascites, n (%) | | | | | 0.391 |
| No | 4 (4.00) | 2 (6.06) | 2 (6.06) | 0 (0.00) | |
| Yes | 96 (96.00) | 31 (93.94) | 31 (93.94) | 34 (100.00) | |
| HE, n (%) | | | | | 0.628 |
| No | 79 (79.00) | 27 (81.82) | 27 (81.82) | 25 (73.53) | |
| Yes | 21 (21.00) | 6 (18.18) | 6 (18.18) | 9 (26.47) | |
| Infections, n (%) | | | | | 0.001 |
| No | 36 (36.00) | 19 (57.58) | 12 (36.36) | 5 (14.71) | |
| Yes | 64 (64.00) | 14 (42.42) | 21 (63.64) | 29 (85.29) | |
| AKI, n (%) | | | | | 0.040 |
| No | 61 (61.00) | 119 (79.33) | 113 (75.84) | 119 (79.87) | |
| Yes | 39 (39.00) | 9 (27.27) | 11 (33.33) | 19 (55.88) | |
| Gastrointestinal hemorrhage, n (%) | | | | | 1.000 |
| No | 93 (93.00) | 31 (93.94) | 31 (93.94) | 31 (91.18) | |
| Yes | 7 (7.00) | 2 (6.06) | 2 (6.06) | 3 (8.82) | |
| CTP score | 12.00 (11.00-13.00) | 11.00 (10.00-12.00) | 12.00 (11.00-13.00) | 13.00 (12.00-13.00) | 0.001 |
| MELD score | 18.00 (14.66-22.67) | 16.42 (12.81-19.77) | 17.32 (15.23-20.67) | 21.13 (16.48-28.10) | 0.005 |
| MELD-Na score | 21.03 (16.29-29.25) | 18.33 (12.85-24.28) | 21.83 (17.07-28.29) | 23.59 (18.79-33.94) | 0.006 |

Continuous variables are presented as mean±standard deviation or median (IQR); categorical variables are presented as n (%).

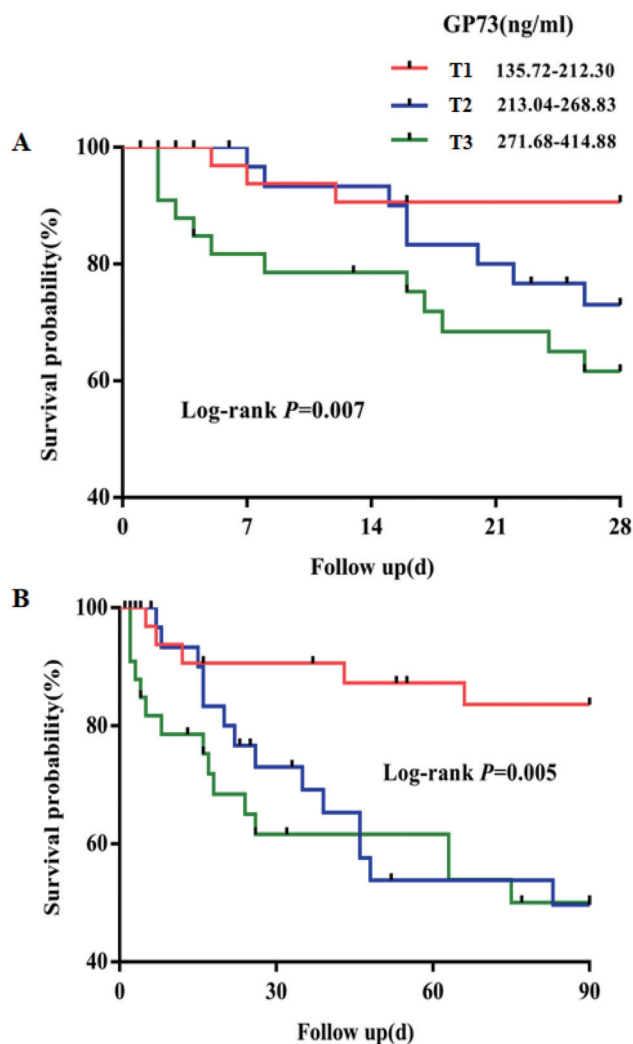


Fig. 2. Kaplan-Meier curves showing the short-term probability of 28-day (A) and 90-day (B) survival among patients with ALD-ACLF stratified into tertiles in accordance with the serum GP73 level.

