

# Serum Nitric Oxide Level Serves as a Potential Prognostic Biomarker in ACLF Patients

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**Background and Aim:** Fewer than 50% of patients with acute-on-chronic liver failure (ACLF) recover spontaneously, and without liver transplantation, ACLF is associated with high death rates. Nitric oxide (NO) has a role in the pathogenesis of various liver disorders. We investigated if serum NO level could be used as a biomarker to predict the severity and prognosis of patients with ACLF.

**Methods:** Between January 2018 and September 2020, a retrospective cohort of 120 ACLF patients, as well as healthy and cirrhotic controls, was investigated. The serum NO levels were measured using a commercial ELISA kit, and Kaplan–Meier survival analysis was conducted.

**Results:** ACLF patients had significantly higher serum NO levels than healthy and cirrhotic controls. Multivariate analysis indicated that the serum NO level (HR=1.078, 95% CI 1.031–1.126,  $P<0.01$ ), as well as the Model for End-stage Liver Disease (MELD) score, may be an affordable, easily available, and significant independent predictive marker for mortality. In ACLF patients, a serum NO level of  $> 53.5 \mu\text{mol/L}$  was associated with a significant increase in the risk of mortality or liver transplantation. A combination of serum NO level and MELD score to assess the severity and prognosis of ACLF patients showed enhanced performance.

**Conclusion:** Based on serum NO levels at the time of hospital admission, ACLF patients may be divided into high-risk and low-risk groups. The combination of serum NO level and MELD score is more closely linked to the patient's outcome than either value alone. This method might be used to evaluate patient prognoses and select candidates for liver transplantation.

**Keywords:** acute on chronic liver failure, autophagy, nitric oxide, reactive oxygen species, prediction

## Introduction

Acute-on-chronic liver failure (ACLF) is defined as acute deterioration of liver function in patients with chronic liver disorders, and it is linked with multiorgan failure and high short-term mortality rates.<sup>1,2</sup> ACLF has different etiologies in different parts of the world. Viral infections (particularly hepatitis B virus [HBV] infection) are more frequent in Asia, but alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD) are more common in America and Europe.<sup>3</sup>

Hepatocyte death is the most common symptom of liver failure; injured/dead hepatocytes cause oxidative stress, which induces further hepatocyte death and prevents regeneration, creating a vicious cycle.<sup>4</sup> Autophagy is an intracellular recycling mechanism that helps to maintain cellular homeostasis and regulates the cellular adaptive response during stressful situations.<sup>5</sup> Autophagy has been implicated in the regulation of resident liver cells, including hepatocytes, hepatic stellate cells (HSCs) and macrophages.<sup>6</sup> Although the link between autophagy and cell death is complicated, a growing body of evidence suggests that autophagy enhances cell longevity by removing reactive oxygen species (ROS). Nitric oxide (NO) accelerates HSCs death by generating ROS.<sup>7</sup>

The cause of many clinical issues linked with liver failure is severe peripheral and splanchnic vasodilation, which is a key hallmark of individuals with liver failure. Hepatocytes, Kupffer cells, HSCs, and hepatic sinusoidal endothelial cells (SECs) may all create NO, a gaseous chemical with significant vasodilator effects. NO synthase (NOS) employs a system of redox

processes to produce NO.<sup>8,9</sup> Endogenous NO has a very short half-life after being synthesized by NOS, about 1 second. As a result, NOS controls endogenous NO levels.<sup>10</sup> NO may play a role in ACLF according to several recent studies. During acute liver failure, NO inhibits autophagy in HSCs, promoting cell death,<sup>11</sup> and autophagy deficit in SECs reduces intrahepatic NO bioavailability owing to both decreased production and increased scavenging, impairing the antioxidant response.<sup>9</sup>

A number of grading systems have been established to measure the severity and predict the prognosis of ACLF. All of these methods are focused on compromised liver function, but limited studies have investigated disease causation.<sup>12</sup> Because NO plays a role in ACLF, we investigated if serum NO levels might be used to predict the severity, mortality, and prognosis of patients with ACLF.

## Methods

### Patients

We included 120 ACLF patients treated between January 2018 and September 2020 at the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China. This retrospective investigation was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained, and the study was approved by the Research Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China. Blood samples were collected at admission and stored at  $-80^{\circ}\text{C}$  within 2 h. All organs were donated voluntarily with written informed consent, and that the transplants were conducted in accordance with the Declaration of Istanbul.

