Risk stratification in cirrhosis and acute-on-chronic liver failure : exploration of invasive and non-invasive prognostic markers

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Copeptin in patients with acute decompensation of liver cirrhosis: relationship with acute-on-chronic liver failure and survival

In revision

ABSTRACT

Background: Acute-on-chronic liver failure (ACLF) is characterized by the presence of acute decompensation of cirrhosis (AD), organ failure and a high short-term mortality rate. Systemic hemodynamic dysfunction and activation of endogenous vasoconstrictor systems are thought to contribute to the pathogenesis of ACLF. We explored whether copeptin, a stable cleavage product of the vasopressin precursor, is a potential marker of hemodynamic dysfunction and outcome in a population of patients admitted for AD or ACLF.

Methods: 198 randomly selected patients hospitalized for AD of cirrhosis from the CANONIC database were included. Presence of ACLF was defined according to the CLIF-consortium organ failure (CLIF-C OF) score. Serum copeptin was measured in samples collected within 2 days after admission. Cox proportional hazard regression analysis was used to evaluate the impact of serum copeptin, laboratory and clinical data on survival.

Results: Serum copeptin concentration was found to be significantly higher in patients with ACLF as compared to those without ACLF at hospital admission [49 (22-76) vs. 15 (9-31) pmol/L, $p<0.001$] and was inversely correlated to the mean arterial blood pressure ($r=-0.172$, $p=0.016$). Copeptin predicted mortality or liver transplantation, independently of MELD, MELD-sodium and CLIF-C OF scores.

Conclusions: Serum copeptin, as an indirect marker of hemodynamic dysfunction, is significantly elevated in patients with ACLF as compared to those with 'mere' acute decompensation of cirrhosis. Copeptin is independently associated with outcome in patients admitted for AD or ACLF.

INTRODUCTION

Acute decompensation of liver cirrhosis (AD) is characterised by the occurrence of major complications of the underlying liver disease and is the main cause of hospitalization in cirrhotic patients. Acute-on-chronic liver failure (ACLF) is a life-threatening syndrome that occurs in patients with AD and is characterised by organ failures.[1,2] Several, non-evidence based working definitions have been proposed for ACLF.[1,3,4] In order to define clear diagnostic criteria for ACLF, the European Association for the Study of the Liver- Chronic Liver Failure (EASL-CLIF) consortium recently performed the Acute-on-Chronic Liver Failure in Cirrhosis (CANONIC) study.[2] In that study, a large cohort of patients hospitalized for AD were prospectively followed and ACLF was found to be a distinct entity in patients with AD, as it was characterized by the presence of organ failure and a high short-term mortality rate.[2] The activation of endogenous vasoconstrictor systems as an adaptive response to a decreased effective circulating blood volume in cirrhotic patients with a hyperdynamic circulation is thought to be associated with the development of organ failure in ACLF.[5,6] Conventional prognostic scoring systems in cirrhosis, such as the Model for End-stage Liver disease (MELD) and Child-Pugh score, do not include variables concerning hemodynamic derangement and extra-hepatic organ failure. Therefore, we hypothesized that it would be particularly interesting to explore biomarkers reflecting the degree of hemodynamic dysfunction as potential prognostic markers in AD and ACLF. Arginine vasopressin (AVP) is a hypothalamic neurohormone, which is secreted into the blood stream by the neurohypophysis upon stimuli such as hyperosmolarity, arterial hypotension and hypovolemia. Due to its role in hemodynamic homeostasis, we hypothesize that AVP may reflect the degree of hemodynamic dysfunction in AD and ACLF and may be of prognostic significance in this specific patient group. However, serum AVP measurements are not applicable in clinical practice, due to its instability in serum and poor reproducibility.[7] Copeptin is a stable cleavage product of the C-terminal part of the AVP precursor and is secreted together with AVP in equimolar amounts.[8,9] Copeptin is therefore considered a surrogate marker for AVP. Aim of the present study was to assess copeptin as a marker of hemodynamic derangement in patients with AD or ACLF and to evaluate whether it is an independent prognostic factor in patients admitted for AD.

PATIENTS AND METHODS

Study population

The present study is an ancillary study of the prospective, observational CANONIC study.[2] Informed consent was obtained from each patient included in that study and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected

in a priori approval by the institution's human research committee. Between February and September 2011, 1343 patients hospitalized for AD of cirrhosis in 29 Liver Units in 8 European countries were included in the CANONIC study. The HCB –IDIBAPS Biobank in Barcelona (Spain) manages the CANONIC database and storage of biomaterials. The present study involved a population of 198 representative patients consisting of two equal groups (n=99) of randomly selected patients with and without ACLF at time of study enrolment. ACLF was defined by the CLIF-Consortium Organ Failure (CLIF-C OF) score[10], a simplification of the original Chronic Liver Failure- Sequential Organ Failure Assessment (CLIF-SOFA) score, which has been specifically developed for the use in cirrhotic patients with AD.[2] These patients were centrally randomly selected in 3 different groups according to the grade of ACLF: grade I (single organ failure, n=49), grade II (2 organ failures, n=31) and grade III (≥3 organ failures, n=19). Demographics, clinical characteristics and laboratory measurements were collected at time of study enrolment. The MELD, MELD-sodium (MELD-Na), Child-Pugh and CLIF-C OF scores and Modification of Diet in Renal Disease (MDRD) values, were determined based on these laboratory results and clinical findings. Survival data and events were reported at set time points of 28 days and 3, 6 and 12 months of follow-up.

