Association Between Plasma Level of Superoxide Dismutase and Survival of Patients with Acute-On-Chronic Liver Failure

Zhen Tian (tianzhen@xjtufh.edu.cn)
Xi'an Jiaotong University Medical College First Affiliated Hospital https://orcid.org/0000-0001-7475-9472

Naijuan Yao
Xi'an Jiaotong University Medical College First Affiliated Hospital

Yuchao Wu
Xi'an Jiaotong University Medical College First Affiliated Hospital

Fei Wang
Xi'an Jiaotong University Medical College First Affiliated Hospital

Yingren Zhao
Xi'an Jiaotong University Medical College First Affiliated Hospital

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Abstract

Fewer than 50% patients with acute-on-chronic liver failure (ACLF) recover spontaneously, and ACLF patients have high mortality without liver transplantation. Oxidative stress has been shown to mediate hepatic inflammation during liver failure. The aim of this study is to investigate whether a biomarker of oxidative stress exists in assessing severity and outcomes of patients with ACLF. Between January 2015 and September 2018, a retrospective cohort of 124 ACLF patients, together with healthy individuals, liver cirrhosis and acute liver failure (ALF) patients were analyzed. Plasma Superoxide Dismutase (SOD) level was measured using a commercial ELISA kit, survival analysis was carried out using the Kaplan-Meier method. Results indicated that patients with ACLF had statistically higher plasma SOD levels compared to controls (healthy controls and cirrhosis patients). A level of SOD > 428 U/mL was associated with a statistically increase in risk for mortality or liver transplantation due to ACLF. Combination of plasma SOD level and MELD score had improved performance in assessing severity and outcomes of ACLF. In conclusion, this research revealed that plasma SOD level measured at hospital admission can be used to assign patients with ACLF into high- and low-risk groups. Combination of plasm SOD level and MELD score was more closely associated with patient outcome than either value alone. This system might be used to determine patient prognoses and prioritize patients for liver transplantation.

1. Introduction

Acute-on-chronic liver failure (ACLF) is defined by an acute deterioration in liver function in an individual with pre-existing chronic liver disease, resulting in multisystem organ failure and high short-time mortality[1]. Currently, few effective therapies exist apart from liver transplantation[2]. The aetiologias of ACLF vary between territories, alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD) are common in American and European countries, while viral infections (especially HBV infection) play central roles in Asian countries. With a carrier rate of hepatitis B virus (HBV) surface antigen at approximately 8% in adults, chronic HBV infection (CHB) is the major cause of ACLF in China[3].

Nowadays, oxidative stress is believed to play an important role in liver failure[4]. Injured/dead hepatocytes greatly increase oxidative stress during ACLF, which in turn contributes to further hepatocyte loss and impede regeneration, culminating in a vicious cycle[5, 6]. As a life-threatening disease with high mortality, scoring systems in assessing severity and disease outcomes of ACLF like King's College Criteria, SOFA score and MELD score have already existed[7]. However, all these methods focus on the impaired liver functions, there are few researches concentrate on disease pathogenesis, especially the increased systemic oxidative stress during ACLF. Since ACLF is mediated by reactive oxygen species (ROS), we thus wonder whether a biomarker of oxidative stress may exist in predicting severity, mortality and outcomes of this disease.

SOD transforms toxic superoxide into hydrogen peroxide and thereby limits the detrimental effects of ROS. Our previous study showed increased plasm SOD level in patients with ALF, and the plasma SOD level was associated with disease severity, further SOD staining in liver tissues revealed an increased

expression of SOD2, also known as manganese-dependent SOD (MnSOD)[8]. Since ALF is a disease with high level of systemic oxidative stress, this elevated plasma SOD level may be an adaptive response during disease pathogenesis. However, to the author's knowledge, no studies evaluated disease severity and outcome of ACLF from the perspective of hepatic oxidative stress. The aim of this present study is to identify whether plasma SOD a valid predictive indicator in ACLF patients.

2. Methods

2.1 Patients

From January 2015 to September 2018, a total of 124 ACLF patients were enrolled in our study at the First Affiliated Hospital of Xi'an Jiaotong University, Shaanxi, China. This study was approved by the Research Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University. Blood samples were collected at admission and 14 days after hospital admission, then stored at -80℃ within 2 hours of collection.

Patients were diagnosed with ACLF based on the criteria of Asian Pacific Association for the Study of the Liver (APASL): 1) serum bilirubin ≥ 85 mol/L; 2) INR ≥ 1.5 or prothrombin activity ≤ 40%; 3) any degree of encephalopathy and/or clinical ascites within 4 weeks; 4) and an evidence of ongoing chronic liver diseases[9]. Patients who were diagnosed with ACLF and aged 18 to 75 years were included.