the presence of a dose-response relationship between the level of GP73 and the risk of short-term death in participants. After adjusting for potential confounders, an approximate linear relationship between the serum GP73 level and short-term mortality risk in patients with ALD-ACLF was observed; although, it seemed to represent a segmentation effect (below and above 260 ng/mL) (Fig. 3). Further threshold effect analysis showed that there was no statistical significance between the segments, and the positive linear correlation between the level of GP73 and the risk of short-term death in ALD-ACLF was verified (HR: 1.01 [1.00-1.02], $p=0.0097$ at 28 days; HR: 1.01 [1.00-1.02], $p=0.0074$ at 90 days) (Table 3).

Short-term prognostic value of serum GP73 in patients with ALD-ACLF

GP73 was compared with the existing classical prognostic scores, namely the CTP, MELD, and MELD-Na score. The area under the (AU)ROC for GP73 was 0.739 and 0.711 for the 28-day and 90-day mortality, respectively. As a single

Table 2. Association between the serum GP73 level and short-term mortality in patients with ALD-ACLF

| Items | Crude model | Model 1 | Model 2 | Model 3 |
|------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| 28-day mortality | | | | |
| GP73 in ng/mL | 1.01 (1.00, 1.02) 0.0015 | 1.01 (1.00, 1.02) 0.0014 | 1.01 (1.00, 1.02) 0.0010 | 1.01 (1.00, 1.02) 0.0097 |
| GP73 tertile | | | | |
| T1 | 1 | 1 | 1 | 1 |
| T2 | 2.91 (0.77, 10.97) 0.1150 | 3.00 (0.79, 11.35) 0.1057 | 2.92 (0.77, 11.06) 0.1148 | 2.93 (0.68, 12.69) 0.1504 |
| T3 | 4.87 (1.37, 17.27) 0.0142 | 4.90 (1.38, 17.41) 0.0141 | 5.01 (1.41, 17.86) 0.0129 | 4.29 (0.99, 18.54) 0.0511 |
| P_{trend} | 0.0099 | 0.0100 | 0.0088 | 0.0505 |
| 90-day mortality | | | | |
| GP73 in ng/mL | 1.01 (1.00, 1.01) 0.0014 | 1.01 (1.00, 1.02) 0.0017 | 1.01 (1.00, 1.02) 0.0008 | 1.01 (1.00, 1.02) 0.0074 |
| GP73 tertile | | | | |
| T1 | 1 | 1 | 1 | 1 |
| T2 | 3.53 (1.27, 9.84) 0.0156 | 3.80 (1.35, 10.65) 0.0112 | 3.64 (1.30, 10.20) 0.0141 | 3.82 (1.24, 11.76) 0.0196 |
| T3 | 4.08 (1.48, 11.24) 0.0066 | 4.09 (1.47, 11.36) 0.0069 | 4.23 (1.52, 11.77) 0.0058 | 3.52 (1.15, 10.79) 0.0276 |
| P_{trend} | 0.0063 | 0.0071 | 0.0054 | 0.0351 |

Data are presented as HR (95%CI). Model 1 was adjusted for age and sex; Model 2 was adjusted for Model 1 + pre-existing chronic liver disease; Model 3 was adjusted for Model 2 + WBC and platelet counts, ALB, TBI and PTA levels, and ascites, infection, AKI, and HE presence.

