

# Erythropoietin in Predicting Prognosis in Patients with Acute-on-Chronic Liver Failure

Tamara Alempijevic<sup>1,2</sup>, Simon Zec<sup>1</sup>, Vladimir Nikolic<sup>1</sup>, Aleksandar Veljkovic<sup>3</sup>, Vladimir Milivojevic<sup>2</sup>, Violeta Dopsaj<sup>4,5</sup>, Sanja Stankovic<sup>5</sup>, Tomica Milosavljevic<sup>1,2</sup>

1) School of Medicine,  
University of Belgrade;

2) Clinic for Gastroenterology  
and Hepatology, Clinical  
Center of Serbia;

3) Faculty of Mathematics,  
University of Belgrade;

4) Department of Medical  
Biochemistry, Faculty of  
Pharmacy, University of  
Belgrade;

5) Center of Medical  
Biochemistry, Clinical Centre  
of Serbia,  
Belgrade, Serbia

## ABSTRACT

**Background & Aims:** Acute-on-chronic liver failure (ACLF) is characterized by a rapid progression to multiple organ failure and is associated with a very high mortality rate of 50-90%. Novel therapies are being investigated such as Erythropoietin (EPO). The aim of this prospective cohort study was to analyse the value of EPO in predicting prognosis and determine which patients may benefit most from EPO therapy.

**Methods:** According to the EASL-CLIF criteria, 104 consecutive patients were diagnosed with ACLF and separated into two groups based on the type of insult: bleeding (Group A=31) or non-bleeding (Group B=73). In addition to a complete biochemical work-up and calculation of relevant prognostic scores, levels of EPO were measured on admission and correlated to the type of insult and final outcome.

**Results:** Fifteen patients from Group A (mean age 60.32±9.29 years) had a lethal outcome and higher values of EPO on admission (319.26±326.58 mIU/ml) ( $p<0.005$ ), compared to the 37 patients from Group B (mean age 59.9±10.19 years) with EPO levels at admission of 29.88±34.6 mIU/mL. In Group B, a cut-off EPO value of 30.65 mIU/mL had a sensitivity of 87.5% and a specificity 57.4% in predicting lethal outcome with an AUROC of 0.823. In Group A, a cut-off value of 229.95 mIU/mL had a sensitivity and specificity of 53.3% and 92.7%, respectively. The AUROC for this cut-off was 0.847.

**Conclusions:** Erythropoietin is superior to the standard prognostic scores in predicting 28-day mortality. Lower levels of EPO were detected in patients without bleeding as an insult indicating a possible therapeutic benefit in these patients.

**Key words:** acute-on-chronic liver failure – erythropoietin – prediction of outcome – gastrointestinal hemorrhage – infection.

**Abbreviations:** ACLF: acute-on-chronic liver failure; ALD: alcoholic liver disease; APACHE II: acute physiology and chronic health evaluation II; AUROC: Area Under the Receiver Operating Curve; CLD: chronic liver disease; CLIF: Chronic Liver Failure Consortium; CLIF-SOFA: Chronic Liver Failure Consortium-Sequential Organ Failure Assessment; CTP: Child-Turcotte-Pugh; EPO: erythropoietin; GI: gastrointestinal; HBsAg: hepatitis B surface antigen; MARS: Molecular Adsorbent Recirculating System; MELD: Model for End Stage Liver Disease; MELD Na: Model for End Stage Liver Disease Sodium; SOFA: Sequential Organ Failure Assessment.

### Address for correspondence:

Tamara Alempijevic, MD, PhD  
Clinic for Gastroenterology  
and Hepatology,  
Clinical Center of Serbia,  
2 Dr Koste Todorovica St.,  
11000 Belgrade, Serbia  
tamara.alempijevic@med.bg.ac.rs