A total of 48 patients in our cohort were excluded for the following reasons: 1)manifestation of decompensated liver cirrhosis prior to ACLF diagnosis, such as ascites and variceal hemorrhage; 2) patients with portal hypertension who received a transjugular intrahepatic portosystemic shunt (TIPS); 3) patients pathologically diagnosed with or clinically suspected for hepatocellular carcinoma (HCC); 4) other malignancies such as gastric cancer; 5) pregnancy; 6) HIV or hepatotropic virus infection other than HBV (Fig. 1).

We calculated the Model for End-Stage Liver Disease (MELD) score using the standard formula: 11.2*ln (INR) + 9.57*ln (creatinine, in mg per decilitre) + 3.78*ln (bilirubin, in mg per decilitre), with a lower limit of 1 for all variables.

During the same period, age- and sex-matched healthy participants and patients with liver cirrhosis were recruited as controls. Also, we used data of 30 ALF patients collected from our previous study.

2.2 Estimation of plasma SOD

Serum SOD level was measured using a commercial ELISA kit (#EIASODC, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacture's protocol. Samples and standards were run in duplicate, and the sensitivity of the assay was 0.044U/mL.

2.3 Statistical methods

Results are presented as means and standard deviations (SDs). Demographics were compared for categorical variables using a chi-squared test or Fisher's exact test as appropriate, and for continuous variables using a Wilcoxon rank sum test. ROC curves were generated using logistic regression where the event was death or transplantation within 90 days post-enrollment. Cut-offs for continuous variables were determined as the maximum of the sum of sensitivity and specificity. Kaplan-Meier survival curves to 90 days post-admission were compared using log-rank tests. Data were analyzed using SPSS version 16.0 software (IBM Corporation, Somers, NY, USA). Differences were considered to be of statistical significance when the *P* value < 0.05.

3. Results

3.1 Baseline characteristics

The current study cohort was made up of a population with admission criteria including coagulopathy and encephalopathy as described above. The clinical baseline characteristics of ACLF patients and controls in the present study are shown in Table 1.

Table 1
Demographic data and clinical characteristics of controls and ACLF patients

Parameter	Healthy Control (n = 30)	Cirrhosis (n = 30)	ACLF (n = 124)
Age (yr)	42.54 ± 8.65	45.68 ± 5.39	47.82 ± 11.95
Gender(M/F)	23/7	23/7	94/30
PTA (%)	84.58 ± 15.77	76.36 ± 16.87	37.02 ± 14.78
FIB (g/L)	2.68 ± 0.67	2.23 ± 0.95	1.54 ± 0.69
INR	1.21 ± 0.19	1.37 ± 0.21	2.12 ± 0.71
WBC(1×10^9/L)	5.68 ± 1.54	4.85 ± 1.75	15.28 ± 8.39
PLT (1×10^9/L)	225.97 ± 38.54	115.04 ± 58.21	104.77 ± 61.59
ALT (U/L)	25.21 ± 12.31	25.81 ± 12.32	309.52 ± 413.93
GLU (mM)	4.39 ± 0.68	4.54 ± 0.98	5.94 ± 3.45
TBIL (μM)	13.22 ± 3.79	19.88 ± 7.98	311.61 ± 133.12
CHOL (mM)	3.97 ± 0.69	3.45 ± 0.91	2.61 ± 0.9
CREA (μM)	49.67 ± 11.21	57.61 ± 9.81	64.55 ± 21.81
MELD			25.06 ± 4.31

ALT, alanine aminotransferase; CHOL, cholesterol; CREA, creatinine; FIB, fibrinogen; GLU, glucose; INR, international normalized ratio; PLT, platelet count; PTA, prothrombin activity; TBIL, total bilirubin; WBC, white blood cell count.

3.2 ACLF is associated with high plasma SOD levels

Compared to healthy individuals, patients with ACLF had statistically higher levels of plasma SOD (423.1 ± 13.75 U/mL vs 164.2 ± 3.82 U/mL, $P < 0.01$), however, no significant difference was found in ACLF patients compared to those patients with ALF (423.1 ± 13.75 U/mL vs 444.4 ± 23.58 U/mL, $P = 0.47$) (Fig. 2). We previously found that ALF is accompanied by liver fibrosis, and oxidative stress has been shown to promote HSCs activation[10]. Thus, we wondered whether this increased SOD level was a cirrhotic response. The serum SOD level in patients with liver cirrhosis was then tested, and no significant differences were found compared to heathy controls (169.3 ± 3.69 U/mL vs 164.2 ± 3.82 U/mL, $P = 0.34$). However, a significant increase in the SOD level was observed in patients with ACLF compared to patients with liver cirrhosis (423.1 ± 13.75 U/mL vs 169.3 ± 3.69 U/mL, $P < 0.01$) (Fig. 2).

