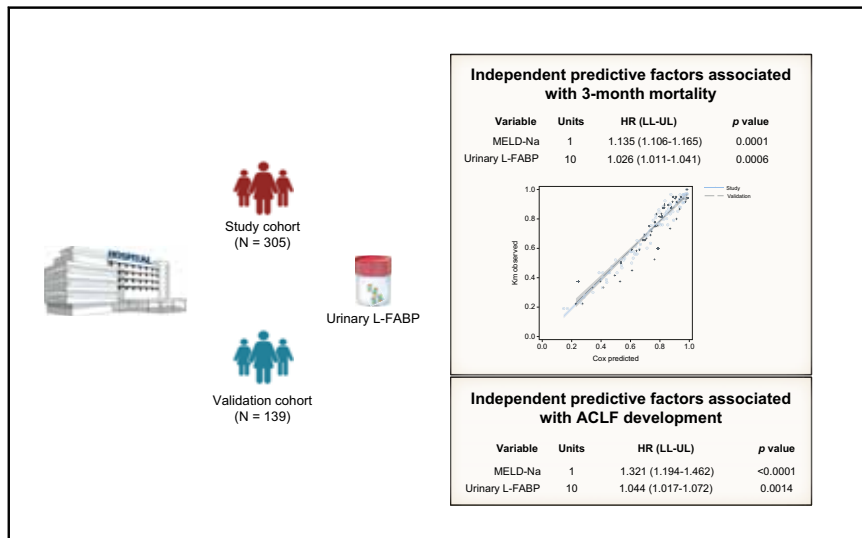


# Urinary L-FABP is a promising prognostic biomarker of ACLF and mortality in patients with decompensated cirrhosis

## Graphical abstract



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## Lay summary

Increased levels of liver fatty acid-binding protein (L-FABP), a protein related to lipid metabolism, have been associated with liver-related diseases. The present study analyzed urinary L-FABP levels in 2 independent groups of patients with decompensated cirrhosis and showed that higher urinary L-FABP levels correlated with increased mortality and risk of acute-on-chronic liver failure development. Therefore, urinary L-FABP levels could be useful as a new tool to predict complications in patients with decompensated cirrhosis.

## Highlights

- L-FABP is a mediator of lipid metabolism and has been associated with liver injury.
- Urinary L-FABP and MELD-Na were associated with 90-day mortality in patients with decompensated cirrhosis.
- Urinary L-FABP correlates with ACLF grade and liver, coagulation and circulatory failures.
- Moreover, urinary L-FABP was related to the development of ACLF.



# Urinary L-FABP is a promising prognostic biomarker of ACLF and mortality in patients with decompensated cirrhosis

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**Background & Aims:** Decompensated cirrhosis (DC) is associated with high mortality, mainly owing to the development of acute-on-chronic liver failure (ACLF). Identifying the patients with DC who are at high risk of mortality and ACLF development is an unmet clinical need. Liver fatty acid-binding protein (L-FABP) is expressed in several organs and correlates with liver and systemic inflammation. Herein, we aimed to assess the prognostic value of L-FABP in patients with DC.

**Methods:** A prospective series of 444 patients hospitalized for DC was divided into 2 cohorts: study cohort (305 patients) and validation cohort (139 patients). L-FABP was measured in urine and plasma samples collected at admission. Neutrophil gelatinase-associated lipocalin (NGAL) was also measured in urine samples for comparison.

**Results:** Urine but not plasma L-FABP correlated with 3-month survival on univariate analysis. On multivariate analysis, urine L-FABP and model for end-stage liver disease (MELD)-Na were the only independent predictors of prognosis. Urine L-FABP levels were higher in patients with ACLF than in those without and also predicted the development of ACLF, together with MELD-Na, during follow-up. In patients with ACLF, urine L-FABP correlated with liver, coagulation, and circulatory failure. Urine L-FABP levels were also increased in patients with acute kidney injury, particularly in those with acute tubular necrosis. The ability of urinary L-FABP to predict survival and ACLF

development was confirmed in the validation cohort. Urine NGAL predicted outcome on univariate but not multivariate analysis.

**Conclusions:** Urinary L-FABP levels are independently associated with the 3-month clinical course in patients with DC, in terms of mortality and ACLF development. Urinary L-FABP is a promising prognostic biomarker for patients with DC.

**Lay summary:** Increased levels of liver fatty acid-binding protein (L-FABP), a protein related to lipid metabolism, have been associated with liver-related diseases. The present study analyzed urinary L-FABP levels in 2 independent groups of patients with decompensated cirrhosis and showed that higher urinary L-FABP levels correlated with increased mortality and risk of acute-on-chronic liver failure development. Therefore, urinary L-FABP levels could be useful as a new tool to predict complications in patients with decompensated cirrhosis.