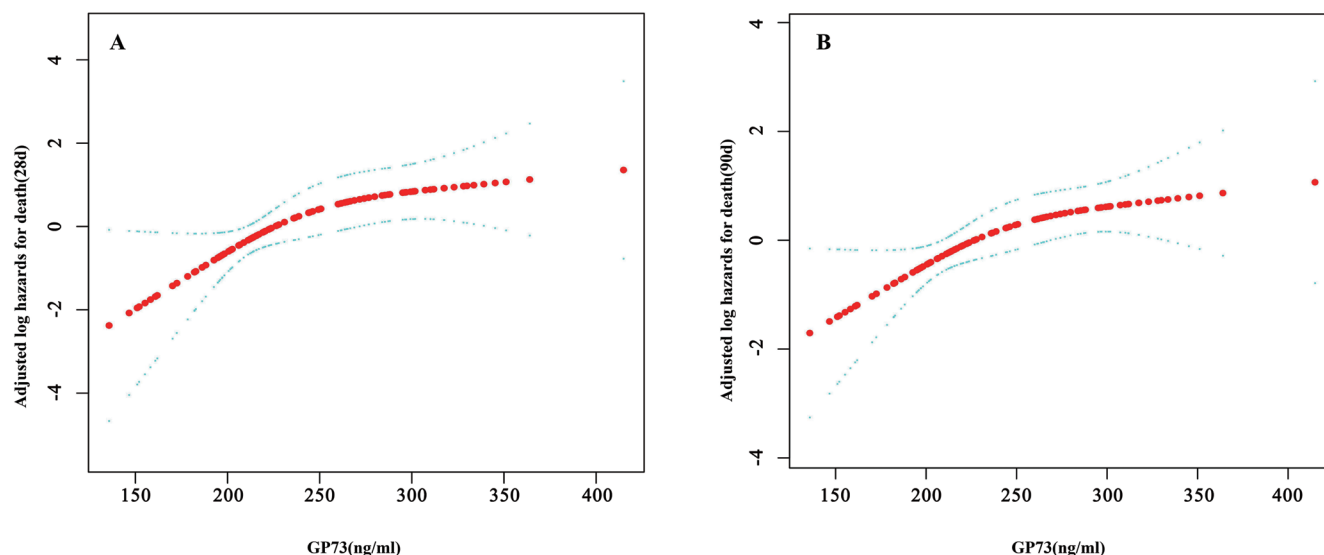


Fig. 3. Association between the serum GP73 level and the risk of 28-day (A) and 90-day (B) mortality among patients with ALD-ACLF. Red line indicates the estimated mortality risk, and blue lines represent pointwise 95% CIs adjusted for age, sex, pre-existing liver disease, WBC and platelet counts, ALB, TBil and PTA levels, and ascites, infection, AKI, and HE presence.

index, GP73 showed a similar or better predictive power than the classical prognostic scores; although, there was no significant difference among them (all $p > 0.05$). Additionally, after combining with GP73, the short-time mortality predictive power of the CTP, MELD, and MELD-Na scores improved significantly (at 28 days: "CTP+GP73" vs. CTP yielded 0.763 vs. 0.666 [$p = 0.1007$], "MELD+GP73" vs. MELD yielded 0.826 vs. 0.783 [$p = 0.2235$], and "MELD-Na+GP73" vs. MELD-Na yielded 0.788 vs. 0.734 [$p = 0.2159$]; at 90 days: "CTP+GP73" vs. CTP yielded 0.731 vs. 0.663 [$p = 0.1865$], "MELD+GP73" vs. MELD yielded 0.737 vs. 0.671 [$p = 0.1980$], "MELD-Na+GP73" vs. MELD-Na yielded 0.755 vs. 0.671 [$p = 0.1950$]) (Fig. 4).

Discussion

The findings of this study show that the serum GP73 level is an independent prognostic factor for short-term mortality in patients with ALD-ACLF. Additionally, the combination of GP73 and classical prognostic scores (CTP, MELD, and MELD-Na) was able to increase the predictive potential of short-term mortality in patients with ALD-ACLF.

In this study, the association between the serum GP73 level and short-term prognosis of patients with ALD-ACLF was investigated from multiple perspectives. First, the serum GP73 level, as both continuous variable and three-tertile ar-

ray, positively correlated with the short-term mortality risk of patients with ALD-ACLF. Thereafter, upon adjusting for potential confounders, including age, sex, pre-existing liver disease, WBC and PLT counts, ALB, TBil and PTA levels, ascites, infection, AKI, and HE, three other models were generated to further verify this positive correlation. This speculation was re-confirmed via subsequent smoothing function analysis and two-piecewise linear regression models. Finally, this study revealed that the mortality risk increased with an increase in the serum GP73 level, indicating that GP73 may be a potential serum marker for the prognosis of ALD-ACLF.