Copeptin measurements

Serum samples for copeptin measurements were obtained ≤2 days after study enrolment and stored at-80^{o}C. Serum copeptin measurements were performed in 50 µl plasma samples using an immunoassay in the chemiluminescence-coated tube format (B.R.A.H.M.S., Kryptor, GmbH, Hennigsdorf, Germany). The reference range of serum copeptin in healthy individuals is 1-12 pmol/L with median values of <5 pmol/L.[11,12]

Statistical analysis

The relation between ACLF grades and serum copeptin was analysed using the Kruskal Wallis test and Wilcoxon signed rank test when appropriate. Spearman's rank order correlation analysis was performed to explore possible correlations between serum copeptin concentration and age, prognostic scoring systems, MDRD, blood pressure and laboratory data. Optimal cut-off points of serum copeptin concentration and MELD, MELD-Na and CLIF-C OF score in predicting mortality or liver transplantation (LT) were determined using the Youden Index. Hereinafter, values exceeding these cut-off points are referred to as 'high' and values below these cut-off points as 'low'. With the use of these cut-off points, survival analysis was performed at 28 days and 3, 6 and 12 months of follow-up using Kaplan Meier analyses and Cox proportional hazard regression models. 'Death or LT' was used as a combined endpoint. Variables with a $p<0.20$ in univariate Cox regression analyses were included in multivariate models. The MELD, MELD-Na and CLIF-C OF score were separately evaluated with copeptin in multivariate models in order to explore whether serum copeptin concentration is associated

with outcome independently of these prognostic scores. Variables with a skewed distribution were log-transformed prior to statistical analysis. Discrete variables are shown as counts (percentage) and continuous variables as mean ± standard deviation (SD). Data with a skewed distribution are expressed as median (interquartile range; IQR). A p-value ≤0.05 was considered statistically significant.

RESULTS

Patient characteristics

Baseline demographics and clinical characteristics are shown in table 1. Serum copeptin at admission was significantly higher in patients with as compared to patients without ACLF [49 (22-76) vs. 15 (9-31) pmol/L, p<0.001]. Significant differences between these patients were also found for mean arterial blood pressure (MAP), diastolic blood pressure (DBP), presence of renal failure, circulatory failure, systemic inflammatory response syndrome (SIRS) and bacterial infection, serum bilirubin, creatinine, sodium and C-reactive protein (CRP) concentrations, white blood cell count (WBC), international normalized ratio (INR), Child-Pugh, MELD, MELD-Na and CLIF-C OF score and the use of vasopressors or dialysis (table 1).

At 28 days after enrolment in the study, 34 (17.2%) patients had died and 7 (3.5%) underwent LT. After 12 months of follow-up 87 (43.9%) patients had died and 32 (16.2%) were transplanted. Out of these, 76 patients (63.9%) had ACLF in association with AD of cirrhosis at enrolment. Patients with ACLF grade III at enrolment displayed the highest serum copeptin concentration at hospital admission as compared to those with ACLF grade I or II [67 (42-126) pmol/L vs. 44 (20-69) pmol/L, p=0.015]. In ACLF grade I and II, serum copeptin was significantly higher in patients with renal failure at hospital admission as compared to patients without renal failure (p=0.046 and p=0.009, respectively). However, no significant difference in serum copeptin was found between patients with and without renal failure in ACLF grade III (p=0.089, figure 1). Serum copeptin was consistently found to be significantly higher in patients who died or were transplanted as compared to those who were still alive at 28 days and 3, 6 and 12 months of follow-up [28 days: 55 (26-92) vs. 21 (10-48), p<0.001; 3 months: 46 (23-76) vs. 17 (9-41) pmol/L, p<0.001; 6 months: 41 (21-75) vs. 17 (9-39) pmol/L, p<0.001 and 12 months: 40 (19-69) vs. 14 (9-32) pmol/L, p<0.001, respectively]. In contrast, MAP and DBP at admission were found to be consistently and significantly lower in patients who died or were transplanted than in those who survived without LT within 28 days and 3, 6 and 12 months of follow-up [MAP: 77±12 vs. 82±14 mmHg, p=0.030, 79±12 vs. 84±14 mmHg, p=0.006, 78±12 vs. 83±14 mmHg, p=0.014, 79±13 vs. 84±14, p=0.005, respectively; DBP: 61±10 vs. 66±13 mmHg, p=0.007), 62±11 vs. 67±12 mmHg, p=0.002, 63±12 vs. 68±12 mmHg, p=0.002 and 63±12 vs. 68±13, p=0.001, respectively]. At enrolment, MAP and DBP

were inversely and significantly correlated to serum copeptin levels. Significant correlations with copeptin were also found for serum sodium and serum creatinine concentration, WBC, MDRD and Child-Pugh, MELD, MELD-Na and CLIF-C OF score (table 2).

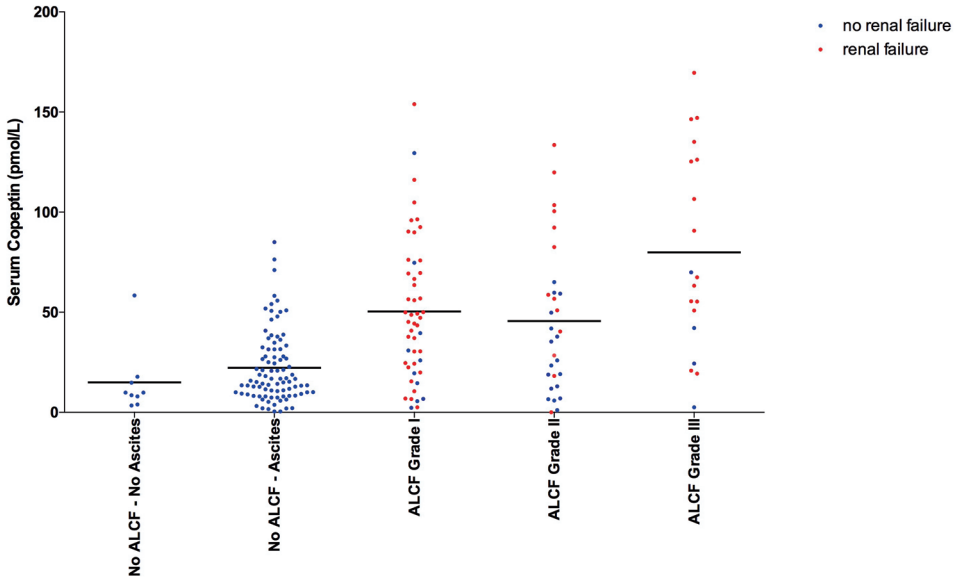

Figure 1. Association of ACLF grades with serum copeptin concentration and the presence of renal failure. Distribution of serum copeptin concentration within subgroups of patients with ACLF and patients with and without ascites and no ACLF at time of admission for acute decompensation of cirrhosis. Dots represent serum copeptin concentrations of individual patients. Horizontal lines denote median values.