Received: 03.07.2016

Accepted: 15.08.2016

## INTRODUCTION

Acute-on-chronic liver failure (ACLF) represents a major challenge for clinicians due to the fact that no single universally accepted diagnostic criterion exists and the illness is characterized by a rapid progression, multiple organ failure and a very high short-term mortality rate of 50-90% [1]. Two definitions currently

predominate: the first consensus definition, of the American Association for the Study of Liver Disease and European Association for the Study of the Liver (AASLD-EASL) states that ACLF is a syndrome incorporating a subgroup of cirrhotic patients who develop organ failure following hospital admission with or without an identifiable precipitating event, resulting in increased mortality rates [2, 3]. The second definition, by the Asia-Pacific Association for the Study of Liver (APASL), characterizes ACLF as an acute hepatic insult manifesting as jaundice (serum bilirubin  $\geq 5$  mg/dL or 85  $\mu$ mol/L) and coagulopathy with an international normalized ratio (INR)  $\geq 1.5$ , or prothrombin activity  $< 40\%$ , complicated within 4 weeks by clinical ascites and/or encephalopathy in a

patient with previously diagnosed or undiagnosed chronic liver disease (CLD) or cirrhosis [3, 4]. Given this lack of consensus, researchers from the EASL-Chronic Liver Failure Consortium (CLIF) prospectively studied patients with liver disease and acute decompensation, and found that patients with acute decompensation who had organ failure and high mortality rates in the short term (28 days) could be diagnosed with ACLF [5]. With no clear definitions, CLD remains the underlying pathology, diagnosed on the basis of prior examination, laboratory, radiological and endoscopic findings [3]. Precipitating events (acute insults) to ACLF may be different, and depend on geographical region [1]. In Western countries, acute drug-induced liver injury, and alcoholic hepatitis are most frequent, while in Eastern countries, infectious etiologies (acute hepatitis B infection or reactivation) predominate. However, AASLD-EASL, APASL and EASL-CLIF acknowledge that the acute insult could not be identified in up to 43% of cases [1, 3-5]. In any case, precipitating events are classically divided into two groups: first, those that directly affect liver function, examples of which are alcoholic hepatitis (most common intrahepatic insult), drug-induced liver injury, new-onset or reactivation of viral hepatitis, and ischemic hepatitis (chiefly Budd Chiari syndrome); second, systemic events with consequent impairment of liver function such as infection (53% of cases) – most commonly spontaneous bacterial peritonitis, pneumonia and sepsis – along with surgery or variceal bleeding [2, 6].

Secondary to the intrinsic effects of the acute insult, the development and progression of ACLF is mediated by a systemic inflammatory response [1]. During ACLF there are increased serum levels of several pro-inflammatory cytokines and their receptors, namely: TNF $\alpha$ , sTNF-aR1, sTNF-aR2, IL-2, IL-2R, IL-4, IL-6, IL-8, IL-10 and interferon- $\alpha$ . This, coupled with a rise in C-reactive protein, an elevated basal oxidative status and decreased phagocytic activity, cumulatively lead to hepatic inflammation, apoptosis and necrosis, alongside cholestasis and fibrosis [1, 7, 8]. These events also lead to multi-system organ failure of the renal, central nervous, cardiovascular, coagulation and pulmonary systems with the clinical outcome largely depending on the extent of multi-system failure [1]. Due to the inherent nature of ACLF many organ failure prognostic scores have been evaluated in predicting outcome. These include the Acute physiology and chronic health evaluation II (APACHE II), Child-Turcotte-Pugh score (CTP), Model for End Stage Liver Disease (MELD), Model for End Stage Liver Disease Sodium (MELD Na), Sequential Organ Failure Assessment (SOFA), and CLIF-SOFA scores [9-13].

Liver transplantation is the only proven treatment with a benefit on survival rate. However, since a large number of patients succumb while waiting on the transplant list [1], novel therapies based on hepatocellular regeneration are being investigated. One possible therapeutical option is the use of Erythropoietin (EPO) [14]. Erythropoietin, a glycoprotein predominantly synthesized by the kidney with a molecular weight of approximately 30kDa, plays a central role in the stimulation of erythropoiesis. In addition to this, EPO also has cytoprotective, anti-apoptotic, anti-inflammatory and antioxidant activity [14, 15]. Erythropoietin achieves its effects by activating the phosphatidylinositol

3-kinase / AKT / endothelial NO synthase pathway [16] and by inhibition of caspase-3 activation [17]. Activation of the phosphatidylinositol 3-kinase-AKT pathway, in turn, mobilizes endothelial progenitor cells and increases their regenerative role [8]. This is the basis of the protective effect of EPO in ACLF.

