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EASL CLIF Consortium, European Foundation for the Study of Chronic Liver Failure (EF Clif)

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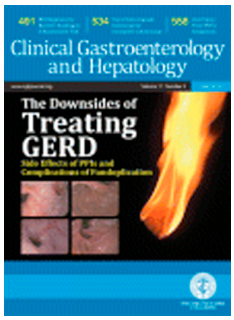

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Title: Association Between Grade of Acute on Chronic Liver Failure and Response to Terlipressin and Albumin in Patients With Hepatorenal Syndrome

Short title: ACLF grade affects terlipressin efficacy in HRS

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List of abbreviations

ACLF, acute-on-chronic liver failure; EASL-CLIF, European Association for the Study of the Liver Chronic Liver Failure consortium; HRS, hepatorenal syndrome; ICA, International Club of Ascites; LT, liver transplantation; MELD, model of end stage liver disease; sCr, serum creatinine.

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Disclosures

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Author Contributions

SP: study design, collection of data, interpretation of data, drafting of the manuscript

HS: study supervision, collection of data, drafting of the manuscript, critical revision for important intellectual content

AA: statistical analysis

XA, AR, AH, ES, MT, MM, BS, CS: collection of data

AG, MB, CA, JT, TG, FN: collection of data, critical revision for important intellectual content

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Abstract

Background & Aims: Type-1 hepatorenal syndrome (HRS) is the most high-risk type of renal failure in patients with cirrhosis. Terlipressin and albumin are effective treatments for type-1 HRS. However, the effects of acute on chronic liver failure (ACLF) grade on response to treatment are not clear. We aimed to identify factors associated with response to treatment with terlipressin and albumin in patients with type 1 HRS (reduction in serum level of creatinine to below 1.5 mg/dl at the end of treatment) and factors associated with death within 90 days of HRS diagnosis (90-day mortality).

Methods: We performed a retrospective analysis of 4 different cohorts of consecutive patients with HRS treated with terlipressin and albumin from February 2007 through January 2016 at medical centers in Europe (total, 298 patients). We analyzed demographic, clinical, and laboratory data collected before and during treatment; patients were followed until death, liver transplantation, or 90 days after HRS diagnosis.

Results: Response to treatment was observed in 53% of patients. Of patients with grade 1 ACLF, 60% responded to treatment; among those with grade 2 ACLF, 48% responded, and among those with grade 3 ACLF, 29% responded ($P<.001$ for comparison between grades). In multivariate analysis, baseline serum level of creatinine (odds ratio, 0.23; $P=.001$) and ACLF grade (odds ratio, 0.63; $P=.01$) were independently associated with response to treatment. Patient age (hazard ratio [HR], 1.05; $P<.001$), white blood cell count (HR, 1.51; $P=.006$), ACLF grade (HR, 2.06; $P<.001$), and no response to treatment (HR, 0.41; $P<.001$) associated with 90-day mortality.

Conclusion: In a retrospective analysis of data from 4 cohorts of patients treated for type-1 HRS, we found ACLF grade to be the largest determinant of response to terlipressin and albumin. ACLF grade affects survival independently of response to treatment. New therapeutic strategies should be developed for patients with type-1 HRS and extrarenal organ failure.

Keywords: Acute kidney injury; cirrhosis; liver transplantation; hepatorenal syndrome.

Introduction

Type-1 hepatorenal syndrome (HRS) is one of the most life-threatening complications in patients with decompensated cirrhosis[1]. It is characterized by a rapidly progressive acute renal failure, frequently triggered by bacterial infections or other precipitating events, and is associated with very high short-term mortality rate.

The pharmacological treatment with terlipressin (or noradrenaline) and albumin is the most effective treatment of type-1 HRS[2–5]. It was introduced in the 1990's, soon after the formulation of the peripheral arterial vasodilation hypothesis, which proposed HRS as the extreme expression of a severe splanchnic arterial vasodilation and impairment in circulatory function[6]. Subsequent studies in patients with spontaneous bacterial peritonitis (SBP) revealed that HRS occurs in the setting of marked reduction in cardiac output proving for the first time that HRS results from a more complex pathogenesis[7]. In addition to arterial vasodilation, left ventricular dysfunction is also a major contributor. Therefore, resolution of HRS after pharmacological treatment was proposed to be due to a combined effect of terlipressin on systemic vascular resistance and of albumin on effective circulating blood volume and cardiac output. This strategy enables a resolution of HRS in 40-70% of patients. Important predictors of complete response and mortality seem to be serum bilirubin, serum creatinine (sCr) concentration and the early response to treatment[8,9].

The characterization of Acute-on-Chronic Liver Failure (ACLF) by the Canonic study in 2013, has deeply modified our concepts on the pathogenesis of HRS[10]. ACLF is a syndrome defined by acute decompensation of cirrhosis, single or multiple (2 or more) organ failures, severe systemic inflammation and very high short-term mortality rate[10].Type-1 HRS is, therefore, a special form of ACLF. Patients with decompensated cirrhosis without ACLF present severe chronic systemic inflammation probably as

consequence of continuous translocation of bacteria and bacterial products from gut microbiota to the systemic circulation[11]. ACLF develops when there is a further acute increase in systemic inflammation in response to pro-inflammatory triggers (i.e. bacterial infections, acute liver injury or, in patients without identifiable triggers, probably an acute burst of bacterial translocation). The current belief is that systemic inflammation is the common mechanism of acute decompensation and organ dysfunction/failure(s) in patients with decompensated cirrhosis[11]. As such, a higher degree of systemic inflammation was associated with a higher number of organ failures and, thus, with a higher grade of ACLF[10,12]. This profound change of the pathophysiological background may affect the efficacy of the treatment of type-1 HRS by terlipressin plus albumin in patients with ACLF. However, the potential negative impact of the degree of ACLF on the response to the treatment with terlipressin and albumin in patients with type-1 HRS has been evaluated only in a very small series of patients with sepsis induced HRS[13].