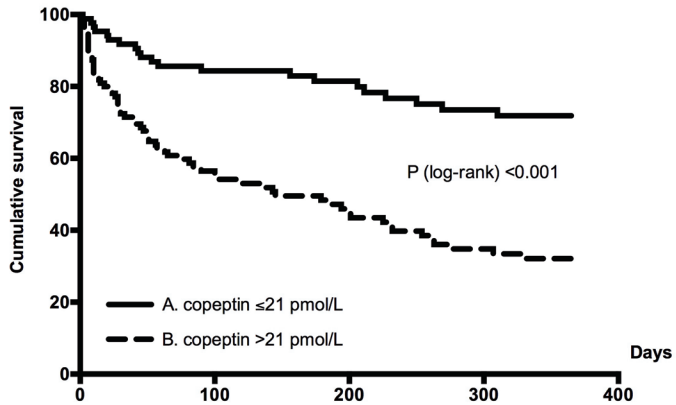

Figure 2. Association of serum copeptin concentration with outcome. One-year survival analysis using mortality or liver transplantation as a composite endpoint, stratified according to serum copeptin concentration (pmol/L) in 198 cirrhotic patients hospitalized for acute decompensation.

Table 1. Baseline characteristics of 198 cirrhotic patients hospitalized for acute decompensation of cirrhosis.

Variable	All patients (n= 198)	No ACLF (n= 99)	ACLF (n= 99)	p-value[a]
Age (years)	56.9 ± 11.5	56.4 ± 11.7	57.3 ± 11.4	0.953
Gender (male), n (%)	121 (61.1)	61 (60.6)	60 (60.6)	0.884
Background, n (%)				
Diabetes	52 (26.7)	29 (55.8)	23 (44.2)	0.353
Coronary heart disease	12 (6.6)	3 (3.3)	9 (10.0)	0.067
Etiology, n (%)				
Alcohol	129 (66.2)	60 (61.9)	69 (70.4)	0.207
Hepatitis B	8 (4.3)	5 (5.4)	3 (3.3)	0.479
Hepatitis C	61 (32.8)	38 (40.9)	23 (24.7)	0.019
NAFLD	10 (5.4)	3 (3.2)	7 (7.5)	0.194
PBC	3 (1.6)	2 (2.2)	1 (1.1)	0.561
Cryptogenic	11 (6.0)	5 (5.4)	6 (6.5)	0.770
Other	16 (8.7)	12 (13.0)	4 (4.3)	0.034
Physical exam				
SBP (mmHg)	113 ± 19	114 ± 18	111 ± 21	0.266
DBP (mmHg)	65 ± 12	69 ± 11	62 ± 13	< 0.001
MAP (mmHg)	81 ± 13	84 ± 12	78 ± 14	0.003
Clinical features, n (%)				
Ascites	138 (69.7)	63 (63.6)	75 (75.8)	0.064
Bacterial infection	57 (28.9)	18 (18.2)	39 (39.8)	< 0.001
SIRS	35 (17.7)	6 (6.1)	29 (29.3)	< 0.001
HE	105 (53.0)	50 (50.5)	55 (55.6)	0.477
Renal failure	66 (33.3)	0 (0.0)	66 (66.7)	< 0.001
Circulatory failure	18 (9.1)	0 (0.0)	18 (19.2)	< 0.001
Laboratory data				
Copeptin (pmol/L)	26 (12-55)	15 (9-31)	49 (22-76)	< 0.001
WBC (x 10^9/L)	6.1 (4.2-9.5)	5.0 (3.0-7.0)	8.0 (5.0-12.0)	< 0.001
CRP (mg/L)	20.0 (7.4-43.0)	15.8 (6.0-29.2)	27.0 (9.3-56.0)	0.024
Bilirubin (mg/dL)	3.7 (1.9-9.1)	2.9 (1.8-4.9)	5.9 (2.1-20.1)	< 0.001
Prothrombine time (s)	24 (18-36)	25 (17-41)	24 (18-36)	0.641
INR	1.5 (1.3-2.2)	1.5 (1.3-1.7)	1.8 (1.4-2.6)	< 0.001
Creatinine (mg/dL)	1.3 (0.8-2.4)	0.9 (0.7-1.3)	3.3 (1.5-6.0)	< 0.001
Sodium (mmol/L)	135 ± 17.7	136 ± 5	133 ± 7	0.009
Scores				
Child –Pugh	10.0 ± 2.0	9.4 ± 1.9	10.7 ± 2.3	< 0.001
MELD	22 ± 8	17 ± 5	28 ± 7	< 0.001
MELD-Na	25 ± 8	19 ± 6	30 ± 7	< 0.001
CLIF-OF	8.7 ± 2.6	7.0 ± 0.9	10.5 ± 2.5	< 0.001
MDRD	64 ± 43	84 ± 34	44 ± 42	< 0.001
Treatments, n (%)				
Diuretics	37 (19.1)	17 (17.5)	20 (20.6)	0.584
Vasopressors	22 (11.3)	2 (2.1)	20 (20.6)	< 0.001
Dialysis	5 (2.5)	0 (0.0)	5 (5.1)	0.024

Data are presented as mean ± standard deviation, median (interquartile range) or numbers and percentage.