The Asian Pacific Association for the Study of the Liver (APASL) criteria were used to diagnose patients with ACLF: (1) serum bilirubin  $\geq 85$  mol/L; (2) international normalized ratio (INR)  $\geq 1.5$  or prothrombin activity  $\leq 40\%$ ; (3) any degree of encephalopathy and/or clinical ascites within 4 weeks; and (4) signs of persistent chronic liver disease.<sup>13</sup>

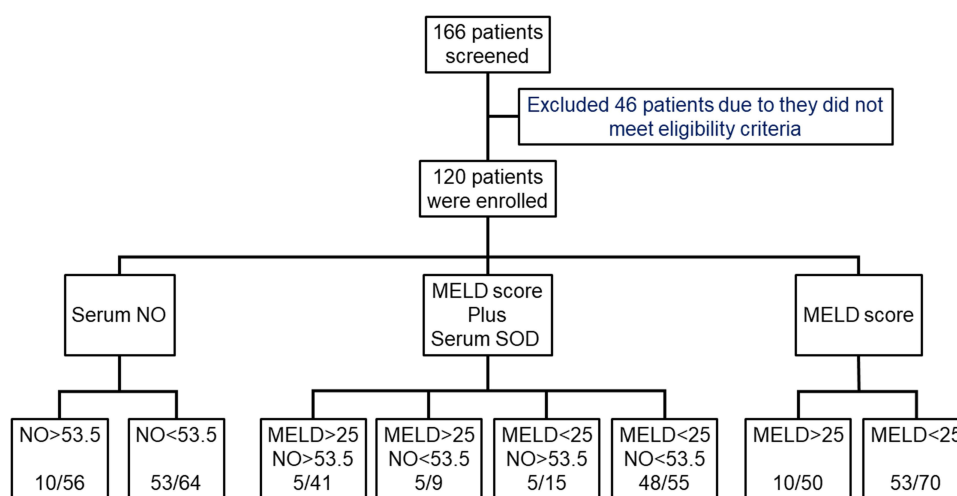
The patients diagnosed with ACLF were aged 18 to 75 years.

In total, 46 participants were excluded for the following reasons: (1) decompensated liver cirrhosis prior to ACLF diagnosis, such as ascites and variceal hemorrhage; (2) portal hypertension and placement of a transjugular intrahepatic portosystemic shunt (TIPS); (3) pathological diagnosis or clinical suspicion of other malignancies, except hepatocellular carcinoma (HCC); (4) pregnancy; or (5) infection with HIV or hepatotropic virus other than HBV (Figure 1).

Controls were recruited throughout the same time period and were age- and sex- matched healthy people and patients with liver cirrhosis.

### Nitric Oxide Concentration Detection

A Griess test (#G2930; Qiagen, Hilden, Germany) was used to assess serum NO levels in patients and healthy and cirrhotic controls, according to the manufacturer's instructions. Experiments were performed in duplicate.



**Figure 1** Patients disposition throughout the study.

## Statistical Methods

The data are presented as means and standard deviations (SDs). For categorical data, a chi-squared test or Fisher's exact test was used, and for continuous variables, a Wilcoxon rank sum test was used. The predictive value of NO levels for illness severity and prognosis was determined using ROC analysis. Cut-off values for continuous variables were calculated with the maximum of the sum of sensitivity and specificity. To compare Kaplan–Meier survival curves up to 90 days following admission, Log rank tests were performed. After univariate Cox regression analysis, covariables with a *P*-value of <0.05 were incorporated into a forward multivariate analysis. The data were analyzed using SPSS version 16.0 (IBM Corporation, Somers, NY, USA). When *P*<0.05, differences were judged as being statistically significant.

## Results

### Baseline Characteristics

The clinical baseline characteristics of ACLF patients and controls in this study are shown in Table 1.

Only 120 of 166 ACLF patients satisfied the inclusion criteria. The most common causes of underlying chronic liver disease were HBV infection (62.50%) and alcohol use (17.50%); the most common causes of acute hepatic insult were HBV reactivation (52.50%), alcoholic hepatitis (13.33%), and bacterial infection (27.50%) (Table 2).

The median age was 47.29±1.09 years, and males made up 82.50% of the population. The existence of complications was used to define clinical events. Hepatic encephalopathy (HE) was the most prevalent complication (34.17%), and 31.67% of patients satisfied acute kidney injury (AKI) criteria at the time of admission. Baseline clinical parameters, laboratory parameters, and in-hospital complications are summarized in Table 2.

### ACLF Patients Have High Mortality Rate and Serum NO Levels

Three individuals in the ACLF group received a liver transplant, whereas 54 died without liver transplantation. ACLF patients had a survival rate of 52.50% (Figure 2A). We examined serum NO levels in ACLF patients. Patients with the progression stage of ACLF had significantly higher NO concentrations than healthy controls ( $53.11 \pm 1.68$  vs  $31.03 \pm 0.64$ , *P*<0.01) and cirrhotic patients ( $53.11 \pm 1.68$  vs  $32.18 \pm 0.70$ , *P*<0.01) (Figure 2B). There was no significant difference in serum NO levels between the cirrhotic patients and healthy controls ( $32.18 \pm 0.70$  vs  $31.03 \pm 0.64$ , *P*>0.05). Furthermore, NO levels were lower in the remission stage than in the progression stage ( $29.10 \pm 1.04$  vs  $53.11 \pm 1.68$ ,