The aim of this study was to analyse and compare the serum levels of EPO in patients with ACLF precipitated by an acute bleeding or non-bleeding event, and in accordance with the EPO levels at admission, to determine the value of EPO as a predictor of prognosis and analyse which group of patients would have the greatest potential benefit from treatment with EPO.

## PATIENTS AND METHODS

We carried out a prospective cohort study in 104 consecutive patients diagnosed with ACLF at the Emergency Unit, Department of Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade, Serbia from January 1st 2014 to July 1st 2015. All the patients were previously diagnosed with CLD or cirrhosis based on prior liver histology, clinical features, laboratory tests, and imaging studies [18]. Patients were diagnosed with ACLF according to the EASL-CLIF criteria in accordance with the CLIF-SOFA organ failure score: liver failure: serum bilirubin  $\geq$  12 mg/dl; renal failure: serum creatinine  $\geq$  2 mg/dl; cerebral failure: grade III-IV hepatic encephalopathy (West-Haven classification); coagulation failure: international normalized ratio (INR)  $\geq$  2.5; circulatory failure: use of vasoconstrictors to treat severe arterial hypotension (use of vasoconstriction for the treatment of type 1 hepato-renal syndrome in patients without severe hypotension not included); respiratory failure: PaO<sub>2</sub>/FiO<sub>2</sub>  $\geq$  200 or SpO<sub>2</sub>/FiO<sub>2</sub>  $\geq$  214. Renal dysfunction was diagnosed when serum creatinine ranged between 1.5 and 1.9 mg/dl; cerebral dysfunction was diagnosed in patients with grade I or grade II hepatic encephalopathy. Type I ACLF is defined by the presence of renal failure alone or of any other type of single failure if associated with renal dysfunction and/or cerebral dysfunction. Type II ACLF and type III ACLF are defined by the presence of 2 and 3 to 6 organ failures, respectively [9].

Exclusion criteria were as follows: age < 18 years, absence of any CLD, presence of hepatocellular carcinoma, presence of severe chronic extra-hepatic disease, admission due to other chronic illness, human immunodeficiency virus infection, chronic decompensation of end-stage liver disease, less than 28 days of follow-up, and incomplete data [19]. All patients gave written informed consent for inclusion in the study.

Alcoholic liver disease (ALD) was considered as the underlying CLD if there was a positive history of alcohol consumption of at least 50 g per day for the past five years. Positive hepatitis B surface antigen (HBsAg) or anti-hepatitis C antibodies defined viral etiology. Autoimmune etiology including, autoimmune hepatitis, primary biliary cholangitis, and primary sclerosing cholangitis, was diagnosed using specific antibodies. The remaining study cases had liver cirrhosis of other etiology, including non-alcoholic steatohepatitis, Wilson's disease,  $\alpha$ -1 antitrypsin deficiency, hemochromatosis and cryptogenic, and were thus classified as other.

Based on the precipitating insult, patients were separated into two groups. Group A consisted of patients with gastrointestinal (GI) bleeding and Group B included those patients with a non-GI bleeding insult (infection, acute drug-induced liver injury, alcoholic hepatitis, reactivation of viral hepatitis, acute vascular liver disease, etc.) [6]. The control group consisted of age and gender matched patients with CLD of various etiology, who did not have signs or symptoms of acute deterioration.

Components of various prognostic scores, such as body temperature, cardiac status (heart rate, blood pressure, mean arterial pressure), respiratory rate, neurological status (Glasgow coma scale) and blood parameters (routine blood tests with white blood cell and platelet counts, hematocrit, coagulation profiles including prothrombin time and INR, serum electrolyte levels, renal and liver function tests and arterial blood gas analysis) were analyzed [20].

All patients were evaluated based on various prognostic scores, including APACHE II, CTP, MELD, MELD Na, SOFA, and CLIF-SOFA, which were calculated as per standard definitions at the time of admission [9-13].