[a] p-value of comparisons between patients with and without ACLF.

Table 2. Associations of clinical parameters and prognostic scoring systems with serum copeptin concentration.

Variable	Correlation coefficient with serum copeptin (r)	p-value
Age	0.21	0.003
Scores		
Child-Pugh	0.26	< 0.001
MELD	0.42	< 0.001
MELD-Na	0.42	< 0.001
CLIF-C OF	0.39	< 0.001
MDRD	-0.60	< 0.001
Laboratory data		
WBC*	0.28	< 0.001
Bilirubin*	0.09	0.193
Prothrombin time*	0.02	0.869
INR*	0.08	0.260
Creatinine*	0.59	< 0.001
Sodium	-0.19	0.048
Blood pressure		
SBP	-0.10	0.154
DBP	-0.21	0.004
MAP	-0.17	0.016

*Variable was log-transformed prior to statistical analysis.

Survival analysis

The optimal cut-off point of serum copeptin in predicting mortality or LT was 21 pmol/L. For MELD, MELD-Na and CLIF-C OF score, optimal cut-off points were 22 points, 26 points and 10 points, respectively. Patients with a high serum copeptin concentration at hospital admission showed a significantly higher risk for death or LT at 28 days and 3, 6 and 12 months (figure 2) of follow-up as compared to patients with a high serum copeptin concentration (Log-rank: $p<0.001$). Survival curves in figure 3A, 3B and 3C display the risk for mortality or LT stratified for serum copeptin and MELD score, serum copeptin and MELD-Na score and serum copeptin and CLIF-C OF score at time of hospital admission, respectively. Patients with both a low serum copeptin concentration and MELD, MELD-Na or CLIF-C OF score displayed the best outcome at 12 months as compared to patients with 1) both a high serum copeptin concentration and MELD, MELD-Na or CLIF-C OF score and 2) a low serum copeptin concentration and high MELD, MELD-Na or CLIF-C OF score and 3) a high serum copeptin concentration and low MELD, MELD-Na or CLIF-C OF score at time of admission for AD of cirrhosis (table 3A). This finding suggests that copeptin could possibly add prognostic information to these prognostic scores. Figure 4 shows the estimated probability of death after 12 months of follow up by using the CLIF-C OF score and stratified according to serum copeptin concentration, showing that high copeptin concentrations have a negative impact on mortality risk.

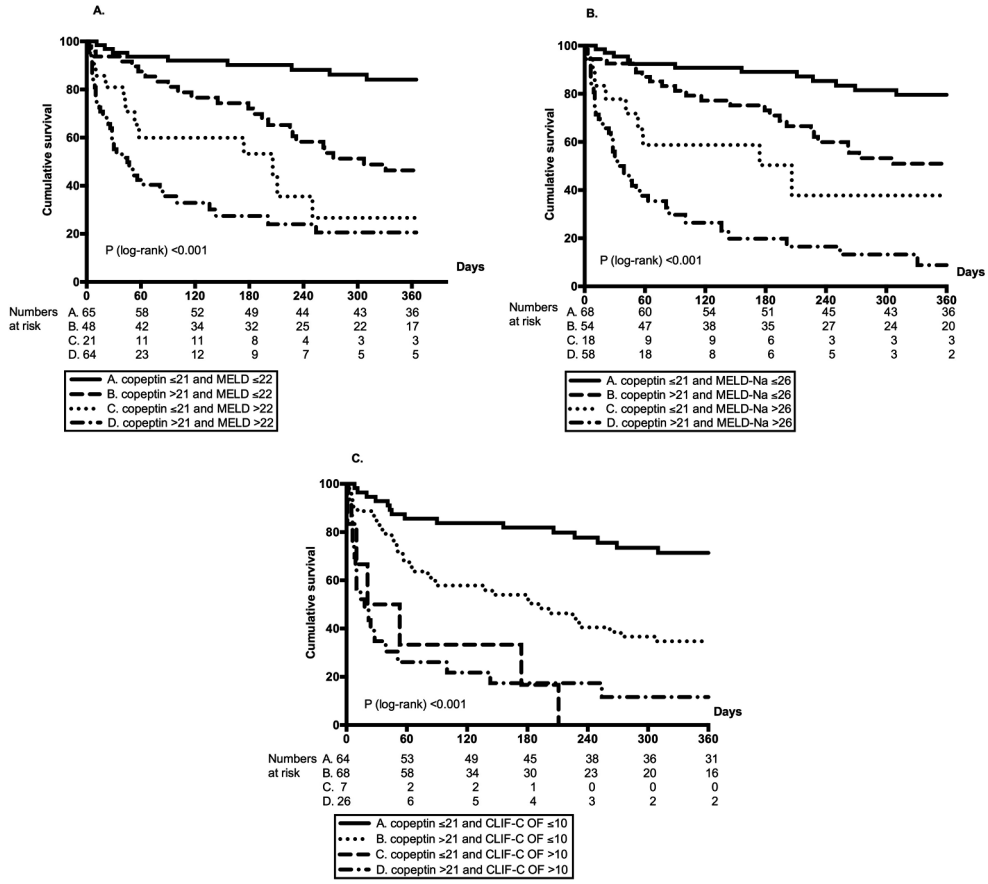

Figure 3. Association of serum copeptin concentration and MELD, MELD-Na and CLIF-C OF score with mortality or liver transplantation. One-year survival analysis of 198 cirrhotic patients admitted for acute decompensation, stratified according to serum copeptin concentration (pmol/L) and MELD score (A), serum copeptin concentration and MELD-Na score (B) and serum copeptin concentration and CLIF-C OF score (C) at time of hospitalization.

Results of univariate analysis of clinical and laboratory data in relation to LT or mortality awaiting LT at 28 days and 12 months are shown in table 4. Results of univariate analysis of the same factors at 3 months are comparable to those at 28 days and results at 6 months are comparable to those at 12 months. A strong association was found for serum copeptin concentration at admission with the composite endpoint at all time-points.