**Table 1** Demographic Data and Clinical Characteristics of Controls and ACLF Patients

Variables	Healthy Control (n=30)	Cirrhosis (n=30)	ACLF (n=120)
Age (yr)	31.13 ± 1.24	38.33 ± 0.93	47.29 ± 1.09
Sex (M/F)	24/6	24/6	99/21
PT (%)	86.17 ± 2.12	76.40 ± 3.18	37.96 ± 1.34
Fb (g/L)	2.53 ± 0.12	2.16 ± 0.09	1.58 ± 0.07
INR	1.15 ± 0.03	1.06 ± 0.01	2.08 ± 0.07
WBC ( $1 \times 10^9/L$ )	5.54 ± 0.26	4.04 ± 0.28	6.93 ± 0.35
PLT ( $1 \times 10^9/L$ )	211.97 ± 7.10	115.23 ± 13.64	101.58 ± 5.65
ALT (U/L)	22.20 ± 2.80	34.53 ± 3.88	294.15 ± 36.85
GLU (mM)	4.19 ± 0.06	4.30 ± 0.15	5.86 ± 0.31
TBIL (μM)	10.13 ± 0.70	18.99 ± 1.81	320.62 ± 136.81
CHOL (mM)	3.72 ± 0.12	3.37 ± 0.14	2.59 ± 0.08
CREA (μM)	54.67 ± 2.31	54.54 ± 2.27	65.58 ± 2.02
NO (μmol/mL)	32.18 ± 0.70	31.03 ± 0.64	53.11 ± 1.68
MELD			25.07 ± 0.40

**Abbreviations:** PT, prothrombin activity; Fb, fibrinogen; INR, international normalized ratio; WBC, white blood cell count; PLT, platelet count; ALT, alanine aminotransferase; GLU, glucose; TBIL, total bilirubin; CHOL, cholesterol; CREA, creatinine; NO, nitric oxide.