In addition to the standard work-up, serum EPO levels were also measured for all patients at the time of admission, using IMMULITE 2000 Systems Analyzers (Siemens) with an analytical sensitivity of 1.0 mIU/mL (WHO 2nd IRP 67/343) and a calibration range up to 200 mIU/mL.

Patients were followed for 28 days to determine short-term mortality, and were defined as either survivors or non-survivors based on in-hospital death within the follow-up period. Values of EPO and prognostic scores at admission were analyzed in correlation to the type of insult (GI bleeding versus non-GI bleeding) and outcome at 28 days. The diagnostic accuracy, sensitivity and specificity of EPO in predicting short-term mortality was assessed by calculation of an area under the receiver operating curve (AUROC).

For normal variables, means and standard deviations were calculated.  $\chi^2$  test and independent-sample *t*-test were used to assess the differences between the groups. *P* values less than 0.05 were considered statistically significant. Statistical analyses were performed using SPSS 22.0 (SPSS Inc. Chicago, IL, USA).

This study was approved by the Ethics Committee of the Clinical Center of Serbia (18.11.2014; 1393/9) in keeping with the principles of the Declaration of Helsinki (2000 revision of Edinburgh).

## RESULTS

Demographic and clinical characteristics of all patients included in the study are shown in Table I. In both study groups the majority of the patients were male (74.2% in Group A, and 73.9% in Group B). The mean age in Group A (n=31) was 60.32±9.29 years, range 39-75, and in Group B (n=73) 59.90±10.19 years, range 36-82. The mean age in the control group (n=20) was 61.1±8.3 years, range 32-64. The underlying etiology of disease was ALD in the majority of the patients. Lethal outcome at 28 days was seen in 48.4% of patients from Group A and 50.7% from Group B.

Mean values of EPO on admission, along with CTP, MELD, MELD Na, SOFA, APACHE II, CLIF-SOFA scores, and the type

**Table I.** Demographics and characteristics of the study groups and controls

	Group A n=31	Group B n=73	Controls n=20
Mean age in years (range)	60.32 ± 9.29 (39-75)	59.90 ± 10.19 (36-82)	61.1 ± 8.3 (32-64)
Gender			
Male	23 (74.2%)	54 (73.9%)	6 (30%)
Female	8 (25.8%)	19 (26.1%)	14 (70%)
Etiology of CLD			
ALD	25 (80.6%)	56 (76.7%)	15 (75%)
Viral	3 (9.7%)	6 (8.2%)	1 (5%)
Autoimmune	2 (6.5%)	6 (8.2%)	3 (15%)
Other	1 (3.2%)	5 (6.9%)	1 (5%)
Outcome at 28 days			
Lethal	15 (48.4%)	37 (50.7%)	0 (0%)
Survivors	16 (51.6%)	36 (49.3%)	20 (100%)

Group A: Bleeding insult, Group B: Non-bleeding insult, ALD: alcoholic liver disease; CLD: chronic liver disease

of ACLF for the study groups and controls are presented in Table II. There was a statistically significant difference in EPO levels between the two study groups ( $p < 0.001$ ). The bleeding group had higher levels at the time of admission with a mean of 293.62±282.76 mIU/mL, whereas the non-bleeding group had a mean value of 41.59±50.91 mIU/mL. Within the control group, the mean levels of EPO at admission were lower than in the study groups (14.27±13.45 mIU/mL). The prognostic scores were slightly lower in Group A; however, this was not statistically significant.

The values of EPO and the prognostic scores, according to the outcome are presented in Table III. In Group A, the bleeding group, survivors at 28 days had lower levels of EPO at admission, than those with a lethal outcome; however, this was not statistically significant ( $p = 0.633$ ). In Group B, the EPO values were lower in patients with a lethal outcome 29.88±34.6 mIU/mL, compared to that of survivors 37.77±49.2 mIU/mL,  $p = 0.046$ . The prognostic scores were similar in both groups.