GP73 is a Golgi transmembrane glycoprotein that is cleaved and secreted into the extracellular space, and is detectable in serum or cell culture supernatants.^{25,26} Initially, GP73 was considered an HCC marker. As such, GP73 upregulation has been assessed previously in tissues affected by HCC through immunohistochemical analysis, suggesting that GP73 is involved in cell proliferation,^{18,19,27} concurrent with the findings of previous studies.^{15,28-32} It has been reported that the serum GP73 level in healthy children decreased with age, with infants displaying the highest levels and indicating that GP73 is potentially associated with active hepatocyte proliferation or differentiation.³³ Furthermore, GP73 is expressed in activated hepatic stellate cells³⁴ and cirrhotic liver tissues,^{18,19} indicating that GP73 is expressed during cell proliferation. However, serum GP73 is reportedly associated with hepatocellular injury and positively correlated with the severity of liver necroinflam-

Table 3. Threshold effect analysis of GP73 on short-term mortality among patients with ALD-ACLF

| Outcomes | 28-day mortality | | 90-day mortality | |
|---------------------------------------|-------------------|---------|-------------------|---------|
| | HR (95% CI) | p-value | HR (95% CI) | p-value |
| One-line linear regression model | 1.01 (1.00, 1.02) | 0.0097 | 1.01 (1.00, 1.02) | 0.0074 |
| Two-piecewise linear regression model | | | | |
| GP73 <260 ng/mL | 1.02 (1.00, 1.04) | 0.0452 | 1.01 (1.00, 1.03) | 0.0611 |
| GP73 ≥260 ng/mL | 1.01 (0.99, 1.02) | 0.4800 | 1.01 (0.99, 1.02) | 0.5899 |
| Log likelihood ratio test | 0.336 | | 0.584 | |

Adjusted for age, sex, pre-existing chronic liver disease, WBC and platelet counts, ALB, TBil and PTA levels, and ascites, infection, AKI, and HE presence.