3.3 Plasma SOD levels and MELD scores associated with mortality or liver transplantation in patients with ACLF

In the current cohort, 3 patients had liver transplantation, there were 63 deaths without transplantation. Using ROC methodology, the maximum sensitivity and specificity for plasm SOD level as a predictor of death or transplantation within 90 days was 428 U/mL, and 25 for MELD score (Fig. 3a, c). Kaplan-Meier analysis showed that patients with a SOD value above this had increased risk of death or transplantation ($P < 0.05$) when censored at 90 days (Fig. 3b). Previously, we found that a MELD score > 25 was associated with high mortality risk in patients with ALF[11], here, we assessed the predictive value of MELD score in ACLF patients, and found that patients with MELD score > 25 in the present study also had significantly greater mortality (Fig. 3d). Furthermore, a positive correlation was observed ($Y = 21.03*X-106.2$, $R^2 = 0.3749$) when the SOD level was correlated with the MELD score (Fig. 3e).

3.4 Combination of plasma SOD level and MELD score had improved prognosis performance

Performance characteristics were improved when plasma SOD and MELD score were combined. 89.29% of patients with MELD < 25 and SOD < 428 spontaneously recovered and survived at 90 days. Patients whose SOD > 428 and MELD < 25 together with those whose SOD < 428 and MELD > 25 had relatively diminished survival, patients exceeding both SOD and MELD thresholds demonstrated the poorest survival at 90 days (11.36%) (Fig. 4). Thus, plasma SOD level is additionally informative in the prediction of transplantation or death when combined with MELD score.

3.5 Association of plasma SOD levels with Oxidative Stress

We previously found that the cytokine and chemokine levels vary during ALF and observed a significantly decreased SOD level during the remission stage compared to the progression stage in ALF patients[8]. However, the same result was not observed in these ACLF patients (Fig. 5a). In the present study we found that patients with ACLF based on ALD and NAFLD showed increased plasma SOD with disease progression (14 days after hospital admission), while, ACLF patients due to chronic hepatitis B (CHB) infection tend to recover with decreased SOD level. We collected another 12 ACLF patients based on HCC and found increased plasma SOD at 14 days (Fig. 5b). Kaplan-Meier analysis showed different risk of death or transplantation among these patients (Fig. 5c).

Recent studies have shown that ALD, NAFLD and HCC are all disorders with increased systemic oxidative stress. Of the current cohort, 21 of these ACLF patients were ALD, 16 were NAFLD, and another 12 ACLF patients based on HCC. Although significantly higher than healthy controls, no significant differences were found in plasma SOD levels among patients with ACLF caused by CHB, ALD, NAFLD and HCC (431.4 ± 16.91 U/mL vs 430.7 ± 23.24 U/m vs 371.9 ± 44.91 U/mL vs 479.5 ± 39.76 U/mL) at time of hospital admission (Fig. 5d).

4. Discussion

ACLF is characterized by massive cell death and a sudden deterioration in liver function, which leads to high short-time mortality. An acute insult based on chronic liver diseases lead to rapid and progressive liver failure and result in high mortality of approximately 50–90% at 90 days[12]. Here, we found a 59.93% mortality rate at 90 days among all these ACLF patients. King's College Criteria, SOFA score and MELD score, together with liver volume, platelet to white cell ratio, albumin-bilirubin score and $\log_{10}$AFP have been shown simple models in evaluating the severity and disease outcomes of patients with ACLF[7]. However, most of these prognostic markers focus on the impaired liver function and have been characterized by high specificity but low sensitivity. We thus wonder whether a marker reflecting disease pathogenesis and evaluating the severity and prognosis of ACLF exists.

Many human diseases, including ACLF, are always associated with excessive inflammation, inflammasome activation servers as a double-edged sword, contributing to both protective antimicrobial responses and cell death when excessive active[13]. ACLF patients often present with endotoxemia and increased LPS levels due to increased gut permeability[14], hepatic stellate cells (HSCs) together with sinusoidal endothelial cells and Kupffer cells constitute the liver sinusoids, these cells facing the sinusoidal lumen and in direct contact with the portal circulation serve as the gate against inflammatory stimuli, they produce inflammatory cytokines when stimulated by the gut microbiota and microbial byproducts like LPS in septic liver injury[15]. Inflammatory cytokines are relayed to the parenchyma, resulting in hepatocytes damage, injured/dead hepatocytes then greatly increase oxidative stress during ACLF. As previous studies have revealed that ROS are crucial for NLRP3 inflammasome activation[16]. Increased oxidative stress contribute to inflammasome activation and lead to further hepatocyte loss and impede regeneration, culminating in a vicious cycle.