The aim of the current study was, therefore, to assess the impact of severity of ACLF on the renal response to terlipressin and albumin in patients with HRS. Secondary aim of the study was to assess predictors of 90-day mortality

Patients and methods

Patients

The study includes four different cohorts of consecutive patients with HRS treated with terlipressin and albumin. Some patients were included in prospective investigations already published[4,5,10,13]. The first cohort includes patients evaluated from February 2007 to January 2016 at the University Hospital of Padova (Italy), the second cohort includes patients evaluated from July 2009 to December 2015 at the Liver Unit,

Hospital Clinic of Barcelona (Spain), the third cohort includes patients recruited from February to September 2011 in the Canonic study and the fourth cohort included patients treated from 2010 to 2015 at the University Hospital of Munster (Germany). The protocol was approved by the local ethics committee at each center.

Inclusion criteria were: a) diagnosis of cirrhosis according to histological, clinical, biochemical, ultrasonographic and/or endoscopic findings; b) diagnosis of type-1 HRS according to the International Club of Ascites (ICA) criteria[14]; c) age ≥18 years old. Exclusion criteria were: a) hepatocellular carcinoma beyond Milan criteria; b) extrahepatic malignancy; c) septic and/or hypovolemic shock; d) proteinuria>500 mg/24 hours; e) hematuria>50 red blood cells per high-resolution field; f) abnormal renal ultrasound; g) severe extrahepatic disease (congestive heart failure stage NYHA≥2, chronic obstructive pulmonary disease stage GOLD≥2); h) Treatment of HRS with midodrine, octreotide and albumin or norepinephrine and albumin; h) previous liver transplant.

Definitions

Type-1 HRS was defined according to ICA criteria[14]: a) an increase in sCr of at least 100% in the previous 14 days up to values above 2.5 mg/dl in a patient with cirrhosis and ascites; b) no response (sCr>2 mg/dl) after diuretic withdrawal and albumin expansion at the dose of 1gr/kg/day for 2 days; c) absence of signs of parenchymal kidney damage (24-hour proteinuria<500 mg; red blood cells in urine<50 per high resolution field; normal renal ultrasonography); d) absence of shock and/or treatment with nephrotoxic drugs (i.e. iodine contrast media, non-steroidal anti-inflammatory drugs, aminoglycosides).

Resolution of HRS (or response to treatment) was defined as a reduction of sCr to <1.5 mg/dl during treatment.

ACLF was defined according to the European Association for the Study of the Liver-Chronic Liver Failure (EASL-CLIF) consortium[10]. Extrahepatic organ failures were defined by the CLIF organ failure score, which is a simplified version of the original CLIF-SOFA score[15]. More in detail, liver failure was defined as a bilirubin ≥12 mg/dl; coagulation failure as a INR ≥2.5; brain failure as hepatic encephalopathy grade ≥3 (West Haven criteria); respiratory failure as a SpO2/FiO2 ratio≤214 or a PaO2/FiO2 ratio≤200 or the need for mechanical ventilation; circulatory failure is defined by as the need for vasopressor therapy to achieve an adequate mean arterial pressure.

ACLF was graded as follows: ACLF-1: HRS alone; ACLF-2: HRS and 1 extra-renal organ failure; ACLF-3: HRS and 2 or more extra-renal organ failures. Therefore, by definition, all patients with type-1 HRS included had at least ACLF-1.

Design of the study

Terlipressin was administered at a starting dose of 2 to 3 mg/day administered as intravenous boluses (0.5 mg every 4-6 hours) or continuous i.v. infusion. The dose of terlipressin was increased in a stepwise manner up to a maximum of 12 mg/day in patients without early response to treatment (decrease of sCr to more than 25% of pretreatment value after 48-72 of treatment). In patients developing severe side effects potentially related to terlipressin the drug was withdrawn. Albumin was administered at a loading dose of 1 g/kg/day (corresponding to the second day of differential diagnosis of HRS) and 20-40 gr/day the subsequent days. The treatment was continued until resolution of HRS (sCr<1.5 mg/dl) and/or the appearance of severe side effects

(pulmonary edema, cardiac arrhythmia or severe ischemic complications) up to a maximum of 15 days.

Non-responders to treatment were managed according to standard of care. Renal replacement therapy was considered an option when indicated only in patients who were candidates to liver transplantation (LT).

Clinical and laboratory characteristics were collected on the day of first administration of terlipressin and albumin (baseline data), on the 3rd day of treatment and at the end of treatment. Patients were followed up until death, LT or for a maximum of 90 days.

Statistical analysis

Results are presented as frequencies and percentages for categorical variables, means and SDs for normally distributed continuous variables and median and interquartile range for not normally distributed continuous variables. Not normally distributed variables were log-transformed when necessary. In univariate analyses, Chi-square test was used for categorical variables, Student's t-test or ANOVA for normal continuous variables and Mann-Whitney or Kruskal Wallis test for not normally distributed continuous variables. To assess the response to terlipressin and albumin treatment, logistic regression models were carried out. Factors showing a clinically and statistically significant association to the outcome in univariate analyses were selected for the initial model. The final models were fitted by using a step-wise forward method based on Likelihood Ratios with the same significance level ($p<0.05$) for entering and dropping variables. Using the final model an estimated probability of response after treatment was calculated. The proportional-hazards model for Competing-Risks proposed by Fine and Gray[16] was used to assess the presence of independent factors of mortality. This model was chosen in order to account for LT as an event 'competing'

with mortality. In all statistical analyses, significance was set at $p<0.05$. Analyses were done with SPSS (version 23.0; SPSS, Inc. Chicago, IL) and SAS (version 9.4; SAS Institute Inc.; Cary, NC) statistical packages.

Results

Study population

Two-hundred and ninety-eight patients with cirrhosis and HRS were included in the study. Supplementary Table 1 illustrates the clinical characteristics of the patients. HRS was associated with bacterial infections in 169 patients (68 with SBP). The most common extra-renal organ failure was liver failure (27%), followed by coagulation failure (13%), brain failure (7%), and respiratory failure (2%). One-hundred and seventy-nine patients (60%) had ACLF 1, 91 (31%) ACLF 2, and 28 (9%) ACLF 3. The characteristics of the 4 cohorts of patients studied are shown in Supplementary Table 2.

Resolution of HRS

Patients were treated for a median of 8 days and the median cumulative dose of terlipressin was 24 mg (IQR=14–42 mg). Resolution of HRS was observed in 53% of the patients. Responders showed significantly lower baseline serum bilirubin (3.3 vs 4.8 mg/dl; $p=0.003$), INR (1.6 vs 1.8; $p=0.004$) and sCr (2.7 vs 3.2 mg/dl; $p<0.001$) than non-responders (Table 1). Response rate showed a stepwise decrease from grade 1 to grade 3 ACLF (60, 48 and 29% for ACLF 1, ACLF 2 and grade ACLF 3, respectively; $p<0.001$).