A high serum copeptin and high MELD, MELD-Na and CLIF-C OF score were found to be significantly associated with the combined endpoint 'LT or death awaiting LT' in univariate analysis at all time-points (table 3A). In multivariate analyses, a high serum copeptin predicted outcome at 3, 6 and 12 months of follow-up, independently of high MELD, MELD-Na and CLIF-C OF scores (table 3B).

Table 3. Parameters associated with transplant-free survival time. Univariate (A) and multivariate (B) Cox regression analyses using optimal cut-off points of serum copeptin and MELD, MELD-Na and CLIF-SOFA score in predicting liver transplantation or mortality awaiting liver transplantation in 198 cirrhotic patients hospitalized for acute decompensation of cirrhosis.

Time of follow-up	**28 days**		**3 months**		**6 months**		**12 months**	
Variable	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
A. Univariate								
Copeptin > 21 pmol/L	3.57 (1.65-7.73)	0.001	3.42 (1.99-5.86)	0.001	2.89 (1.83-4.57)	< 0.001	2.92 (1.95-4.37)	< 0.001
MELD score > 22 points	9.42 (3.96-22.42)	< 0.001	7.03 (4.13-11.98)	< 0.001	5.84 (3.73-9.14)	< 0.001	4.94 (3.73-7.24)	< 0.001
MELD-Na score > 26 points	9.65 (4.27-21.80)	< 0.001	7.87 (4.70-13.19)	< 0.001	6.35 (4.11-9.82)	< 0.001	5.50 (3.76-8.04)	< 0.001
CLIF-C OF score > 10 points	8.33 (4.48-15.47)	< 0.001	4.62 (2.84-7.51)	< 0.001	4.33 (2.76-6.78)	< 0.001	4.07 (2.67-6.23)	< 0.001
Copeptin and MELD score								
≤ 21 pmol/L and ≤ 22 points	reference		reference		reference		reference	
> 21 pmol/L and ≤ 22 points	1.39 (0.28-6.90)	0.685	2.19 (0.85-5.64)	0.106	1.89 (0.90-4.00)	0.095	2.73 (1.48-5.04)	0.001
≤ 21 pmol/L and > 22 points	5.71 (1.36-23.88)	0.017	5.76 (2.19-15.14)	0.001	4.66 (2.12-10.22)	< 0.001	5.67 (2.85-11.29)	< 0.001
> 21 pmol/L and > 22 points	13.00 (3.96-42.63)	< 0.001	12.79 (5.77-28.39)	< 0.001	9.85 (5.23-18.57)	< 0.001	9.93 (5.63-17.52)	< 0.001
Copeptin and MELD-Na score								
≤ 21 pmol/L and ≤ 26 points	reference		reference		reference		reference	
> 21 pmol/L and ≤ 26 points	1.74 (0.39-7.78)	0.468	1.95 (0.80-4.76)	0.145	1.94 (0.96-3.92)	0.067	2.30 (1.30-4.08)	0.004
≤ 21 pmol/L and > 26 points	7.13 (1.70-29.83)	0.007	5.71 (2.20-14.82)	< 0.001	5.05 (2.30-11.09)	< 0.001	4.99 (2.49-10.01)	< 0.001
> 21 pmol/L and > 26 points	14.80 (4.50-48.65)	< 0.001	13.75 (6.46-29.29)	< 0.001	11.19 (6.01-20.84)	< 0.001	10.67 (6.22-18.32)	< 0.001
Copeptin and CLIF-C OF								
≤ 21 pmol/L and ≤ 10 points	reference		reference		reference		reference	
> 21 pmol/L and ≤ 10 points	2.25 (0.69-7.31)	0.177	2.79 (1.43-5.43)	0.003	2.48 (1.40-4.41)	0.002	2.64 (1.60-4.34)	0.001
≤ 21 pmol/L and > 10 points	11.80 (2.95-47.26)	< 0.001	6.68 (2.34-19.02)	< 0.001	6.12 (2.40-15.57)	< 0.001	6.95 (2.95-16.38)	< 0.001
> 21 pmol/L and > 10 points	16.63 (5.60-49.39)	< 0.001	8.94 (4.33-18.46)	< 0.001	7.70 (4.05-14.63)	< 0.001	7.09 (3.93-12.78)	< 0.001
B. Multivariate								
Model 1								
Copeptin > 21 pmol/L	2.02 (0.91-4.45)	0.083	2.21 (1.27-3.84)	0.005	2.02 (1.27-3.24)	0.003	2.15 (1.42-3.26)	< 0.001
MELD score > 22 points	7.74 (3.18-18.84)	< 0.001	5.82 (3.37-10.05)	< 0.001	5.02 (3.17-7.93)	< 0.001	4.17 (2.81-6.17)	< 0.001
Model 2								
Copeptin > 21 pmol/L	1.98 (0.89-4.28)	0.093	2.22 (1.28-3.86)	0.005	2.09 (1.31-3.34)	0.002	2.22 (1.47-3.36)	< 0.001
MELD-Na score > 26 points	8.01 (3.46-18.50)	< 0.001	6.62 (3.91-11.23)	< 0.001	5.54 (3.55-8.64)	< 0.001	4.47 (3.21-6.98)	< 0.001
Model 3								
Copeptin > 21 pmol/L	2.44 (1.11-5.39)	0.027	2.83 (0.64-4.91)	< 0.001	2.43 (1.52-3.88)	< 0.001	2.50 (1.66-3.78)	< 0.001
CLIF-C OF > 10 points	6.88 (3.65-12.98)	< 0.001	3.69 (2.25-6.05)	< 0.001	3.52 (2.23-5.56)	< 0.001	3.22 (2.09-4.95)	< 0.001

Table 4. Parameters associated with transplant-free survival. Univariate Cox regression analysis of factors in relation to liver transplantation or mortality awaiting liver transplantation in 198 cirrhotic patients hospitalized for acute decompensation.