In the present study, we assessed for the first time the prognostic value of plasm SOD level, which increases as an adaptive response to elevated systemic oxidative stress during ACLF. We found that circulating SOD level is markedly elevated in ACLF patients compared to those in the healthy control or in liver cirrhosis groups, regardless of whether it is due to CHB, ALD or NALFD. Plasma SOD level has also been shown associated with disease severity, as ACLF patients with SOD > 428 U/mL have significantly increased mortality.

This present study suggested that plasma SOD level could serve as an independent predictor of mortality in ACLF patients. MELD score has been widely used as predictive criterial in assessing severity of ACLF but is limited by the inner-laboratory variability in 3 components, creatinine and bilirubin related to renal and hepatic function, together with INR[7], which can be affected by the usage of coagulation products. However, plasm SOD level can be easily tested by ELISA. In addition, this study assessed the value of very early testing of plasm SOD level as a predictor of ACLF outcome.

This present study reported that combination of plasm SOD level and MELD score had a higher predicting power than either plasm SOD level or MELD score alone. Clinically, the most applicable use of this information is in predicting who will not die from ACLF without liver transplantation. Patients with ACLF who have SOD < 428 U/mL and MLED score < 25 are very likely to have spontaneous recovery and survive. In contrast, ACLF patients with SOD > 428 U/mL and MLED score > 25 have a survival rate less than 12% at 90 days. Thus, the addition of a simple, objective blood measurement, SOD, can be considered a practical and significant adjunct to decision making for these extremely ill ACLF patients.

The present study showed that ACLF patients based on ALD, NAFLD and HCC showed higher plasma SOD levels than those based on CHB when censored at 14 days. ACLF is characterized by ROS production, ROS actives the NLRP3 inflammasomes in regulating various physiological responses and plays important roles in hepatic failure. Mitochondria is the main source of ROS in hepatocytes acutely and/or chronically exposed to a “damage” injury (viruses, alcohol, environmental drugs, therapeutical drugs, etc.)[17]. To NAFLD, mitochondria derived oxidative stress initiates a vicious cycle of exacerbated mitochondrial dysfunction and increased hepatocellular oxidative damage[18], moreover, excessive production of ROS was shown in HCC, which exceeds the capacity of the cells to move therefore plays a crucial role in the occurrence and development of liver cancer[19]. Since oxidative stress plays a central role during ACLF, the increased oxidative stress during ACLF progression due to combination of ALD, NAFLD or HCC leaded to much higher mortality rates compared to ACLF patients due to chronic HBV infection.

Results in the present study are derived from a large consortium over a 3 year of time, underscoring the generalizability of our findings, however, the limitations of the current study include that common to retrospective analysis with potential biases such as selection bias.

Declarations

Funding: This work was financially supported by the National Natural Science Foundation of China (81800548). The sponsors had no role in the study design and in the collection, analysis, and interpretation of data.

Conflict of interest: None.

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

Authors' contributions: Zhen Tian contributed to the study conception and design. Data collection was performed by Naijuan Yao, Fei Wang. Data analysis was performed by Zhen Tian and Yuchao Wu. The first draft of the manuscript was written by Zhen Tian and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics declarations: 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. Requirement for individual patient consent forms was waived due to the retrospective, observational nature of the study.