Mean arterial pressure increased significantly during treatment (79 vs 86 mmHg at day 3; $p<0.001$), but no difference was found in delta increase of MAP at day 3 between responders and non-responders. Unexpectedly, the delta MAP at day 3 increased in a

stepwise fashion from grade 1 to grade 3 ACLF (5, 6 and 13 mmHg, respectively; p=0.014). The increase in MAP was retained in responders while MAP dropped in non-responders at the end of treatment. The delta decrease in sCr at day 3 was significantly higher in responders than in non-responders (Table 1).

Complete sequential data on serum albumin concentration were available in 175 patients. Although serum albumin increased significantly (p<0.001), the median delta increase was very small (0.2 g/dl) (Supplementary Figure 1) and the delta increase of albumin on day 3 was not significantly different between responders and non-responders.

In the multivariate analysis, only baseline sCr (OR=0.23; p=0.001) and ACLF grade (OR=0.63; p=0.01) were found to be independent predictors of response to treatment. Figure 1 shows the expected probability of HRS resolution according to baseline sCr concentration and ACLF grade. The probability of HRS resolution decreased stepwise from ACLF-1 to ACLF-3 at any level of sCr. According to the final model, the probability of response to terlipressin plus albumin can be estimated by the following equation:

$$P = 1/(1 + e^{-(2.114 - 0.367*\text{sCr} - 0.549*\text{ACLF grade})})$$

We also explored predictors of response to treatment using variable available on day 3. Again, ACLF grade on day 3 (OR=0.55; 95% CI=0.35-0.86; p=0.009) and the delta sCr (OR=0.43; 95% CI=0.28-0.67; p<0.001) were found to be the only independent predictors of response to terlipressin and albumin. Interestingly, the expected probability of HRS resolution was very low (≈10%) in patients with ACLF grade 3 and no improvement in sCr (Supplementary Figure 2).

Survival

During the 90-day follow up 119 patients died (40%), 50 were transplanted (17%), 123 completed follow up (41%) and 6 patients were lost to follow up (2%). The cumulative incidence of 90-day mortality was 41%. As expected, non-survivors were older and had a significant worse liver function than survivors (Table 2). Scores of liver disease such as MELD, Child Pugh and CLIF-C ACLF score were significantly higher and serum sodium was significant lower in non-survivors than in survivors. There was a non-significant trend towards a higher white blood cells count in non-survivors than in survivors. The cumulative incidence of 90-day mortality increased in a stepwise fashion according to ACLF grade (30, 50 and 79% for ACLF 1, ACLF 2 and ACLF 3, respectively; p<0.001) (Figure 2A). In univariate analysis, response to treatment was also a main determinant of survival. Indeed, the cumulative incidence of mortality was significantly lower in responders than in non-responders (28 vs 57%; p<0.001) (Figure 2B). More in detail, in patients with ACLF grade 1 and 2, responders had a significant decrease in 90-day mortality than non-responders (20 vs 47%, p<0.001; 40 vs 60%, p=0.018; for grade 1 and 2, respectively), while no difference was observed in patients with ACLF grade 3 (71 vs 80%; p=0.201). However, a trend towards an improvement in 28-day mortality was observed in responders vs non-responders with ACLF grade 3 (43 vs 80%; p=0.063). Interestingly, mortality rate increased according to ACLF grade both in responders and non-responders (Figure 3 panel A and panel B).

In multivariate analysis, age [HR (95% CI): 1.05 (1.03-1.07); p<0.001], white blood cell count [1.51 (1.12-2.02); p=0.006], ACLF grade [2.06 (1.54-2.75); p<0.001] and response to treatment (yes/no)[0.41 (0.29-0.60); p<0.001] were found to be independent predictors of 90-day mortality. When CLIF-C ACLF score was included in the model, it was found to be an independent predictor of survival (HR=1.09; p<0.001) together with the response to treatment (HR=0.44; p<0.001).

Side effects

Detailed information about side effects were available in 241 patients (Table 3). One-hundred and ten patients (46%) developed side effects likely related to treatment with terlipressin and albumin. The most common side effects were diarrhea and abdominal pain, followed by intestinal ischemia, peripheral ischemia, pulmonary edema and angina. The treatment with terlipressin and albumin was withheld in 48 patients (20%).

Discussion

Our study, which represents the largest investigation on the pharmacological treatment of HRS, confirms the two major findings so far described. The first is that the combination of terlipressin and albumin is effective resolving HRS in approximately 50% of patients. The second is that baseline sCr and/or early improvement in sCr are accurate predictor of treatment response and patient survival. It has been proposed that once severe HRS develops, ischemic tubular lesions and/or intrarenal pathways worsening renal hypo-perfusion (i.e. imbalance between the intrarenal synthesis vasoconstrictor and vasodilator molecules) maintain or worsen renal failure independently of potential positive effects of treatment in cardiovascular function[17]. On this basis, current clinical guidelines propose that early detection and treatment of HRS would increase the rate of treatment response and survival in patients with ongoing HRS[18].

The most outstanding observation of our study is that severity of ACLF, as estimated by the number of extra-renal organ failures, is an additional important factor predicting the pharmacological treatment response in patients with HRS. The rate of resolution of HRS was 60% in patients without extra-renal organ failure (ACLF-1), of 48% in

patients with 1 extra-renal organ failure (ACLF-2), and of only 29% in patients with 2 or more extra-renal organ failures (ACLF-3).

Beyond the hypothesis that cholestasis may be responsible for a direct tubular damage in patients with liver failure[19], a recent study by Clària et al.is of special relevance for the interpretation of these data[12]. They measured the plasma levels of cytokines and chemokines as markers of systemic inflammation and the plasma concentration of renin and copeptin (a precursor of antidiuretic hormone) as markers of systemic circulatory dysfunction in a large series of patients with decompensated cirrhosis with and without ACLF. There was a significant increase in the plasma levels in both types of biomarkers in patients with ACLF as compared with patients without ACLF[12]. However, the strength of the association of ACLF with biomarkers of systemic inflammation was significantly higher. Furthermore, the plasma cytokine levels, but neither renin nor copeptin, increased stepwise with the grade of ACLF. Finally, the clinical course (resolution, improvement, steady course or worsening of ACLF) correlated significantly with systemic inflammation but not with circulatory dysfunction[12]. Such associations were also observed when HRS instead of ACLF was considered.