**Table 2** Baseline Characteristics of ACLF Patients

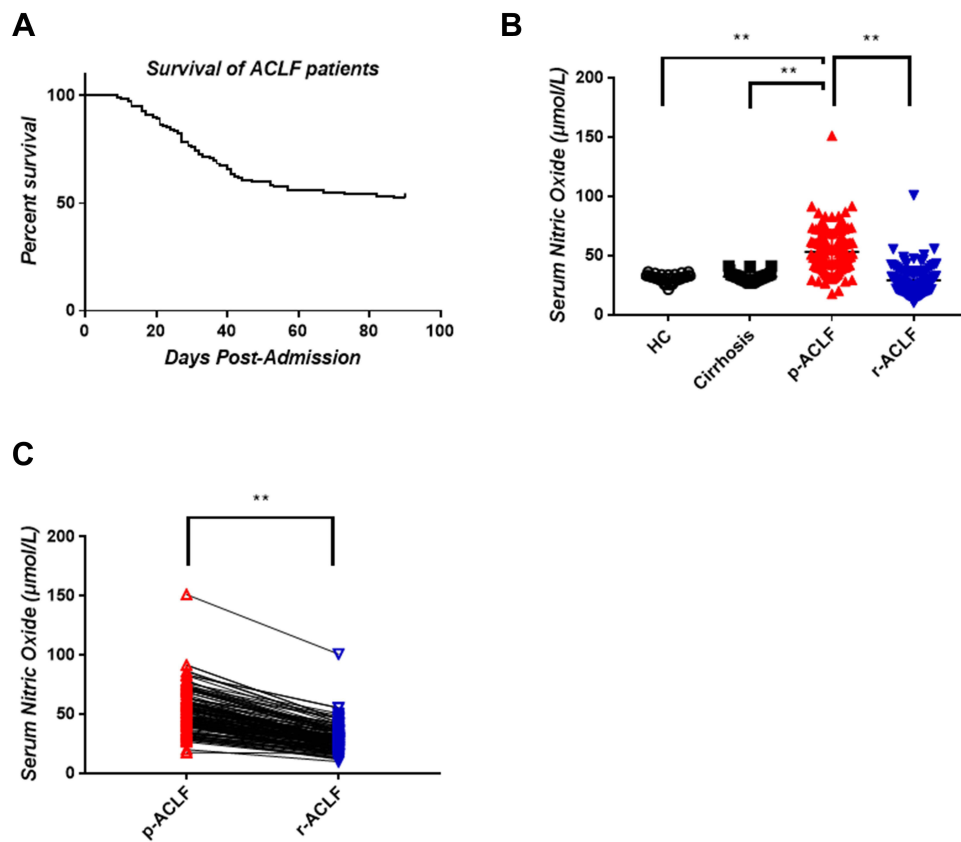
Variables	n=120	Non-Survivors n=57	Survivors n=63	P value
Age (yr)	47.29 ± 1.09	48.84 ± 1.43	45.89±1.62	0.178
Sex (M/F)	99/21	47/10	52/11	0.990
ACLF etiology				
Acute hepatic insult, n (%)				
Alcoholic hepatitis	16 (13.33%)	7 (12.28%)	9 (14.29%)	0.747
HBV reactivation	63 (52.50%)	32 (56.14%)	31 (49.21%)	0.448
Bacterial infection	33 (27.50%)	16 (28.07%)	17 (26.98%)	0.894
Others	8 (6.67%)	2 (3.51%)	6 (9.52%)	0.187
Underlying CLD, n (%)				
CHB	75 (62.50%)	30 (52.63%)	45 (71.43%)	0.034
ALD	21 (17.50%)	12 (21.05%)	9 (14.29%)	0.330
NAFLD	16 (13.33%)	10 (17.54%)	6 (9.52%)	0.197
HCC	8 (6.67%)	5 (8.77%)	3 (4.76%)	0.379
Clinic events at presentation, n (%)				
Ascites	120 (100%)	57 (100%)	63 (100%)	
Jaundice	120 (100%)	57 (100%)	63 (100%)	
AKI	38 (31.67%)	29 (50.88%)	9 (14.29%)	<0.01
HE	41 (34.17%)	28 (49.12%)	13 (20.63%)	<0.01
Acute variceal bleed	15 (12.50%)	9 (15.79%)	6 (9.52%)	0.300
SBP	9 (7.50%)	6 (10.53%)	3 (4.76%)	0.231
Parameter				
PT (%)	37.96 ± 1.34	37.02 ± 2.33	38.81±1.44	0.570
Fb (g/L)	1.58 ± 0.07	1.62 ± 0.12	1.54±0.07	0.535
INR	2.08 ± 0.07	2.38 ± 0.11	1.82±0.05	<0.01
WBC (1×10 <sup>9</sup> /L)	6.93 ± 0.35	7.83 ± 0.60	6.11±0.35	0.012
PLT (1×10 <sup>9</sup> /L)	101.58 ± 5.65	92.68 ± 6.70	109.6±8.82	0.135
ALT (U/L)	294.15 ± 36.85	237.87 ± 47.38	345.08 ± 55.16	0.147
GLU (mM)	5.86 ± 0.31	6.21 ± 0.52	5.54±0.36	0.281
TBIL (μM)	320.62±136.81	398.64 ± 16.68	239.79 ± 11.77	<0.01
CHOL (mM)	2.59 ± 0.08	2.54 ± 0.12	2.65±0.12	0.507
CREA (μM)	65.58 ± 2.02	66.04 ± 2.923	65.17±2.80	0.829
NO (μmol/mL)	53.11 ± 1.68	63.82 ± 2.49	43.41 ± 1.44	<0.01
Organ failure				
Kidney, n (%)	11 (9.17%)	6 (10.53%)	5 (7.94%)	0.623
Cerebral, n (%)	19 (15.83%)	15 (26.32%)	4 (6.35%)	<0.01
Coagulation, n (%)	40 (33.33%)	31 (54.39%)	9 (14.29%)	<0.01
Circulation, n (%)	14 (11.67%)	8 (14.04%)	6 (9.52%)	0.442
Lung, n (%)	32 (26.67%)	22 (38.60%)	10 (15.87%)	<0.01
Prognostic score				
MELD	25.07 ± 0.40	27.54 ± 0.53	22.83±0.42	<0.01

**Abbreviations:** CLD, chronic liver diseases; CHB, chronic hepatitis B; ALD, alcoholic liver disease; NAFLD, nonalcoholic fatty liver disease; HCC, Hepatocellular carcinoma; AKI, acute kidney injury; HE, hepatic encephalopathy; SBP, Spontaneous peritonitis.

$P<0.01$ ) (Figure 2B and C). In summary, NO levels were significantly higher in patients with ACLF, and NO levels were related to disease severity.

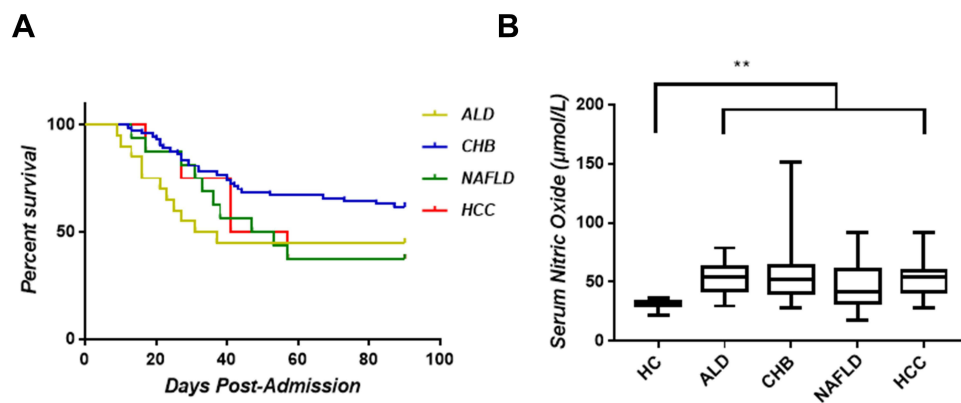
## ACLF on the Background of Different Diseases