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Figures

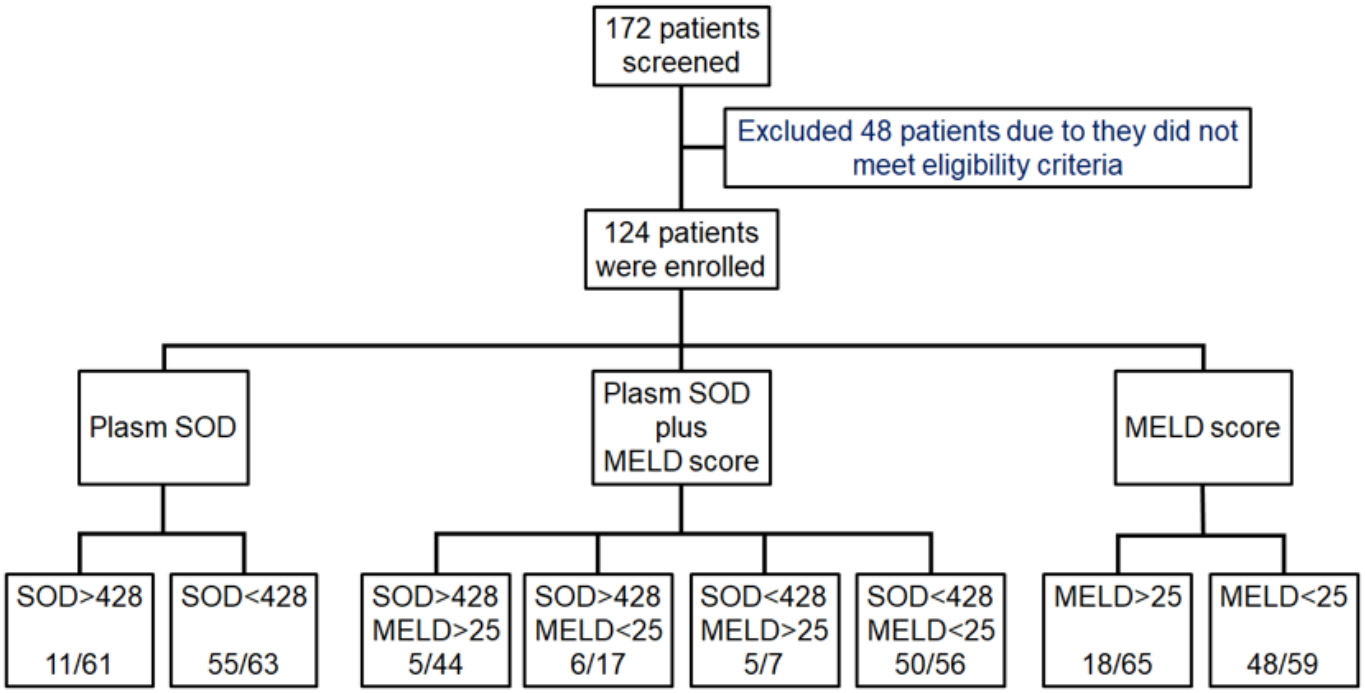

Figure 1

Patient disposition throughout the study.