The authors' interpretation of these findings was that, although both systemic inflammation and systemic circulatory dysfunction participates in the pathogenesis of ACLF (and HRS), systemic inflammation is the main driver of the syndrome. It is well known that in addition of reducing organ perfusion as consequence of arterial vasodilation and impaired left ventricular function, systemic inflammation can impair renal and extra-renal organ function through direct deleterious effects of inflammatory mediators on essential tissue and cell homeostatic mechanisms, including local microcirculation, mitochondrial function and apoptosis[20].

Our results suggest that this pathophysiological interpretation may also offer a rational explanation for our findings. In patients with HRS and ACLF-1, who have moderate increase in systemic inflammation over the chronic inflammation of decompensated cirrhosis, cardio-circulatory dysfunction and renal hypo-perfusion may have a significant role in ACLF development and, therefore, they are more likely to respond to terlipressin and albumin. In contrast, in patients with ACLF-2 and particularly with ACLF-3, in whom the increase in systemic inflammation is more intense, the predominant mechanism would be a generalized increase in microcirculatory and mitochondrial function and cell death, disorders that cannot be sufficiently reversed by improving systemic circulatory function. Indeed, despite the increase in mean arterial pressure on day 3 was progressively higher moving from patients with ACLF-1 to those with ACLF-3, the rate of HRS reversal decreased.

The combination of the two major predictors of response into two prognostic diagrams (Figure 1 and Supplementary Figure 2), disclosed the second important finding of our study. It showed that severity of ACLF influences treatment response independently on whether sCr was moderately or severely increased, thus supporting the important role played by the pathophysiological process leading to ACLF on the effectiveness of terlipressin and albumin treatment. These data as well as the effects of HRS reversal on survival may have important implications for clinical practice, in particular in patients with ACLF grade-3. In fact, the reversal of HRS had a modest short-term survival benefit in these patients. Thus, it can be relevant for patients who are eligible to liver transplantation while it may be pointless in those who are not. Therefore, the decision to treat or not a patient with ACLF grade 3 who is not eligible to liver transplantation should be individualized taking into account several factors, including the patient's will.

As in previous studies, early renal response to treatment (delta sCr concentration at day 3) was an accurate predictor of HRS resolution. We, however, did neither observe significant differences between changes in delta mean arterial pressure at day 3 nor in delta serum albumin concentration at day 3 and at the end of treatment in patients who responded and who did not respond to treatment. Another interesting observation was that the increase of serum albumin concentration at the end of treatment was minor in the two groups despite of a significant administration of exogenous albumin (an initial dose of 1 g per Kg of body weight, followed by 20-40/g/day during treatment). Therefore, it is very likely that the current pharmacological treatment of HRS is underpowered in relation with the albumin dosage, which, in contrast to terlipressin, is not adjusted to response.

As expected and confirming previous studies, age and response to treatment were independent predictors of survival in patients with HRS[2,4,5]. Other predictors were the baseline white blood cells count, which is a sensitive marker of systemic inflammation in patients with ACLF, and the ACLF grade. Interestingly, mortality rate increased from ACLF-1 to ACLF-3 in patients responding and not responding to treatment, indicating that severity of ACLF predict survival more accurately than treatment response[21]. Remarkably, patients with ACLF grade 2 and 3 have a very detrimental prognosis even if they respond to terlipressin and albumin, highlighting the need to develop new treatments for these patients.

In summary, severity of renal and of ACLF grade are independent predictors of therapeutic response to terlipressin and albumin in patients with HRS. Age, baseline white blood cells count, baseline ACLF grade, and therapeutic response are independent predictors of survival. These data indicate that the grade of systemic inflammation and the presence and number of extra-renal organ failure(s) have a great impact in the

clinical course in patients admitted with HRS. New treatments should be explored for patients with type-1 HRS looking at systemic inflammation as a potential target.

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Figure Legend

Figure 1. Probability of response to treatment with terlipressin and albumin according to baseline serum creatinine in patients with ACLF grade 1 (green line), grade 2 (yellow line) and grade 3 (red line).

Legend: ACLF, acute-on-chronic liver failure.

Figure 2. Cumulative incidence of mortality at 90-day according to ACLF grade (Panel A) and response to treatment with terlipressin and albumin (panel B).

Legend: ACLF, acute-on-chronic liver failure.

Figure 3. Cumulative incidence of mortality at 90-day according to ACLF grade in responders (Panel A) and nonresponders (panel B) to treatment with terlipressin and albumin.

Legend: ACLF, acute-on-chronic liver failure.

Table 1. Characteristics of responders versus non-responders to treatment with terlipressin and albumin

	Non-responders (N=139)	Responders (N= 159)	P-value
Age (years) – m ±SD	58±9	57±10	0.403
Gender (Male)– n (%)	103 (74.1)	103 (64.8)	0.082
Clinical Features			
Spontaneous bacterial peritonitis – n (%)	21 (16.9)	47 (32.9)	0.003
Other infection– n (%)	43 (32.1)	58 (40.0)	0.170
Gastrointestinal bleeding– n (%)	13 (9.7)	32 (22.1)	0.005
Tense/large ascites – n (%)	75 (57.7)	104 (67.5)	0.087
Hepatic encephalopathy – n (%)	58 (43.3)	86 (54.1)	0.065
Mean arterial pressure (mmHg) – m ± SD	78±10	79±14	0.455
Delta MAP on day 3 (mmHg) – m ± SD	5±12	7±10	0.316
MAP at end of treatment (mmHg) – m ± SD	81±13	86±13	0.003
Heart Rate (bpm) – m ± SD	75±12	76±14	0.355
Laboratory data			
Leukocyte count (x 10^9/L)– M (IQR)	8.2 (4.7-12.5)	8.2 (5.6-11.6)	0.723
Platelet count (x 10^9/L)– M (IQR)	81 (48-115)	104 (65-167)	<0.001
Bilirubin (mg/dl) – M (IQR)	4.8 (2.2-20.1)	3.3 (1.5-8.4)	0.003
Albumin (g/dl) – M (IQR)	3.0 (2.7-3.4)	3.0 (2.6-3.4)	0.353
Delta albumin on day 3 (g/dl) – M (IQR)	0.1 (-0.1 – 0.5)	0.3 (0 – 0.6)	0.113
International normalized ratio– M (IQR)	1.8 (1.5-2.2)	1.6 (1.4-1.9)	0.004
Serum creatinine (mg/dl) – M (IQR)	3.2 (2.6-3.8)	2.7 (2.4-3.4)	<0.001
Delta creatinine on day 3 (mg/dl)– M (IQR)	-0.4 (-0.9-0.1)	-1.0 (-1.6/-0.7)	<0.001
BUN (mg/dl) – M (IQR)	74 (56-97)	57 (45-76)	<0.001
Serum sodium (mmol/L) – m ± SD	133±6	132±7	0.666
Organ Failures– n (%)			
Liver	49 (35.3)	31 (19.5)	0.002
Kidney	139 (100.0)	159 (100.0)	-
Brain	9 (6.5)	13 (8.2)	0.575
Coagulation	26 (18.7)	14 (8.8)	0.012
Circulation	0 (0.0)	0 (0.0)	-
Respiratory	4 (2.9)	2 (1.3)	0.321
Number of organ failures – M (IQR)	1 (1-2)	1 (1-2)	0.002
ACLF Grade – n (%)			0.005