At 90 days after admission, the survival rate of ACLF patients was 52.50%; it was 60.00% for ACLF patients on the background of chronic hepatitis B (CHB), 42.86% for ACLF patients on the background of ALD, 37.50% for ACLF patients on the background of NAFLD, and 37.50% for ACLF patients on the background of HCC. Figure 3A shows the



**Figure 2** High level of NO in the blood was linked to a bad prognosis for ACLF. The period from admission to the time of peak MELD score was defined as the progression stage of ACLF, whereas the period from the peak MELD score to the lowest in-hospital MELD score was designated as the remission stage. **(A)** Kaplan-Meier analysis of ACLF patients' survival; **(B)** When compared to healthy controls or cirrhosis patients, serum NO levels in the progression stage of ACLF (p-ACLF) were significantly higher; **(C)** Serum NO levels fell during the remission stage of ACLF (r-ACLF). \*\* $P < 0.01$ .

survival curve of ACLF patients against the background of various liver disorders. Serum NO levels were greater in ACLF patients than in healthy controls, and there were no significant differences between ACLF patients with different types of liver disorders (Figure 3B).



**Figure 3** Serum NO levels in ACLF patients with different liver diseases. **(A)** Kaplan-Meier survival analyses for ACLF patients with various liver disorders; **(B)** Admission serum NO levels for ACLF patients with various liver conditions. \*\* $P < 0.01$ .

## Serum NO Level Was an Independent Risk Factor for Mortality in Patients with ACLF

Using univariate and multivariate Cox regression models, we next identified possible risk variables for death among ACLF patients. INR (HR=7.079, 95% CI 2.760–18.158,  $P<0.01$ ), model for end-stage liver disease (MELD) score (HR=1.442, 95% CI 1.252–1.661,  $P<0.01$ ), and serum NO level (HR=1.113, 95% CI 1.070–1.158,  $P<0.01$ ) were all shown to be substantially linked with death in ACLF patients in Table 3. We therefore performed a forward multivariate analysis. MELD score (HR=1.231, 95% CI 0.924–1.640,  $P<0.01$ ), and serum NO level (HR=1.078, 95% CI 1.031–1.126,  $P<0.01$ ) were shown to be independent risk factors for death in ACLF patients.

## Serum NO Levels are Associated with Mortality in ACLF Patients

Of the 120 individuals in the present study, 54 patients died and three required liver transplantation. In ACLF patients, a MELD score of more than 25 was linked to a higher risk of death (Figure 4A and B). We divided the ACLF patients into two groups, a low-risk group (MELD<25) and a high-risk group (MELD>25), and compared their serum NO levels. NO levels were higher in the high-risk group than in the low-risk group ( $64.49 \pm 2.59$  vs  $44.98 \pm 1.63$ ,  $P<0.01$ ) (Figure 4C). Furthermore, the serum NO level was significantly correlated with the MELD score ( $R^2=0.3642$ ,  $P<0.01$ ) (Figure 4D). As serum NO levels were significantly elevated in ACLF patients, we investigated whether it can be utilized to predict disease outcomes. The greatest sensitivity and specificity for NO as a predictor of mortality within 90 days were obtained with a cutoff value of 53.5  $\mu\text{mol/mL}$ , according to ROC methodology (Figure 4E). Patients with higher NO levels had a higher risk of mortality ( $P<0.01$ ), according to our Kaplan–Meier analysis (Figure 4F).