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## Introduction

Fatty acid-binding proteins (FABPs) are a group of intracellular chaperones that are involved in lipid-mediated processes. FABPs are thought to be critical mediators of metabolism and inflammatory pathways.<sup>1-3</sup> FABPs are 14-15 kDa proteins that bind hydrophobic ligands, such as fatty acids. Different FABPs have been described, which have a specific tissue expression pattern, but there is no FABP exclusively expressed in a single tissue.<sup>2</sup> Liver FABP (L-FABP), also known as FABP1, is abundantly expressed in the liver, but also in other tissues such as the kidney, intestine, lungs and pancreas.<sup>2</sup> L-FABP function in the liver is not completely understood. It has been hypothesized that L-FABP participates in the intracellular storage and transport of fatty

Keywords: Liver; Cirrhosis; Acute-On-Chronic Liver Failure; Organ Failure; Mortality; L-FABP; Fatty Acid-Binding Proteins.

Received 27 May 2021; received in revised form 11 August 2021; accepted 30 August 2021; available online 13 September 2021

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<https://doi.org/10.1016/j.jhep.2021.08.031>



acids. L-FABP is also able to bind potentially cytotoxic molecules besides fatty acids, such as heme group.<sup>4</sup>

In previous studies, increased L-FABP levels have been described in the setting of liver tissue injury in different conditions, including liver inflammation after surgical resection,<sup>5,6</sup> acetaminophen-induced acute liver failure,<sup>7</sup> liver transplant rejection,<sup>8</sup> non-alcoholic fatty liver disease<sup>9</sup> and chronic hepatitis C.<sup>10</sup> In this regard, the role of L-FABP as a biomarker in cirrhosis has been assessed but information is very limited.<sup>11,12</sup> Moreover, the potential role of L-FABP as a biomarker in the setting of ACLF has not been investigated.

On this background, we hypothesized that L-FABP could be a biomarker of prognosis and disease progression in cirrhosis, not only by reflecting liver injury but also multiorgan failure and lipid-related metabolic pathways potentially involved in the pathophysiology of ACLF.<sup>13</sup> Despite extensive research, the number of good biomarkers in decompensated cirrhosis (DC) and ACLF remains limited.<sup>11,14–17</sup> Therefore, further research in this field is warranted. In this context, the aim of the present study was to investigate the usefulness of plasma and urinary L-FABP for the prediction of survival and ACLF development in patients with DC. Neutrophil gelatinase-associated lipocalin (NGAL), a previously reported biomarker of prognosis in cirrhosis and ACLF,<sup>15</sup> was also evaluated for comparison.

## Patients and methods

### Study population

The study was performed in a prospective series of 444 patients consecutively admitted for DC, divided into 2 cohorts: a cohort of 305 patients (study cohort) and another cohort of 139 patients (validation cohort). Exclusion criteria were: i) hemodialysis before admission; ii) liver and/or kidney transplantation; iii) admission for elective diagnostic or therapeutic procedures; iv) advanced hepatocellular carcinoma beyond Milan criteria; and v) severe extrahepatic diseases with poor short-term prognosis. All patients signed written informed consent and the protocol was approved by the Institutional Review Board of the Hospital Clinic of Barcelona.

### Study design

Demographic and clinical data and standard liver and kidney function tests were collected at admission in all patients. Urine and plasma samples were also collected at the time of admission. Complications of cirrhosis were managed according to international guidelines.<sup>18</sup> Patients were followed-up for at least 3 months. Presence of ACLF at admission or its development during follow-up were carefully assessed in all patients.

### Definitions

Cirrhosis was diagnosed on the basis of a liver biopsy or by a combination of clinical, laboratory, and ultrasonographic findings, according to current guidelines.<sup>18</sup> ACLF was defined using criteria of the CANONIC study.<sup>19</sup> Acute kidney injury (AKI) was defined according to the current definition of the International Club of Ascites.<sup>20</sup>

### Samples and laboratory measurements

Plasma samples collected at admission were centrifuged at 2,000 rpm for 10 minutes and the supernatant stored at  $-80^{\circ}\text{C}$  until analysis. Urine samples were centrifuged at 1,000 rpm for

10 minutes within the first 4 hours and stored at  $-80^{\circ}$  until analysis.