Grade I	72 (51.8)	107 (67.3)	
Grade II	47 (33.8)	44 (27.7)	
Grade III	20 (14.4)	8 (5.0)	
Scores			
MELD– m ± SD	30±7	27±6	<0.001
MELD-Na– m ± SD	32±6	29±6	<0.001
Child-Pugh– m ± SD	10.2±1.9	10.5±2.0	0.240
CLIF-C OF– m ± SD	9±2	9±1	0.005
CLIF-C ACLF– m ± SD	47±8	45±7	0.050

Legend: n, number; m, mean; SD, standard deviation; M, median; IQR, interquartile range MAP, mean arterial pressure; ACLF, acute-on-chronic liver failure; MELD, model of end stage liver disease; CLIF-C OF, CLIF Consortium organ failure score; CLIF-C ACLF, CLIF consortium acute-on-chronic liver failure score.

Table 2. Comparison of characteristics of patients who survived and those who died during the 90-day follow up*

Variables	Survivors (N=123)	Dead (N=119)	P-value
Age (years) – m ±SD	56±9	60±10	0.002
Gender (Male)– n (%)	84 (68.3)	82 (68.9)	0.918
Clinical Features	82 (68.3)	88 (74.6)	0.287
Spontaneous bacterial peritonitis – n (%)	32 (29.9)	27 (24.3)	0.354
Other infection– n (%)	32 (28.6)	50 (43.1)	0.022
Gastrointestinal bleeding– n (%)	22 (19.6)	16 (13.8)	0.236
Tense/large ascites – n (%)	84 (70.6)	64 (58.2)	0.050
Hepatic encephalopathy – n (%)	56 (45.5)	63 (55.3)	0.134
Mean arterial pressure (mmHg) – m ± SD	79±14	78±12	0.477
Heart Rate (bpm) – m ± SD	76±12	77±15	0.529
Laboratory data			
Leukocyte count(x 10^9/L)– M (IQR)	7.8 (5.2-11.6)	8.8 (5.6-13.8)	0.095
Platelet count (x 10^9/L)– M (IQR)	116 (64-181)	83 (53-120)	<0.001
Serum bilirubin (mg/dl) – M (IQR)	3.2 (1.4-6.0)	6.7 (2.4-20.0)	<0.001
Serum albumin (g/dl) – M (IQR)	3.1 (2.7-3.5)	2.9 (2.6-3.3)	0.153
International normalized ratio– M (IQR)	1.5 (1.3-1.8)	1.8 (1.5-2.4)	<0.001
Serum creatinine (mg/dl) – M (IQR)	2.8 (2.5-3.7)	3.0 (2.5-3.6)	0.547
BUN (mg/dl) – M (IQR)	59 (48-79)	70 (48-89)	0.079
Serum sodium (mmol/L) – m ± SD	134±6	132±7	0.031
Organ Failures– n (%)			
Liver	19 (15.5)	47 (39.5)	<0.001
Kidney	123 (100.0)	119 (100.0)	-
Brain	10 (8.1)	9 (7.6)	0.870
Coagulation	5 (4.1)	28 (23.5)	<0.001
Circulation	0 (0.0)	0 (0.0)	-
Respiratory	0 (0.0)	5 (4.2)	0.022
Number of organ failures – M (IQR)	1 (1-2)	2 (1-2)	<0.001
ACLF Grade – n (%)			
Grade I	92 (74.8)	52 (43.7)	<0.001
Grade II	28 (22.8)	46 (38.7)	
Grade III	3 (2.4)	21 (17.7)	
Scores			
MELD– m ± SD	27±6	31±7	<0.001

MELD-Na– m ± SD	29±6	33±6	<0.001
Child-Pugh– m ± SD	9.9±1.9	10.7±2.0	0.017
CLIF-C OF– m ± SD	9±1	10±2	<0.001
CLIF-C ACLF– m ± SD	44±6	50±8	<0.001
Response to treatment – n (%)			
No response	35 (28.5)	75 (63)	<0.001
Complete response	88 (71.5)	44 (37.0)	