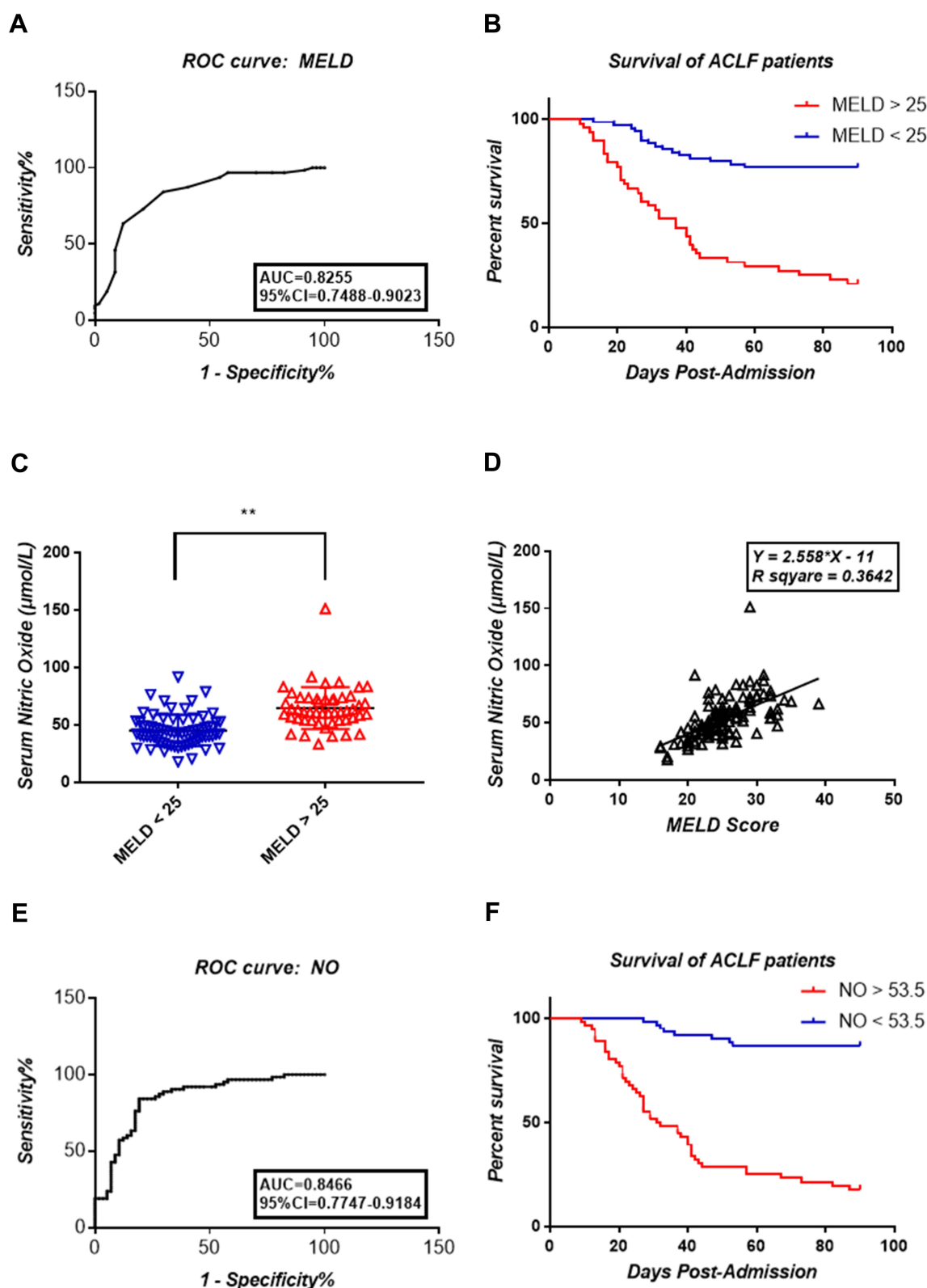
According to the serum NO level upon hospital admission, these 120 ACLF patients were separated into two groups: the low NO group (NO<53.5  $\mu\text{mol/L}$ ) and the high NO group (NO>53.5  $\mu\text{mol/L}$ ). Table 4 shows the differences in clinical and laboratory variables between the two groups. In the high NO group, the MELD score was greater than in the low NO group. Furthermore, patients with higher NO levels had elevated INR, WBC, ALT, TBIL levels, and mortality rates. The PT, Fb, PLT, GLU, CHOL, and CREA levels in the two groups were not significantly different.

## Combination of the Serum NO Level and MELD Score Had an Improved Prognostic Value

When the serum NO levels and MELD scores were combined, performance attributes improved. At 90 days, 87.27% of MELD<25 and NO<53.5  $\mu\text{mol/L}$  patients had spontaneously recovered and lived. Patients with NO>53.5  $\mu\text{mol/L}$  and

**Table 3** Uni- and Multivariate Logistic Analysis of Prognosis Factors Associated with Survival in Patients with ACLF

	Univariate			Multivariate		
	HR	95% CI	P	HR	95% CI	P
Age (yr)	1.006	0.392–2.582	0.990	1.038	0.181–5.939	0.967
Sex (M/F)	1.021	0.990–1.053	0.179			
PT (%)	0.992	0.967–1.017	0.505			
Fb (g/L)	1.174	0.709–1.946	0.533			
INR	7.079	2.760–18.158	<0.01			
WBC ( $1 \times 10^9/\text{L}$ )	1.145	1.024–1.282	0.192			
PLT ( $1 \times 10^9/\text{L}$ )	0.995	0.989–1.001	0.137			
ALT (U/L)	0.999	0.998–1.000	0.157			
GLU (mM)	1.062	0.952–1.185	0.283			
TBIL ( $\mu\text{M}$ )	1.013	1.008–1.017	0.294			
CHOL (mM)	0.872	0.585–1.302	0.504			
CREA ( $\mu\text{M}$ )	1.002	0.986–1.018	0.828			
MELD	1.442	1.252–1.661	<0.01	1.231	0.924–1.640	<0.01
NO ( $\mu\text{mol/mL}$ )	1.113	1.070–1.158	<0.01	1.078	1.031–1.126	<0.01