The EPO levels along with prognostic scores at the time of admission, as predictors of 28-day mortality are shown in Table IV. Overall, EPO and CLIF-SOFA were the best predictors of outcome in both study groups. In Group A, EPO, CLIF-SOFA, and APACHE II were found to be the best predictors of 28-day mortality with AUROCs of 0.847, 0.736, and 0.632, respectively. The AUROC for EPO in Group B (0.823), was superior to that of MELD, MELD Na, and CLIF-SOFA, all of which had similar values above 0.700. The AUROCs for Group A and Group B are shown in Figures 1 and 2, respectively.

In Group B, the non-bleeding group, a cut-off EPO value of 30.65 mIU/mL had a sensitivity of 87.5% and specificity 57.4% in predicting lethal outcome with an AUROC of 0.823. For predicting lethal outcome in the bleeding group, 229.95 mIU/mL proved to be a cut-off value with a sensitivity and specificity of 53.3% and 92.7%, respectively. The AUROC for this cut-off was 0.847.

**Table II.** Mean values of Erythropoietin and prognostic scores, including type of Acute-on-Chronic liver failure at the time of admission in the study groups and controls

	Group A n=31	Group B n=73	Controls n=20	P value
EPO (mIU/mL)	293.62±282.76	41.59±50.91	14.27 ±13.45	<0.001
Prognostic scores				
CTP	10.74±2.02	11.16±1.72	5.25±0.55	0.279
MELD	20.29±5.83	22.37±6.68	9.80±1.20	0.135
MELD Na	22.19±5.97	24.58±6.58	10.45±1.23	0.086
SOFA	9.10±2.59	9.62±2.59		0.351
APACHE II	14.03±4.02	14.47±4.31		0.634
CLIF-SOFA	55.87 ±6.26	55.74 ±8.21		0.943
CLIF type				
I	6	13		
II	15	30		0.692
III	10	30		

APACHE II: Acute physiology and chronic health evaluation II; CLD: Chronic Liver Disease; CLIF-SOFA: Chronic Liver Failure Consortium-Sequential Organ Failure Assessment; CTP: Child-Turcotte-Pugh; EPO: Erythropoietin, MELD: Model for End Stage Liver Disease, MELD Na: Model for End Stage Liver Disease Sodium, SOFA: Sequential Organ Failure Assessment,

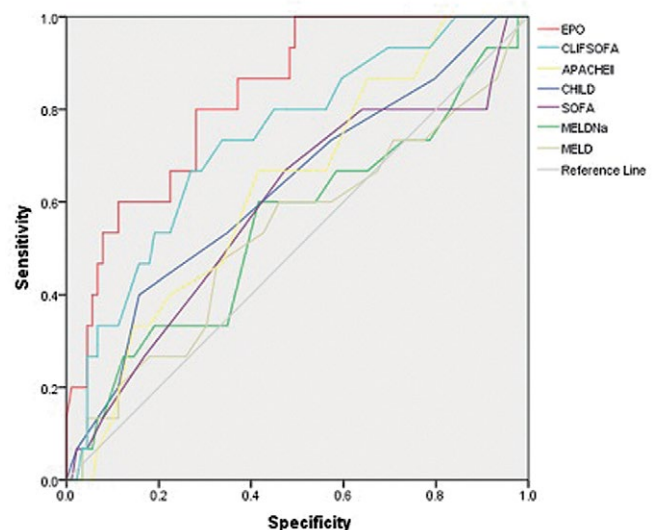
**Table III.** Mean values of Erythropoietin and prognostic scores at the time of admission in relation to outcome at 28 days

	Lethal outcome	Survivors	P value
EPO (mIU/mL)			
Group A	319.26 ±326.58	269.58±243.08	0.633
Group B	29.88±34.60	53.62±61.70	0.046
CTP			
Group A	11.8±1.86	9.75±1.65	0.003
Group B	11.68±1.76	10.64±1.51	0.009
MELD			
Group A	22.6±7.38	18.13±2.60	0.030
Group B	25.78±6.95	18.86±4.13	<0.001
MELD Na			
Group A	24.87±7.27	19.69±2.87	0.013
Group B	27.89±6.38	21.17±4.85	<0.001
SOFA			
Group A	9.93±2.84	8.31±2.12	0.081
Group B	10.19±1.97	9.03±3.02	0.055
APACHE II			
Group A	15.87±3.38	12.31±3.89	0.011
Group B	16.05±3.73	12.83±4.31	0.001
CLIF-SOFA			
Group A	61.80±8.07	50.31±6.54	<0.001
Group B	60.59±7.64	50.75±5.29	<0.001

See Table II for abbreviations.