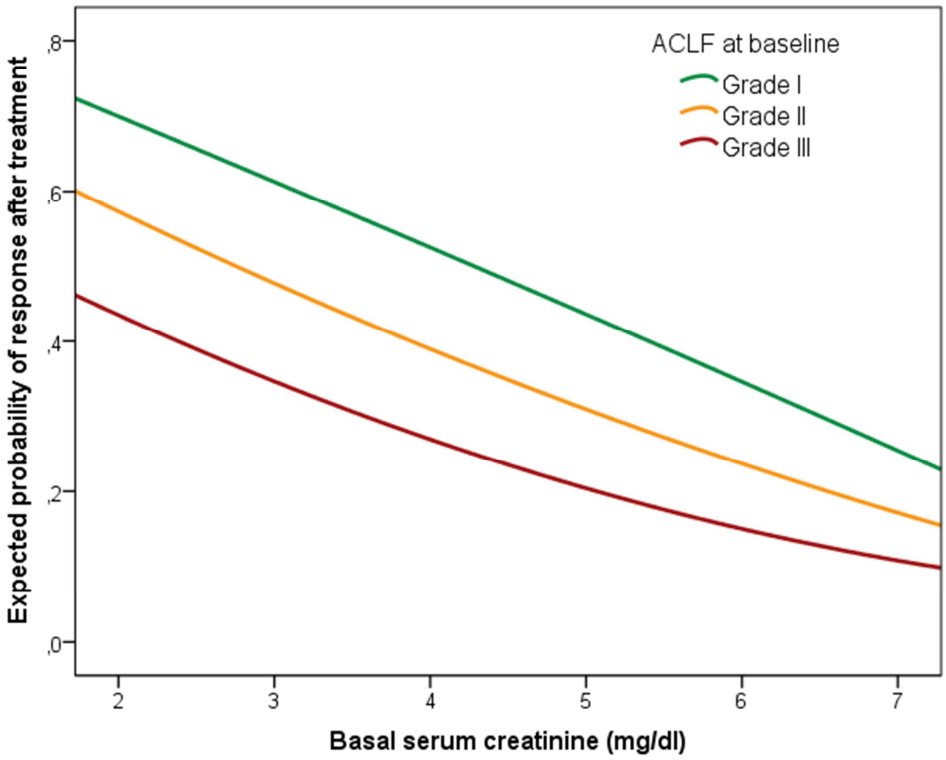
*Legend***:** n, number; m, mean; SD, standard deviation; M, median; IQR, interquartile range ACLF, acute-on-chronic liver failure; MELD, model of end stage liver disease; CLIF-C OF, CLIF Consortium organ failure score; CLIF-C ACLF, CLIF consortium acute-on-chronic liver failure score.

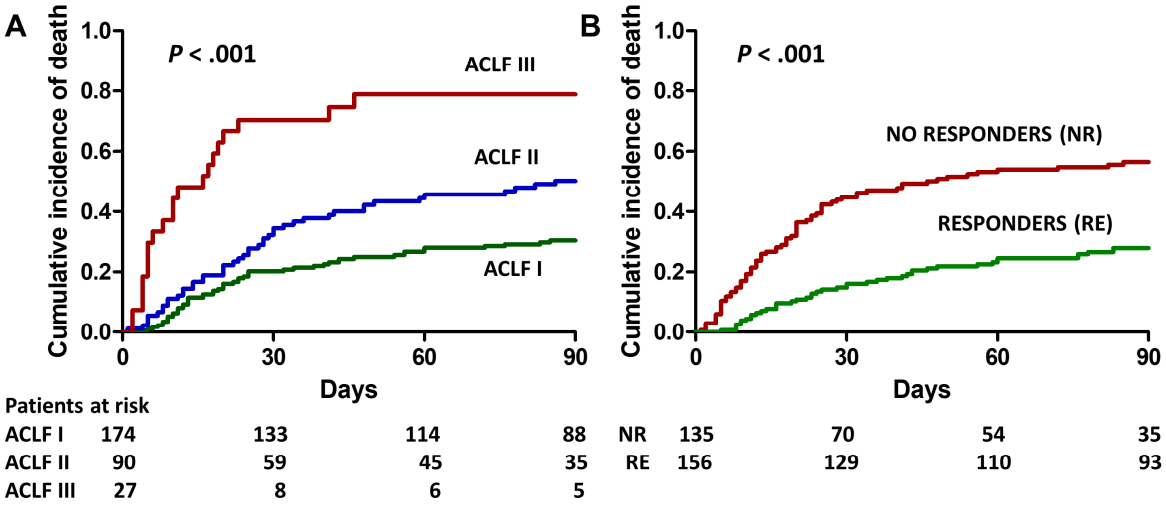
*patients transplanted (N=50) or lost to follow up (N=6) were excluded from this analysis.

Table 3. Side effects of treatment with terlipressin and albumin.

	N=241*
Side effects – n (%)	110(45.6)
Angina	14(5.8)
Peripheral ischemia	22(9.3)
Circulatory overload	20(8.3)
Diarrhea	59(24.6)
Intestinal ischemia	22(9.1)
Abdominal pain	49(20.3)
Hyponatremia#	14 (7.1)
Other	11(4.6)
Withdrawal – n (%)	48(19.9)
Angina	4(8.3)
Peripheral ischemia	3(6.3)
Circulatory overload	8(16.7)
Diarrhea	5(10.4)
Intestinal ischemia	5(10.4)
Abdominal pain	4(8.3)
Other	1(2.1)
Combination	18(37.5)

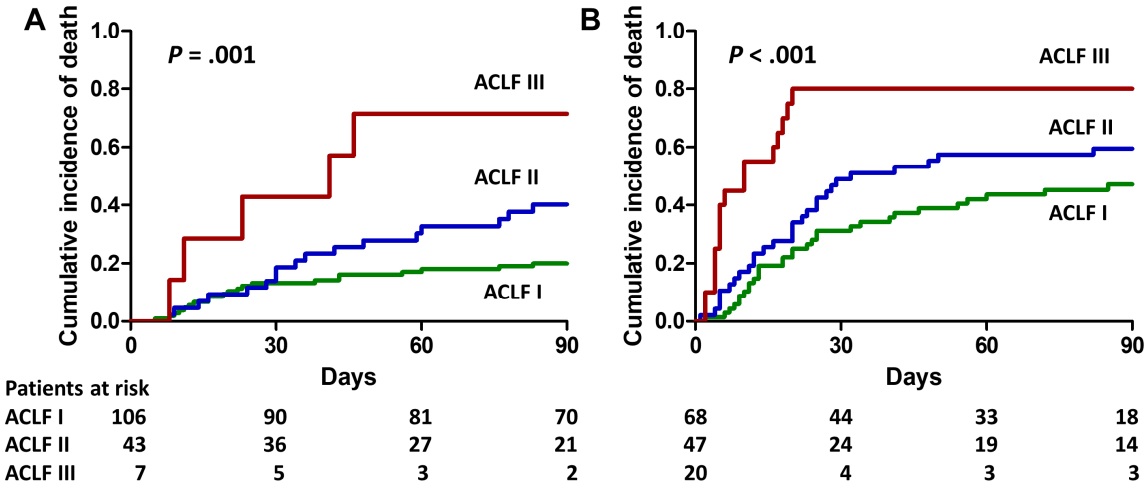
Legend: n, number; *, 57 patients had no information about side effects; #, defined as serum sodium<130 mmol/L in patients with baseline sodium≥130 mmol/L (196 patients). 3 patients developed severe hyponatremia (serum Na<125 mmol/L)


ACLF at baseline
Grade I
Grade II
Grade III
Expected probability of response after treatment
,8
,6
,4
,2
,0
2
3
4
5
6
7
Basal serum creatinine (mg/dl)



Patients at risk				
ACLF I	174	133	114	88
ACLF II	90	59	45	35
ACLF III	27	8	6	5

NR	135	70	54	35
RE	156	129	110	93



Supplementary Table 1. Demographic, clinical and laboratory characteristics of patients.