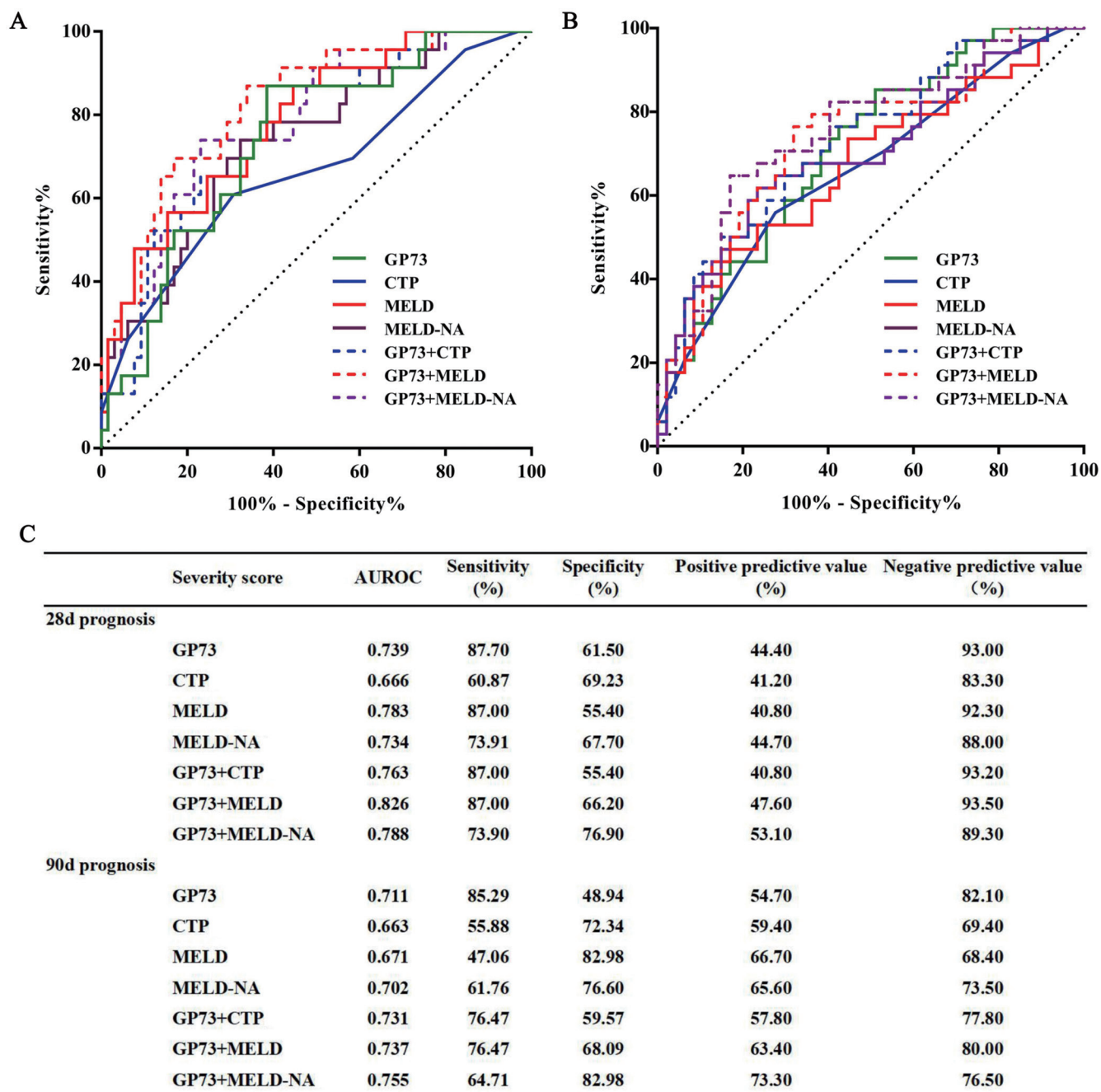


Fig. 4. Discrimination ability of GP73, classic scoring models, and GP73 plus classic scoring models to predict the prognosis of patients with ALD-ACLF at 28 days (A) and 90 days (B). (C) Indicators of predictive ability of prognostic models.

mation,^{17,34-39} potentially indicating that GP73 significantly contributes to cellular necroinflammation. Furthermore, the elevated level of GP73 in patients with HCC is much lower than that in patients with ACLF or hepatitis.

ACLF has a complex pathophysiology, characterized by a short-term massive liver cell necrosis based on chronic liver injury.¹ Serum GP73 in patients with ACLF originates from the leakage or release of intracellular GP73 caused by hepatic tissue necrosis and the activation and proliferation of hepatic parenchymal cells. In contrast to ALT, which exists mainly in the cytoplasm and can remain unchanged

or be mildly elevated when patients suffer from severe hepatic necroinflammation, GP73 is located on the Golgi apparatus, and its elevation indicates more serious damage involving organelles. Therefore, it could be speculated that the increased serum level of GP73 observed in our study's patients with ALD-ACLF is related, at least partially, to the inflammatory state characteristic and the injury of hepatocytes. The higher the serum GP73 level, the more severe the liver damage, and therefore the worse the prognosis.

In the present study, the serum GP73 level positively correlated with the mortality risk in patients with ALD-ACLF,

probably because GP73 baseline upregulation indicates the severity of chronic liver disease. Since serum GP73 is up-regulated in ACLF, it has potential to indicate an increased risk of mortality. Based on this finding, we evaluated the predictive power of GP73 for short-term prognosis in patients with ALD-ACLF. In accordance with previous results, the combination of GP73 with the classical scoring systems of CTP, MELD, and MELD-Na increased the predictive potential for short-term mortality in the patients with ALD-ACLF. Liver transplantation is performed according to the MELD score in many countries.¹¹ Thus, the use of GP73 together with MELD score may help ameliorate clinical decisions about liver allocation and transplantation.

In summary, this study revealed a positive linear association between GP73 and short-term mortality risk in patients with ALD-ACLF. The serum GP73 level may add prognostic value to the classical scores to assess prognosis of patients with ALD-ACLF. These findings suggest that GP73 is a potential prognostic marker for ALD-ACLF and is potentially associated with liver necroinflammation in ALD-ACLF. We believe that the findings of this study are of interest from a clinical perspective. The serum GP73 level could be valuable in monitoring the progression of ALD-ACLF in clinical practice. Moreover, it could help in risk stratification of patients with ALD-ACLF admitted to hospital.

Nevertheless, there were some limitations to the current study. First, there are various methods to detect GP73, such as ELISA and monoclonal antibody-based latex-enhanced immunoturbidimetric assay; further study is needed to find the most suitable method. Second, due to lack of lactic acid data, the APASL-ACLF research consortium (AARC) score could not be calculated and compared with GP73 and other prognostic scores. Finally, the outcomes of this study were derived from patients with ALD-ACLF recruited in a single liver-transplantation center. These findings should be further confirmed in future studies with a larger cohort and in multi-center investigations and experimental studies.

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Conflict of interest

FL has been an editorial board member of *Journal of Clinical and Translational Hepatology* since 2013. Other authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (JH, FL, JT), acquisition of data (JT, MY, XM, LW, XW, XZ, XX, YW, JC, CG), analysis and interpretation of data (JT), drafting of the manuscript (JT), and critical revision of the manuscript (JH, FL,MY).

Data sharing statement

All data are available upon reasonable request.

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