## DISCUSSION

Of the 104 patients included, 50% had a lethal outcome, indicating that ACLF still carries a very high short-term mortality rate. This is higher than that seen in the CANONIC study where the 28-day mortality was 33.9% [21, 22] and may

**Fig. 1.** The area under the receiver operating characteristic curve for the following prognostic scores in patients with Acute-on-Chronic liver failure with bleeding as an insult. See Table II for abbreviations.

be explained by discrepancies in the quality of medical care. Determining prognosis at the time of presentation is therefore essential in the efficient management of these patients. Many studies have compared the various prognostic scores so as to determine which has the best predictive value [13, 23-27]. By dividing our cohort based on bleeding and non-bleeding type of insult, we were able to analyze the sensitivity and specificity of each of the prognostic scores in the context of insult type, something that we could not find in the available literature. The two groups had comparable scores. Based on our results, CLIF-SOFA was the best predictor of mortality in patients with GI bleeding, along with APACHE II. Conversely, in non-bleeding patients, MELD, MELD Na and CLIF-SOFA had similar predictive values, and were superior to APACHE

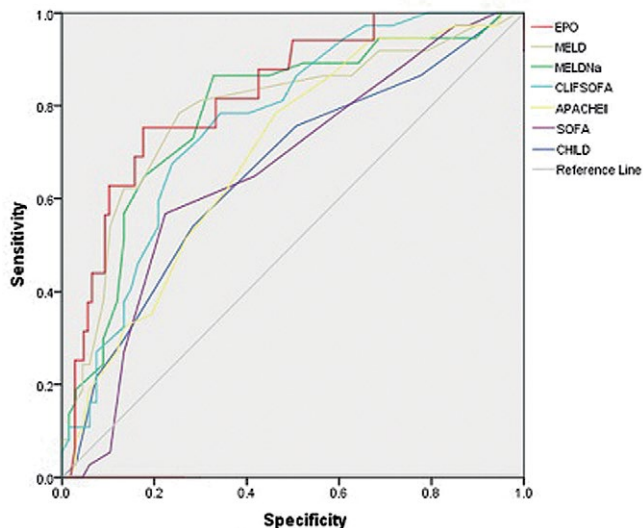


Fig. 2. The area under the receiver operating characteristic curve for the following prognostic scores in patients with acute-on-chronic liver failure with an insult other than bleeding. See Table II for abbreviations.

II, SOFA and CTP. Taking this into account, the best overall predictor was CLIF-SOFA. This confirms the results of recent studies [13, 24, 25]. Older studies [20, 26, 27] which did not include CLIF-SOFA in their analyses found APACHE II as the best predictor of prognosis. Recently, novel prognostic markers including sCD163 and sMR from activated macrophages have been investigated in ACLF, and were suggested to be included alongside current scores to improve the prediction of outcome

[28]. We hypothesized that EPO may also be a predictor of prognosis. Our results indicate that EPO predicts 28-day mortality with high accuracy in ACLF patients. Further studies in this area are needed.

It is well established, both in the literature and in clinical practice, that EPO is beneficial in the treatment of chronic renal and extra-renal anemia [15, 28], and of treatment-associated bone marrow suppression in patients receiving interferon and ribavirin for chronic hepatitis C infection [29]. It was proposed in animal studies that EPO improved overall hepatocyte regeneration and that it also improved survival in patients with fulminant hepatic failure [30, 31]. Only recently it became apparent that EPO therapy is associated with better survival at 12 months in patients with ACLF [32]. Prior to this, the RELIEF trial looked at the therapeutic value of molecular adsorbent recirculating system (MARS) in ACLF, but found no benefit to survival [33]. Bioartificial liver support devices have also been studied, and some have successfully bridged patients to transplant [34]. Granulocyte-colony stimulating factor (G-CSF) was next to be investigated, proving to have a positive effect on short-term mortality [8, 35].