Variables	**(N=298)**
Age (years) – m ±SD	58±10
Gender (Male)– n (%)	206(69.1)
Clinical Features	
Spontaneous bacterial peritonitis – n (%)	68(25.5)
Other infection– n (%)	101(36.2)
Gastrointestinal bleeding– n (%)	45(16.1)
Tense/large ascites– n (%)	179(63.0)
Hepatic encephalopathy– n (%)	144(49.2)
Mean arterial pressure (mmHg)– m ± SD	79±16
Heart Rate (bpm)– m ± SD	76±13
Laboratory data	
Leukocyte count (x 10^9/L)– M (IQR)	8.2(5.2-11.9)
Platelet count (x 10^9/L)– M (IQR)	93(58-144)
Serum bilirubin (mg/dl) – M (IQR)	4.1(1.7-14.3)
Serum albumin (g/dl)– M (IQR)	3.0(2.7-3.4)
International normalized ratio– M (IQR)	1.7(1.4-2.1)
Serum creatinine (mg/dl)– M (IQR)	2.9(2.5-3.6)
BUN(mg/dl) – M (IQR)	64(48-85)
Serum sodium (mmol/L) – m ± SD	133±7
Organ Failures– n (%)	
Liver	80(26.9)
Kidney	298(100.0)
Brain	22(7.4)
Coagulation	40(13.4)
Circulation	0(0.0)
Respiratory	6(2.0)
Number of organ failures – M (IQR)	1(1-2)
ACLF Grade– n (%)	
Grade I	179(60.1)
Grade II	91(30.5)
Grade III	28(9.4)

Scores	
MELD– m ± SD	29±7
MELD-Na– m ± SD	31±6
Child-Pugh– m ± SD	10.3±1.9
CLIF-C OF– m ± SD	9±2
CLIF-C ACLF– m ± SD	46±8
Terlipressin	
Initial dose (mg/day)– M (IQR)	2(2-3)
Days of treatment– M (IQR)	8(6-13)

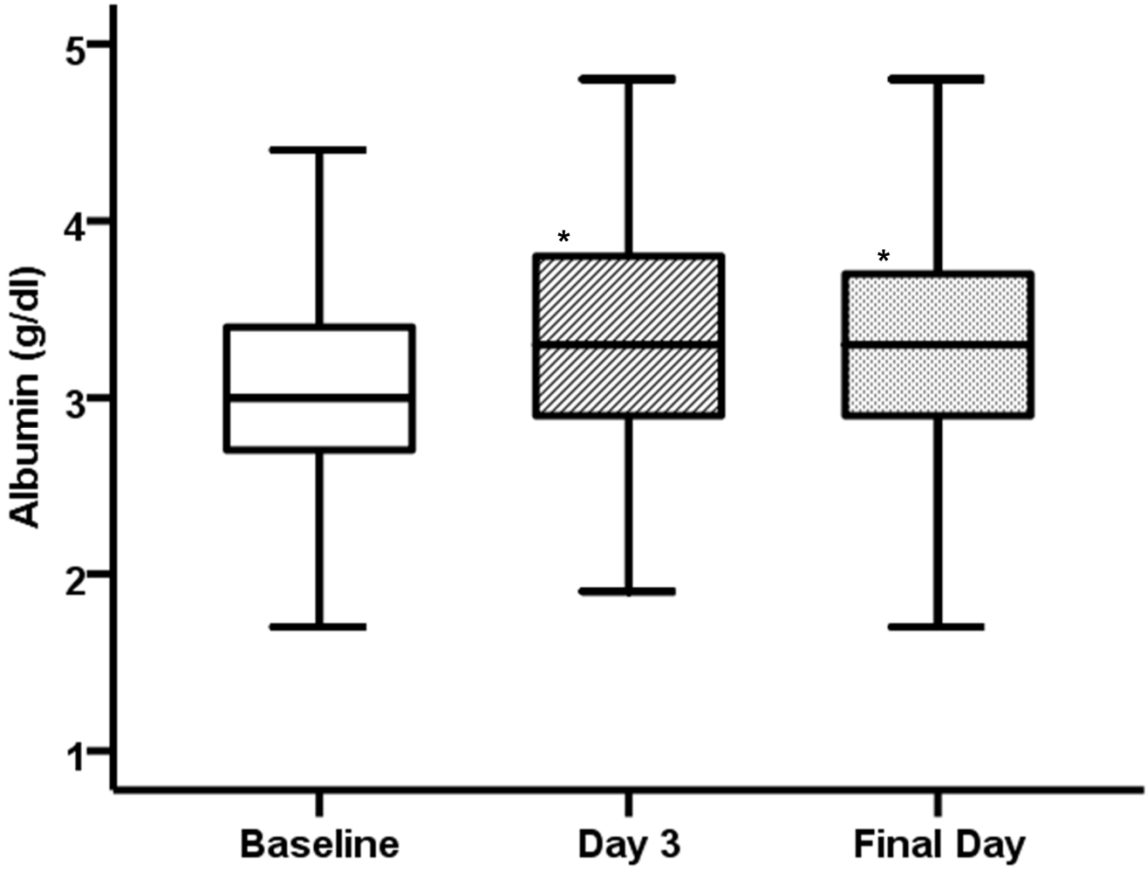
Legend: n, number; m, mean; SD, standard deviation; M, median; IQR, interquartile range ACLF, acute-on-chronic liver failure; MELD, model of end stage liver disease; CLIF-C OF, CLIF Consortium organ failure score; CLIF-C ACLF, CLIF consortium acute-on-chronic liver failure score.