Variable	28 days		12 months	
	HR (95% CI)	p value	HR (95% CI)	p value
Age	1.00 (0.98-1.03)	0.808	1.01 (1.00-1.03)	0.080
Gender (male)	0.70 (0.36-1.35)	0.279	1.02 (0.71-1.48)	0.914
Background				
Diabetes	0.90 (0.44-1.84)	0.773	0.71 (0.45-1.10)	0.124
Coronary heart disease	0.84 (0.20-3.47)	0.804	0.61 (0.25-1.50)	0.280
Etiology				
Alcohol	0.78 (0.41-1.49)	0.455	0.71 (0.49-1.03)	0.074
Hepatitis B	0.58 (0.80-4.23)	0.585	1.03 (0.42-2.53)	0.950
Hepatitis C	0.51 (0.23-1.10)	0.080	1.06 (0.71-1.56)	0.788
NAFLD	2.13 (0.76-5.99)	0.142	1.65 (0.77-3.56)	0.199
PBC	1.68 (0.23-12.21)	0.605	1.42 (0.35-5.76)	0.623
Cryptogenic	0.90 (0.22-3.72)	0.880	0.62 (0.25-1.52)	0.293
Other	0.91 (0.28-2.97)	0.881	1.23 (0.66-2.29)	0.521
Physical exam				
SBP	0.99 (0.98-1.01)	0.306	0.99 (0.98-1.00)	0.063
DBP	0.96 (0.94-0.99)	0.007	0.98 (0.96-0.99)	0.001
MAP	0.97 (0.95-1.00)	0.030	0.98 (0.97-0.99)	0.005
Clinical Features				
ACLF	8.64 (3.39-22.04)	< 0.001	2.94(2.02-4.29)	< 0.001
Ascites	3.36 (1.32-8.55)	0.007	2.09 (1.35-3.23)	< 0.001
Bacterial infection	2.75 (1.49-5.07)	< 0.001	1.44 (0.97-2.13)	0.069
SIRS	5.34 (2.88-9.89)	<0.001	2.55 (1.67-3.91)	< 0.001
HE	2.11 (1.09-4.08)	0.022	1.11(0.77-1.59)	0.577
Renal Failure	3.29 (1.77-6.14)	< 0.001	2.17(1.50-3.13)	< 0.001
Circulatory failure	7.90 (4.05-15.39)	<0.001	3.36 (1.98-5.72)	< 0.001
Laboratory data				
Copeptin*	2.40 (1.65-3.49)	< 0.001	1.79 (1.46-2.20)	< 0.001
WBC*	2.36 (1.47-3.79)	< 0.001	1.72 (1.26-2.34)	< 0.001
CRP	1.01 (1.00-1.02)	0.001	1.01 (1.00-1.01)	0.005
Bilirubin*	2.24 (1.66-3.01)	< 0.001	1.63 (1.38-1.93)	< 0.001
Prothrombin time*	1.84 (0.54-6.30)	0.542	1.41 (0.66-3.01)	0.380
INR*	6.93 (3.37-12.89)	< 0.001	5.84 (3.54-8.48)	< 0.001
Creatinine*	2.61 (1.70-4.03)	< 0.001	2.04 (1.58-2.63)	< 0.001
Sodium	0.95 (0.91-1.00)	0.037	0.93 (0.91-0.96)	< 0.001
Scores				
Child-Pugh	1.60 (1.35-1.89)	< 0.001	1.31 (1.20-1.44)	< 0.001
MELD	1.16 (1.12-1.21)	< 0.001	1.14 (1.11-1.16)	< 0.001
MELD-Na	1.18 (1.12-1.24)	< 0.001	1.15 (1.12-1.18)	< 0.001
CLIF-C OF	1.42 (1.30-1.55)	< 0.001	1.33 (1.24-1.42)	< 0.001
MDRD	0.98 (0.97-0.99)	< 0.001	0.99 (0.98-0.99)	< 0.001
Treatments				
Diuretics	2.04 (1.04-4.01)	0.034	1.61 (1.04-2.49)	0.033
Vasopressors	4.39 (2.23-8.66)	< 0.001	2.96 (1.80-4.87)	< 0.001
Dialysis	4.59 (1.63-12.90)	0.001	3.01 (1.10-8.25)	0.032

*Variable was log-transformed prior to statistical analysis.

Despite the fact that a high serum copeptin concentration at admission is found to be an independent risk factor for mortality or LT, there were also patients with a low serum copeptin concentration who died or were transplanted during follow-up (28 days: n=8; 3 months: n=17; 6 months: n=25; 12 months: n=33). Patient characteristics of deceased or transplanted patients stratified by low or high serum copeptin concentration at 12 months of follow-up are shown in supplementary table 1. At 12 months of follow-up, deceased or transplanted patients with a high serum copeptin concentration at admission, had a significantly higher age, higher levels of inflammatory markers (CRP, WBC) and serum creatinine, higher CLIF-C OF scores and a lower MDRD as compared to deceased or transplanted patients with a low serum copeptin concentration. Also the presence of ascites, bacterial infection, renal failure and ACLF was significantly more common in the subgroup of patients who died or were transplanted with a high serum copeptin concentration at this time point. MAP and DBP were similar in patients who died or were transplanted with a low and high serum copeptin concentration.