**Figure 4** Kaplan–Meier analyses for survival according to the admission serum NO levels and MELD score. **(A)** ROC curve for the MELD score; **(B)** MELD score (above or below 25) identifies ACLF patients with higher mortality; **(C)** ACLF patients with MELD score >25 displayed higher serum NO level than those with MELD score <25; **(D)** Serum NO was correlated with the MELD score; **(E)** ROC curve for serum NO; **(F)** Serum NO (above or below 53.5  $\mu\text{mol/L}$ ) identifies ACLF patients with higher mortality. \*\* $P < 0.01$ .



**Table 4** Clinical and Laboratory Characteristics Among Patients with Different NO Levels at Hospital Admission

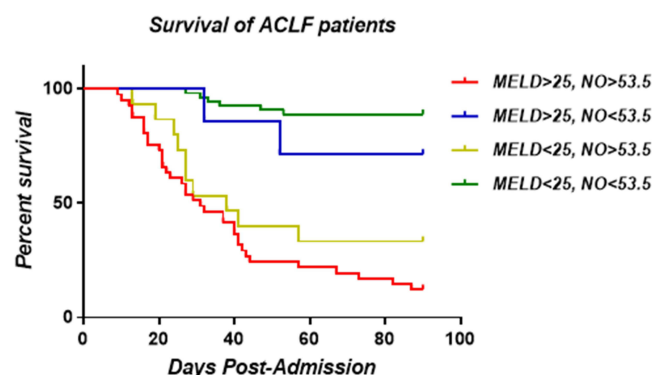
	Low Group (NO>53.5, n=56)	High Group (NO<53.5, n=64)	P
Age (yr)	47.98 ± 1.49	46.69 ± 1.59	0.557
Sex (M/F)	44/12	55/9	NA
PT (%)	37.50 ± 2.44	38.36 ± 1.34	0.749
Fb (g/L)	1.62 ± 0.11	1.54 ± 0.07	0.534
INR	2.41 ± 0.11	1.80 ± 0.04	<0.01
WBC (1×10 <sup>9</sup> /L)	7.82 ± 0.60	6.15 ± 0.35	0.015
PLT (1×10 <sup>9</sup> /L)	97.36 ± 7.65	105.28 ± 8.24	0.486
ALT (U/L)	214.75 ± 44.73	363.63 ± 55.84	0.043
GLU (mM)	5.58 ± 0.48	6.09 ± 0.40	0.410
TBIL (μM)	420.71 ± 13.86	222.96 ± 10.26	<0.01
CHOL (mM)	2.49 ± 0.13	2.69 ± 0.10	0.241
CREA (μM)	66.61 ± 2.90	64.68 ± 2.82	0.636
MELD	27.98 ± 0.48	22.52 ± 0.40	<0.01
NO (μmol/mL)	68.09 ± 2.04	40.00 ± 0.98	<0.01

MELD<25, or patients with NO<53.5 μmol/L and MELD>25 had worse survival rates. Patients who exceeded both the NO and MELD limits had the lowest survival rate at 90 days (12.20%) (Figure 5).

## Discussion

ACLF is characterized by a high risk of short-term mortality due to sudden and extensive cell death and a rapid deterioration in liver function. Acute liver injury on the background of chronic liver disease causes rapid and progressive liver failure, with a death rate of 50–90% within 90 days.<sup>14</sup> At 90 days, we discovered that all of these ACLF patients had a death rate of 47.50%. The King's College Criteria, SOFA score, and MELD score, in combination with the platelet to white cell ratio, the albumin-bilirubin score, and log<sub>10</sub>AFP, have been demonstrated to be straightforward indicators for assessing the severity and outcome of ACLF patients.<sup>12</sup> The majority of these prognostic indicators, however, are focused on decreased liver function and have a high specificity but low sensitivity. As a result, we pondered if a marker reflecting illness etiology and assessing severity might be developed.

Although the link between autophagy and cell death is complicated, a growing body of evidence suggests that autophagy improves cell survival by removing ROS.<sup>15</sup> NO accelerates HSCs apoptosis by producing ROS.<sup>16</sup> NO controls a wide range of physiological reactions. NO's biological significance was originally discovered in blood vessel relaxation and vascular toning. Several studies have focused on the link between NO and autophagy. In the etiology of

**Figure 5** Assignment of ACLF patients into low-, intermediate- or high-risk for 90-day survival according to the combination of serum NO and MELD score.



hepatocellular cancer, NO inhibits autophagy and increases apoptosis;<sup>17</sup> in HSCs during acute liver failure, increasing NO levels promote apoptosis by inhibiting autophagy.<sup>11</sup>

Because of the rarity of ACLF and the severity of the clinical manifestations, the serum NO level has not been studied in ACLF patients. Therefore, in the present study, we evaluated the predictive value of serum NO levels. We found that serum NO levels are considerably higher in ACLF patients than in healthy controls and liver cirrhosis patients, regardless of whether the disease was based on CHB, ALD, NAFLD, or HCC. Serum NO levels were shown to be closely linked to the severity of ACLF, with patients with NO levels >53.5  $\mu\text{mol/L}$  having a considerably higher fatality rate.