Supplementary Table 2. Comparison between the 4 groups

	PADOVA N=76	CLINIC N=44	CANONIC N=57	MUNSTER N=121
Age (years) – m (SD)	58±10	59±8	56±11	58±9
Gender (Male) – n (%)	54 (71.1)	36 (81.8)	39 (68.4)	77 (63.6)
Precipitating events – n (%)	43 (56.6)	30 (68.2)	40 (75.5)	96 (79.3)
SBP – n (%)	17 (22.4)	10 (22.7)	2 (4.4)	39 (38.2)
Other infection – n (%)	29 (38.2)	18 (40.9)	10 (17.5)	44 (43.1)
GI bleeding – n (%)	2 (2.6)	2 (4.6)	3 (5.3)	38 (37.3)
Other PE – n (%)	7 (9.2)	6 (13.6)	4 (7.0)	5 (4.9)
WBC – M (IQR)	7.3 (5.2-10.7)	6.7 (4.0-9.4)	9.8 (5.9-15.0)	8.7 (6.2-12.4)
Platelet – M (IQR)	88 (58-127)	71 (45-96)	75 (44-119)	113 (71-181)
Bilirubin – M (IQR)	4.6 (2.2-14.6)	6.1 (2.8-24.6)	4.9 (2.3-14.2)	2.5 (1.3-10.3)
Albumin – M (IQR)	3.1 (2.7-3.5)	3.1 (2.7-3.4)	3.1 (2.7-3.5)	2.9 (2.6-3.3)
INR – M (IQR)	1.7 (1.5-2.1)	2.0 (1.7-2.8)	1.7 (1.4-2.1)	1.5 (1.3-1.8)
Creatinine – M (IQR)	3.0 (2.6-3.7)	2.9 (2.5-3.4)	3.2 (2.5-3.9)	2.7 (2.2-3.5)
Urea – M (IQR)	65 (48-81)	81 (48-101)	79 (53-120)	58 (47-72)
Na – m ± SD	131±6	129±7	133±6	134±6
MAP (mmHg) – m ± SD	77±11	73±11	78±10	81±15
Heart Rate (bpm) – m ± SD	75±13	77±12	78±16	74±12
Ascites grade (large) – n (%)	70 (92.1)	14 (35.9)	21 (43.8)	74 (61.2)
Encephalopathy – n (%)	33 (43.4)	16 (41.0)	21 (36.8)	74 (61.2)
MELD – m ± SD	30±6	32±6	30±6	26±7
MELD-Na – m ± SD	32±5	34±5	32±6	28±6
Child-Pugh – m ± SD	10.7±1.9	10.5±1.8	8.8±1.9	10.8±1.6
ACLF Grade – n (%)				
Grade 1	45 (59.2)	23 (52.3)	34 (59.7)	77 (63.6)
Grade 2	23 (30.3)	12 (27.3)	17 (29.8)	39 (32.2)
Grade 3	8 (10.5)	9 (20.5)	6 (10.5)	5 (4.1)

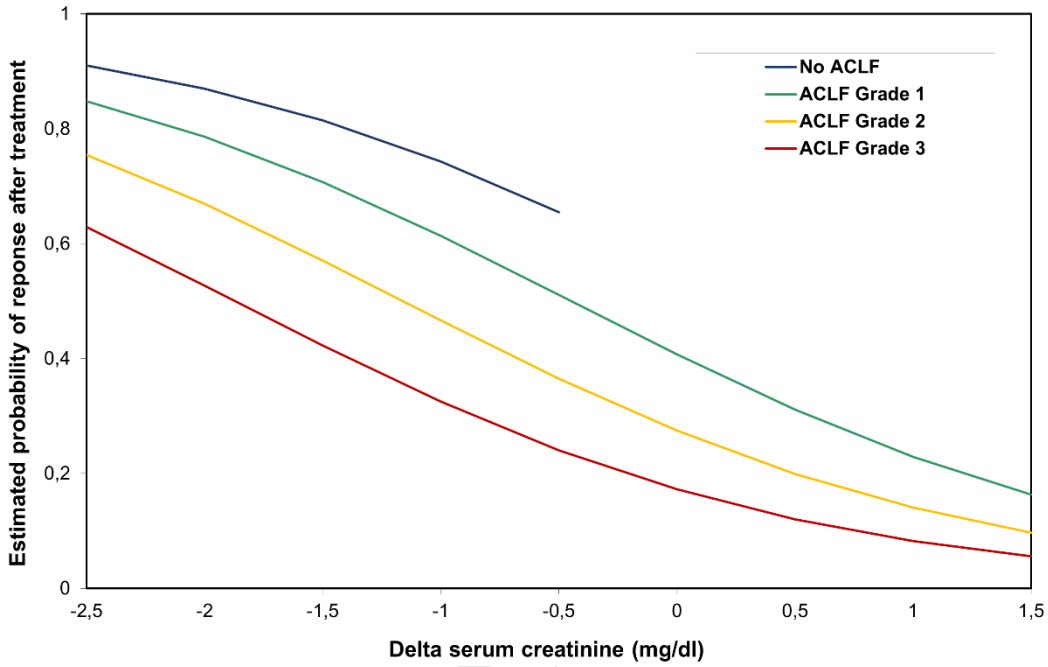
Legend: N, number; m, mean; SD, standard deviation; M, median; IQR, interquartile range SBP, spontaneous bacterial peritonitis; GI, gastrointestinal; PE, precipitating events; MAP, mean arterial pressure; WBC, white blood cells; INR, international normalized ratio; ACLF, acute-on-chronic liver failure; MELD, model of end stage liver disease; CLIF-C OF, Chronic Liver Failure Consortium organ failure score; CLIF-C ACLF, Chronic Liver Failure Consortium acute-on-chronic liver failure score.

Supplementary Figure 1. Albumin concentration at baseline, at day 3 and end of treatment with terlipressin and albumin.



Legend: *, p<0.001 vs baseline

Supplementary Figure 2. Probability of response to treatment with terlipressin and albumin according to delta changes in serum creatinine on day 3 in patients without ACLF (blue line), ACLF grade 1 (green line), grade 2 (yellow line) and grade 3 (red line) on day 3.



Legend: ACLF, acute-on-chronic liver failure;. Delta serum creatinine=serum creatinine on day 3 - serum creatinine on day 0.