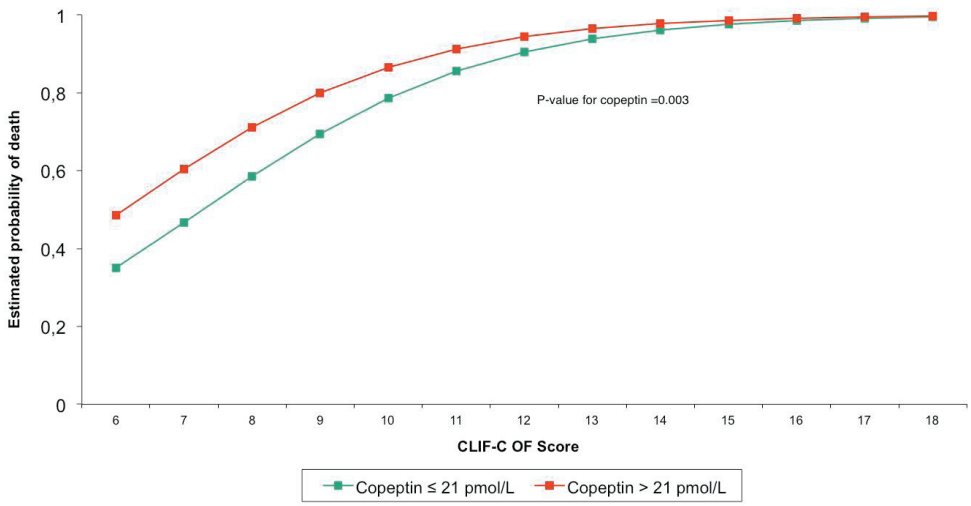

Figure 4. Association of the estimated probability of death using the CLIF-C OF score at 12 months of follow-up, stratified according to serum copeptin concentration.

DISCUSSION

This is the first study to describe the role of copeptin in the setting of AD and ACLF. The results demonstrate that the presence of ACLF is accompanied by significantly higher serum copeptin levels and a significantly lower MAP and DBP as compared to patients without ACLF. The role of copeptin as an indicator of hemodynamic dysfunction was shown by a significant

inverse correlation with MAP and DBP in a population of patients admitted for AD. In addition, serum copeptin is found to be significantly associated with outcome in patients admitted for AD, independently of the MELD, MELD-Na and CLIF-C OF score.

Advanced liver cirrhosis is associated with the presence of a hyperdynamic circulation, manifesting by an increased cardiac output and heart rate and a decreased systemic vascular resistance. This is resulting in an increased blood flow per time unit.[6] Decreased systemic vascular resistance is a stimulus for the secretion of AVP into the bloodstream by the neurohypophysis[13], next to other stimuli such as hyperosmolarity, stress, pain and nausea.[14] A decrease of 5-7% in MAP is, in general, sufficient to cause detectable increases in serum AVP levels.[15] This leads to increased renal sodium and solute-free water retention and splanchnic vasoconstriction.[16] These hemodynamic changes are thought to contribute to the development of multi-organ failure in ACLF, which is the most common cause of death in patients with AD.[2,3,5] ACLF occurs in approximately 30% of the hospitalized cirrhotic patients.[2] Previous studies have shown that ACLF is associated with severe portal hypertension, elevated intrahepatic resistance and decreased liver blood flow.[17,18] The hepatic venous pressure gradient (HVPG) has been found to be an independent predictor of clinical decompensation in patients with portal hypertension and compensated liver disease.[17] In addition, HVPG at time of hospital admission for ACLF has been found to be an independent predictor of mortality in these patients.[19] A lower MAP has previously been reported to be a determinant of ACLF development, whereas a higher MAP is found to be protective for the development of ACLF.[20] In the current study, we have indeed found that patients with ACLF at hospital admission had a significantly lower MAP and DBP as compared to patients without ACLF. This is in consistence with previous findings reported in studies investigating systemic hemodynamics in ACLF.[21] Remarkably, serum copeptin did not consistently increase through ACLF grade I-III. This implicates that, although ACLF occurs in the setting of hemodynamic dysfunction, it may not be directly associated with the severity of ACLF.

Due to the key role of AVP in hemodynamic homeostasis, we hypothesized that it may be a potential prognostic marker in patients with AD or ACLF. In clinical practice, a prognostic biomarker reflecting the degree of circulatory derangement may be of importance as it may help to distinguish between patients who are at a higher risk of developing organ failure and short-term mortality. These patients may require more intensive surveillance and treatment. It may as well add prognostic information to conventional prognostic scoring systems in cirrhosis, such as the MELD and Child-Pugh score, which do not take into account hemodynamic derangement and extra-hepatic organ failure. However, measurements of serum AVP are not useful in clinical practice due to its instability in serum and poor reproducibility. Therefore, we assessed copeptin, a surrogate marker for AVP, as a potential prognostic marker in these

patients. Recent studies have shown an association of high serum copeptin levels with systemic hemodynamics as portal hypertension, a HVPG of >12 mmHg[22] and a decreased cardiac output.[23] In the current study, the role of copeptin in hemodynamic homeostasis was shown by the finding of a significant, but weak inverse correlation between MAP and DBP with copeptin. MAP and DBP at hospital admission were significantly lower in patients who died or were transplanted during follow-up as compared to those who survived without a LT, while serum copeptin concentration was significantly higher in these patients. On the other hand, we did not find any difference in MAP between deceased or transplanted patients with a low and high serum copeptin concentration. We are unable to clarify this finding in this observational study, but it may be postulated that patients who died or were transplanted with a high serum copeptin concentration may be less responsive to the vasopressor effect of AVP.[24] However, further research is needed to clarify this finding.