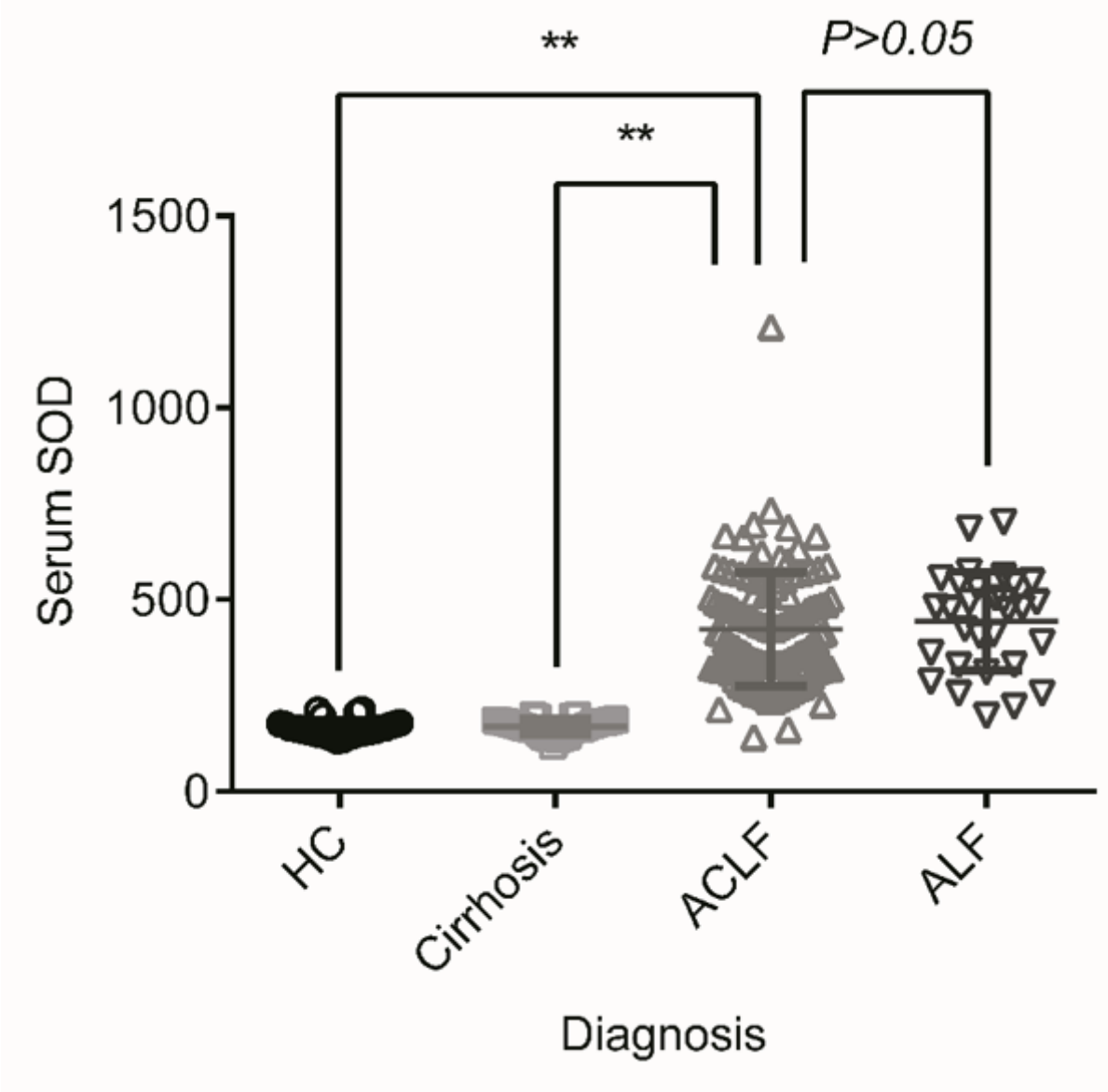

Figure 2

Values of admission plasma SOD (U/mL) in patients with cirrhosis, ACLF and ALF.