According to the present study, serum NO levels might be used as an independent predictor of death in ACLF patients. The MELD score has been widely used to predict the severity of ACLF, but it is restricted by intra-laboratory variability in three components: creatinine and bilirubin, which are related to renal and hepatic function, and INR, which is altered using coagulation products.<sup>18</sup> The serum NO level, however, can be easily measured using an ELISA kit. In addition, the value of very early testing of serum NO levels as a predictor of ACLF outcome was investigated in this study.

The combination of serum NO level and MELD score exhibited a better predictive ability than either serum NO level or MELD score alone. The most clinically useful application of these data is predicting which patients will not die of ACLF without liver transplantation. Patients with ACLF who have serum NO level >53.5  $\mu\text{mol/L}$  and MELD score >25 have a 90-day survival rate of less than 12.50%. A simple, objective blood test can be included to facilitate decision-making in these critically ill ACLF patients.

We previously discovered that cytokine and chemokine levels fluctuate during liver failure and rebound after remission.<sup>19</sup> In the current investigation, we found lower NO levels in ACLF patients during remission compared to the progression stage, demonstrating the predictive usefulness of serum NO levels in determining disease severity.

Furthermore, we discovered that patients with ACLF who had CHB, ALD, NAFLD, or HCC had higher serum NO levels than healthy and cirrhotic controls. We next looked at whether high NO levels were linked to the etiology of chronic liver illnesses such as CHB, ALD, NAFLD, and HCC. NOS uses a series of redox processes to produce NO. Three NOS isoenzymes exist in mammals: neuronal (nNOS or NOS1), inducible (iNOS or NOS2), and endothelial (eNOS or NOS3).<sup>10</sup> Endogenous NO has a very short half-life (about 1 second). As a result, NOS controls endogenous NO levels. HBV infection has been demonstrated to increase NOS2 gene expression and NO generation in human hepatocytes.<sup>20</sup> When compared to healthy controls, ALD patients had a statistically significant rise in serum NO levels.<sup>21</sup> According to a recent study, increased NO generation results in a reduction in intestinal arginase activity, which is important in NAFLD-related intestinal barrier dysfunction.<sup>22</sup> Serum NO levels were discovered to be elevated in HCC patients, and the combination of NO and AFP might be used as a diagnostic marker for HCC.<sup>23</sup>

There are some limitations in the present study. First, the current study is a retrospective analysis, with potential biases such as selection bias. Second, the present study only included a single-center cohort. It could be argued that data originating from other centers might lead to different conclusions. Thus, multicenter, prospective studies of large datasets with longer follow-up periods are needed to validate our research. Moreover, the molecular mechanism by which serum NO is involved in the progression of ACLF is not completely clear. Nevertheless, the present article is the first report to demonstrate that serum NO could serve as a promising predictor of short-term mortality. Our findings could be used to enhance the diagnostic accuracy of the MELD score as a non-invasive biomarker with great clinical significance.

## Conclusion

In conclusion, the current study demonstrated (1) that ACLF patients have increased serum NO levels, and (2) that a combination of serum NO levels and the MELD score is a reliable predictor of ACLF outcomes.

## Abbreviations

ACLF, Acute-on-chronic liver failure; HBV, Hepatitis B virus; ALD, Alcoholic liver disease; NAFLD, Nonalcoholic fatty liver disease; HSCs, Hepatic stellate cells; ROS, Reactive oxygen species; NO, Nitric oxide; SECs, Sinusoidal

endothelial cells; NOS, Nitric oxide synthase; INR, International normalized ratio; TIPS, transjugular intrahepatic portosystemic shunt; HCC, Hepatocellular carcinoma; HE, Hepatic encephalopathy; AKI, Acute kidney injury; CHB, Chronic hepatitis B; MELD, Model for end-stage liver disease.

## Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Ethics Approval and Informed Consent

The study was approved by the Institutional Ethics Committee of The First Affiliated Hospital of Xi'an Jiaotong University and conforms to the ethical guidelines of the Declaration of HELSINKI. Written informed consent was obtained from the patients and/or the legal guardian of patients with hepatic encephalopathy for participation.

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## Author Contributions

ZT contributed to the study conception and design. Data collection was performed by FW and MT. Data analysis was performed by FW, YH and MT. The first draft of the manuscript was written by ZT and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

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