To date, a few studies investigated the prognostic value of copeptin in the setting of liver cirrhosis.[25-28] The results of these studies show that serum copeptin levels increase along with the severity of liver disease[25,27] and predict short- and longer-term transplant-free mortality in patients with various stages of cirrhosis.[25-28] In addition, a prospectively conducted study showed the ability of plasma copeptin to predict the development of cirrhosis related complications and death within 3-months after hospitalization.[28] However, no data have been reported on the prognostic value of serum copeptin in the setting of AD and ACLF. Currently, several scoring systems are in use for risk stratification in critically ill cirrhotic patients, such as the MELD, MELD-Na, Child-Pugh, Sequential Organ Failure Assessment (SOFA) and Acute Physiology, Age and Chronic Health Evaluation (APACHE) scores. The scoring systems accounting for organ failure (SOFA, APACHE) have a better predictive accuracy than scoring systems accounting for the severity of underlying liver disease (MELD, MELD-Na, Child-Pugh) in patients with AD.[1,29,30] However, the incorporation of multiple subscores and ranges in the organ failure scores, do not facilitate clinical use in the setting of ACLF. The CLIF-C OF score was recently developed as a simplified score to diagnose and grade ACLF.[10] Its prognostic accuracy is comparable to the CLIF-SOFA score, but significantly higher than the MELD, MELD-Na and Child-Pugh score. In the current study, it was shown that serum copeptin, predicts the risk for mortality or LT independently of the CLIF-C OF score as well as of MELD and MELD-Na scores. In addition, it was shown that serum copeptin provides additional prognostic information to these prognostic scoring systems. In deceased or transplanted patients, CLIF-C OF scores were significantly higher in those with high serum copeptin levels as compared to patients with low serum copeptin levels. It may be postulated that patients who are unable to increase the release of AVP, and thus of copeptin, die or are transplanted at lower CLIF-C OF scores. This is suggesting that copeptin release is an important protective, adaptive factor.

Some limitations concerning the present study are to be considered. A relatively small population was studied and a single copeptin measurement was performed in samples collected at admission for AD of cirrhosis. To further explore copeptin as a prognostic marker in AD and ACLF, a prospectively conducted larger cohort study in which copeptin measurements are sequentially performed would be interesting. Such measurements of serum copeptin levels over time could possibly also provide more knowledge on the relation of serum copeptin with the course of hemodynamic deregulations and disease progression. Furthermore, when interpreting the results of the current study, we need to take into consideration the fact that the release of AVP, and thus of copeptin, is multifactorial. Next to the presence of decreased systemic vascular resistance in cirrhosis, serum copeptin concentration could be influenced by the presence of other stimuli as hyperosmolarity, stress, pain, nausea and the effects of certain drugs.[14] Further prospectively conducted research is needed to minimize the impact of these factors. Future studies should also focus on the potential causal relationship between renal function and serum copeptin levels, as copeptin is thought to be, at least partly, cleared by the kidneys.[31,32]

In conclusion, serum copeptin levels are significantly more increased in patients with ACLF as compared to those with 'mere' AD. Moreover, high serum copeptin is indicative of poorer outcome in cirrhotic patients admitted for AD, independently of MELD, MELD-Na en CLIF-C OF scores. These findings suggest that serum copeptin is a potential prognostic marker in hospitalized cirrhotic patients with AD.

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Supplementary table 1. Comparison of baseline characteristics of patients who died or were transplanted after 12 months of follow-up with a low or high serum copeptin concentration at time of hospital admission for acute decompensation of cirrhosis.

Variable	Deceased or transplanted patients at 365 days (n=119)		p-value
	≤ 21 pmol/L (n= 33)	> 21 pmol/L (n= 86)	
Age (year)	54 ± 11	60 ± 12	0.015
Gender (Male)	20 (60.6)	53 (61.6)	0.918
Background, n (%)			
Diabetes	8 (24.2)	17 (20.2)	0.634
Coronary heart disease	0 (0.0)	5 (6.3)	0.154
Etiology, n (%)			
Alcohol	20 (62.5)	52 (61.2)	0.896
Hepatitis C	16 (48.5)	22 (28.2)	0.040
Hepatitis B	3 (9.1)	2 (2.6)	0.134
NAFLD	3 (9.1)	4 (5.1)	0.432
PBC	0 (0.0)	2 (2.6)	0.353
Cryptogenic	0 (0.0)	5 (6.4)	0.143
Other	5 (15.2)	6 (7.8)	0.238
Physical exam			
Systolic blood pressure (mmHg)	111 ± 14	111 ± 20	0.914
Diastolic blood pressure (mmHg)	65 ± 13	63 ± 11	0.333
Mean arterial pressure (mmHg)	80 ± 12	79 ± 13	0.535
Clinical features, n (%)			
Ascites	21 (63.4)	72 (83.7)	0.018
Bacterial Infection	4 (12.1)	32 (37.7)	0.007
HE	16 (48.5)	47 (54.7)	0.546
ACLF	15 (45.5)	61 (70.9)	0.010
Renal failure	6 (18.2)	45 (52.3)	<0.001
Circulatory failure	2 (6.1)	14 (16.3)	0.144
Laboratory data			
Copeptin (pmol/L)	10 (7-15)	53 (38-85)	<0.001
WBC (x10^9/L)	5.5 (4.1-7.5)	7.7 (4.4-10.6)	0.035
CRP	11 (7-23)	30 (13-59)	0.016
Bilirubin (mg/dL)	5.7 (2.5-14.3)	4.9 (2.1-14.2)	0.551
Prothrombin time (s)	27 (19-38)	26 (18-36)	0.898
INR	2.1 (1.5-2.6)	1.7 (1.4-2.5)	0.116
Creatinine (mg/dL)	0.9 (0.7-1.3)	1.9 (1.1-3.4)	<0.001
Sodium (mmol/L)	135 ± 6	133 ± 7	0.212
Scores			
Child-Pugh	10.2 ± 1.9	10.9 ± 2.1	0.139
MELD	24 ± 7	26 ± 9	0.206
MELD-Na	26 ± 7	29 ± 8	0.081
CLIF-OF	8.2 ± 2.7	9.9 ± 4.0	0.013
MDRD	83 ± 47	42 ± 33	<0.001
Treatments, n (%)			
Diuretics	8 (25.0)	18 (21.4)	0.680
Vasopressors	4 (12.5)	15 (17.9)	0.486
Terlipressin	4 (12.5)	11 (13.1)	0.932
Dialysis	1 (3.0)	3 (3.5)	0.901

Data are presented as mean ± standard deviation, median (interquartile range) or numbers and precentage.