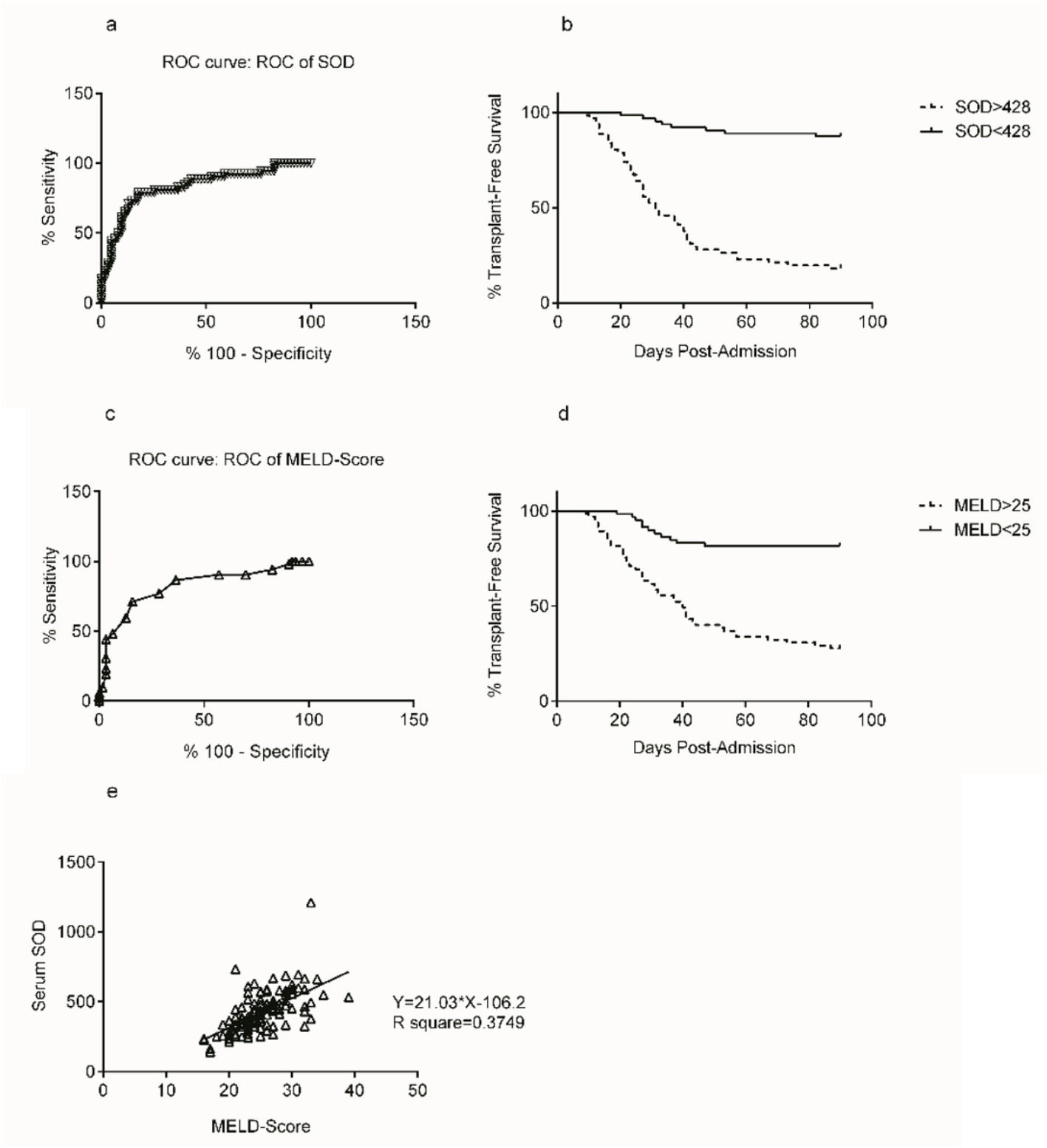

Figure 3

Kaplan-Meier analyses for survival according to admission plasma SOD levels and MELD score. a) ROC curve for plasma SOD. b) Plasma SOD (above or below 428 U/mL) identifies ACLF patients with higher mortality. c) ROC curve for MELD score. d) MELD score (above or below 25) identifies ACLF patients with higher mortality. e) Plasm SOD was correlated with MELD score.

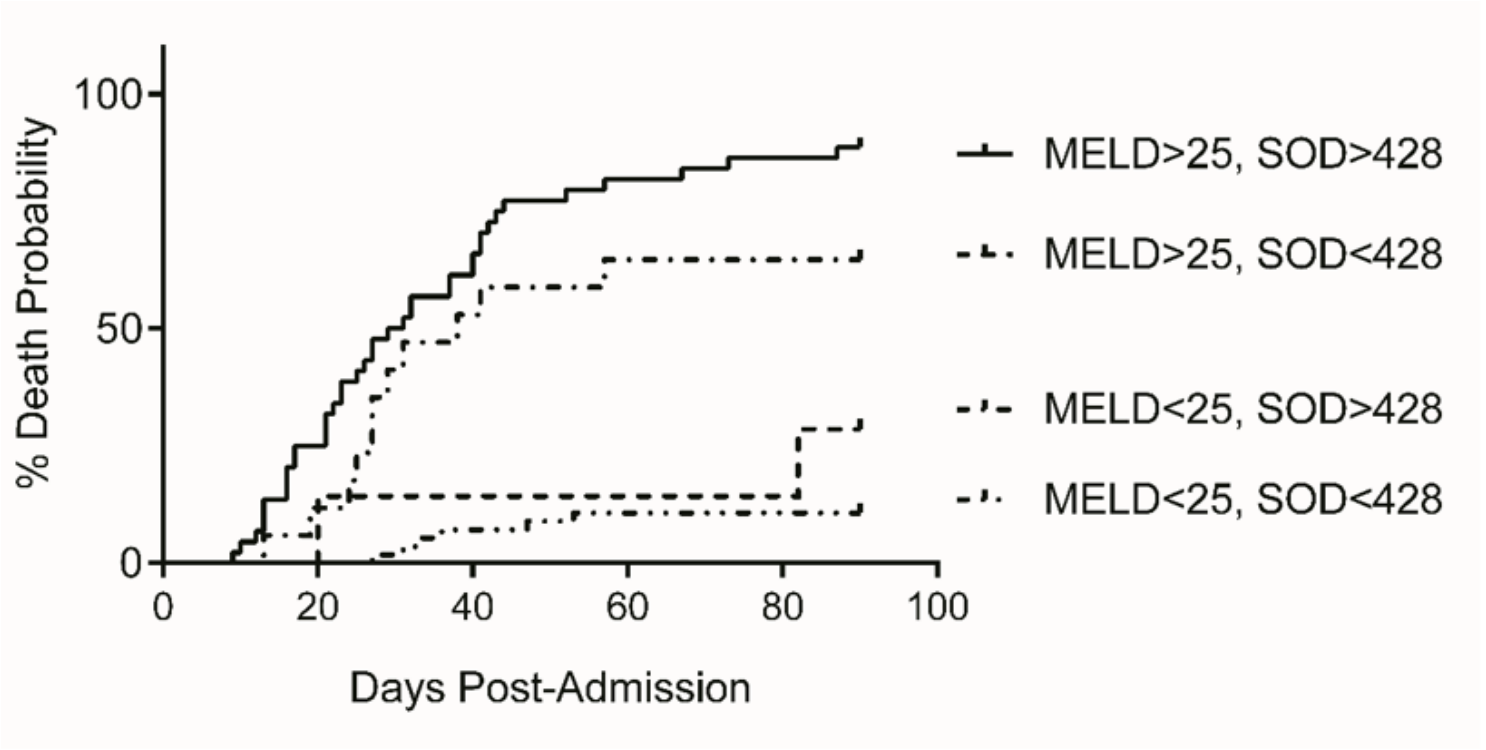

Figure 4

Assignment of ACLF patients into low-, intermediate-, or high-risk for 90-day mortality or transplant according to combination of SOD and MELD.