The first double blind randomized control study examining the effects of EPO therapy in ACLF was performed by Kedarisetty et al., and showed that combination therapy with G-CSF and EPO (in the form of darbopoietin  $\alpha$ ) improved survival in patients with ACLF [32]. One limitation in this study was that the type of insult was not taken into account. We therefore looked to see which patients would potentially benefit most from EPO therapy based on type of insult and subsequent

Table IV. Prediction of outcome at 28 days based of Erythropoietin levels and prognostic scores at the time of admission

	AUROC	Sensitivity	Specificity	CI	P value
EPO (mIU/mL)					
Group A	0.847	53.3%	92.7%	0.757-0.935	<0.001
Group B	0.823	87.5%	57.4%	0.720-0.927	<0.001
CTP					
Group A	0.631	73.3%	42.7%	0.561-0.805	0.106
Group B	0.658	75.7%	50.7%		0.008
MELD					
Group A	0.534	60.0%	42.7%	0.714-0.923	0.677
Group B	0.787	81.1%	70.1%		<0.001
MELD Na					
Group A	0.549	66.7%	41.6%	0.714-0.918	0.548
Group B	0.785	86.5%	67.2%		<0.001
SOFA					
Group A	0.589	66.7%	52.8%	0.576-0.810	0.273
Group B	0.661	64.9%	58.2%		0.007
APACHE II					
Group A	0.632	66.7%	58.4%	0.611-0.835	0.103
Group B	0.695	86.5%	41.8%		0.001
CLIF-SOFA					
Group A	0.736	80.0%	55.1%	0.725-0.904	0.004
Group B	0.765	81.1%	52.2%		<0.001

AUROC: Area Under the Receiver Operating Curve, CI: Confidence Interval. See Table II for other abbreviations.

EPO levels. Previous studies have shown that patients with CLD have increased serum levels of EPO when compared to healthy controls, as was the case in our control group [36, 37]. Degrees of anemia and hepatic dysfunction were strongly correlated with increases in EPO levels. Secondary to chronic anemia, acute bleeding as in the case of variceal hemorrhage, is associated with upregulation of EPO [38]. This was the reasoning behind dividing our cohort based on bleeding versus non-bleeding type of insult. Erythropoietin upregulation resulting from GI hemorrhage held true in our study, where all patients with a bleeding insult had higher levels of EPO at admission, compared to non-bleeding patients. This is expected given the physiological effects of EPO, where acute blood loss in both human studies and animal models demonstrates an increase in EPO production beginning approximately 6 hours after the start of blood loss, and persisting for 24-48 hours thereafter [14-16].

Supporting the results of other studies, we found that patients with a bleeding insult also had lower organ failure scores, however, without statistical significance [39, 40]. Interestingly, Kedarisetty et al. found that G-CSF and EPO combination therapy reduced the incidence of septic shock in their cohort when compared to placebo (6.9% vs 38.5%,  $p=0.005$ ) [32]. We can support this finding partially, as non-bleeding patients in our study had significantly lower EPO values at admission, and a high incidence of infection. Paradoxically, our patients with a bleeding insult and a subsequent lethal outcome at 28 days had the highest levels of EPO. This indicates that a lethal outcome had a stronger correlation with the severity of bleeding than with the degree of ACLF, and also that EPO therapy and its effect on mortality in patients with already elevated EPO levels needs to be further studied. Probably other treatment options are required in this subset of patients.

## CONCLUSION

Based on our findings we can conclude that patients with low levels of EPO at the time of admission, that is, those with an insult other than bleeding, would potentially benefit most from EPO therapy. Also, the prognosis of patients with ACLF may be predicted by EPO levels at the time of admission. Further studies are required to correlate these results.

**Conflicts of interest:** No conflicts to declare.

**Authors' contribution:** T.A. was responsible for the study design, writing, and data analysis. S.Z. wrote the paper and analyzed the data. V.N. collected data, and along with A.V., performed statistical analyses. V.M., V.D. and S.S. contributed to data collection and analysis. T.M. contributed to the study design and gave critical review during the writing of the paper.

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