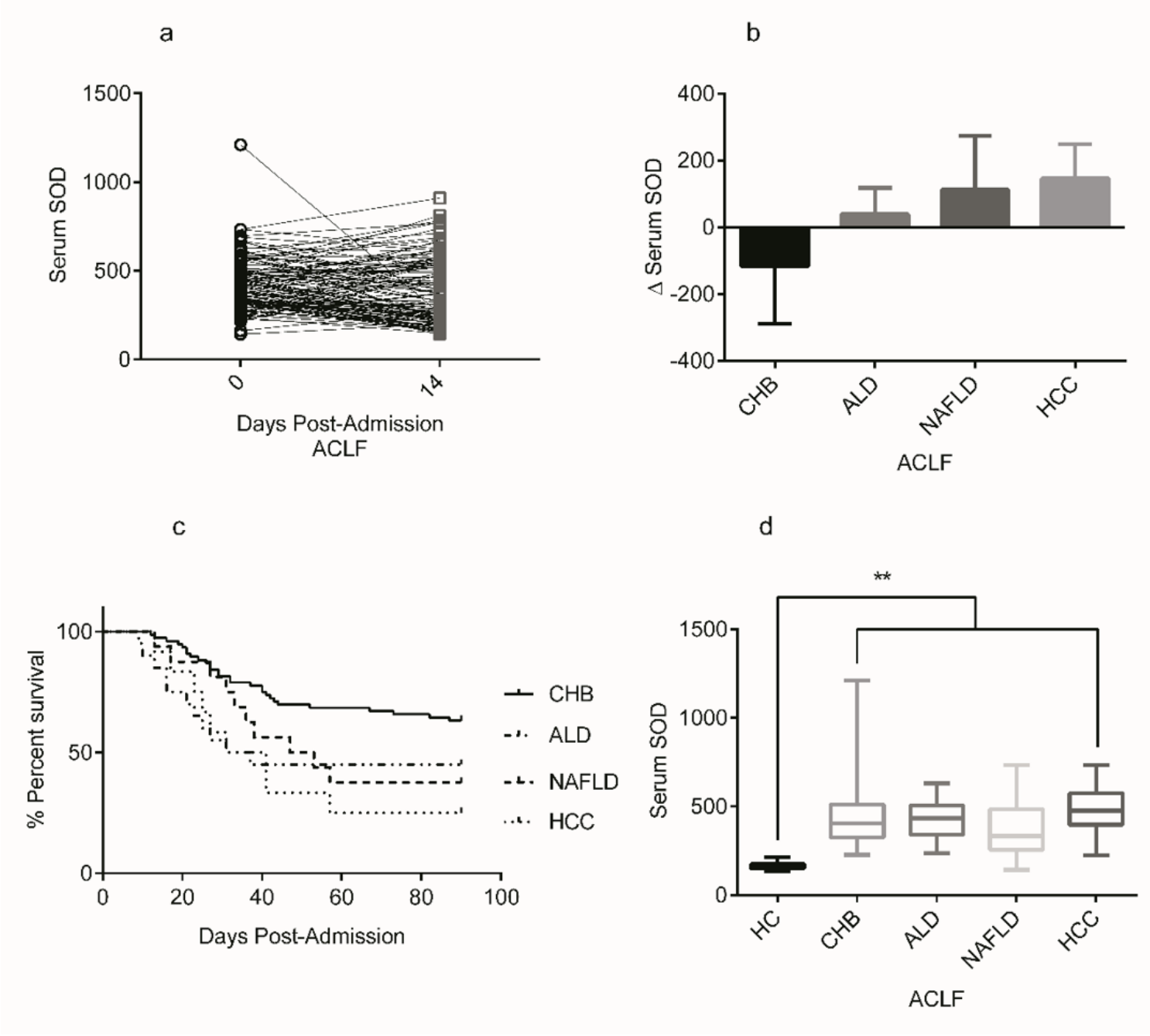

Figure 5

Plasma SOD levels for ACLF patients with different liver diseases. a) Changing of plasma SOD levels for ACLF patients. b) Changing of plasma SOD levels for ACLF patients based on different liver diseases. c) Kaplan-Meier analyses for survival for ACLF patients based on different liver diseases. d) Admission plasma SOD levels for ACLF patients based on different liver diseases.