L-FABP was measured using the Human L-FABP ELISA kit (Hycult Biotech). Coefficients of inter-assay and intra-assay variation for urine and plasma FABPs were lower than 10% and 15%, respectively. Urine L-FABP was expressed in  $\mu\text{g/g}$  creatinine. In addition to L-FABP, NGAL was also measured in urine samples of patients from the study cohort using ELISA, as previously reported.<sup>15,21</sup>

### Statistical analysis

Categorical variables are reported as absolute frequencies and percentages. Quantitative variables are reported as median and interquartile range, or otherwise specified. There was <30% missingness for “CRP” (C-reactive protein) and “Albumin” in the training set, with missing data handled by imputation using the Expectation-Maximization algorithm.<sup>22</sup> This method is valid under the reasonable missing at random assumption (*i.e.* missing data may be predicted from covariates) in this study. No missing data had to be imputed for any other variable in the training or validation sets, and “CRP” and “Albumin” were not used in the predictive models.

Comparisons between variables were carried out using the Fisher exact test for categorical variables and the Mann-Whitney test for continuous variables. Survival function was described using the Kaplan-Meier method.

Factors associated with 90-day mortality were identified in a bivariate analysis. Transplanted patients ( $n = 15$ , 5% of the study cohort;  $n = 6$ , 4% of the validation cohort) were censored at the time of intervention. Factors showing statistically significant association in bivariate analysis were selected for the initial multivariate analysis. Cox regression models were used to select the best subset of predictors having assessed fitting characteristics. Continuous variables were fitted as continuous linear variables and categorical variables (using tertiles and the median cut-offs); deviations from linearity were explored by adding non-linear transformation terms to the model. The proportional hazards assumption was assessed by reviewing the survival function plots. The final model was fitted using a stepwise forward method based on the improvement in model likelihood ratios. Significance levels to enter and drop model variables were adopted as 5% and 10%, respectively. All variables not selected for inclusion ( $p \geq 0.10$ ) were checked against the final model in turn to determine whether their inclusion improved the fit of the model, as evidenced by  $p < 0.10$  or a lower Akaike information criterion value.

The predictive model developed for the study cohort was applied to the validation cohort. The Cox coefficients from the study cohort analyses were fitted to the validation set.

### Calibration and discrimination

Calibration describes how closely the predicted probabilities agree numerically with the actual outcomes.<sup>23</sup> Predicted probabilities from the Cox model were compared against the observed probabilities from the Kaplan-Meier method using the Brier score.<sup>24</sup> A calibration plot assessing whether the observed vs. predicted regression slope was 1 and the intercept 0, as expected from a perfect fit, is shown for the study and validation sets.

Discrimination refers to the ability of the model to correctly distinguish between 2 classes of outcomes such as death and survival.<sup>23,25</sup> The Harrell concordance statistic (95% CI)<sup>26</sup> and

area under the receiver operating characteristic curve (AUROCs<sup>27</sup> at 7, 14, 28, 45, 60 and 90 days) were used to assess discrimination of the model. The predicted Cox and the observed probabilities of death are plotted in a survival plot and compared using the log-rank test.

Statistical analyses were performed using SAS (v9.4; SAS Institute Inc., Cary, NC). This article adheres to the “Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): The TRIPOD statement”.<sup>28</sup>

## Results

### Characteristics of the study population

Demographic and clinical characteristics of the study cohort are shown in Table 1. Most patients were male (65%) and the most common etiology of cirrhosis was alcohol consumption (43%). Patients had moderate to severe impairment of liver function as reflected by a median model for end-stage liver disease (MELD) score of 17 and MELD-Na score of 21. One hundred-and-eleven patients (36%) had ACLF at admission, most of whom had ACLF grade 1 (53 patients, 48% of patients with ACLF). Most patients (77%) had a history of previous complications of cirrhosis before admission.

### Relationship between urine L-FABP levels and mortality

During a 3-month follow-up period, 84 (27%) patients died, 15 (5%) were transplanted and the remaining 206 (68%) were alive at the end of follow-up. Univariate analysis of 3-month survival is shown in Table 2. As expected, patients who died had more marked impairment of liver function compared to those alive at the end of follow-up, as reflected by higher bilirubin levels, international normalized ratio, MELD, and MELD-Na scores. In addition, although there were no significant differences in the

frequency of bacterial infections at admission, patients who died had significantly higher leukocyte count compared to those who were alive at the end of follow-up. Moreover, the presence of AKI and ACLF was significantly more frequent in patients who died compared to those who survived. Finally, urinary NGAL (u-NGAL) and urinary L-FABP (uL-FABP) levels were significantly higher in patients who died than in those who survived. By contrast, there were no significant differences between groups in the plasma levels of L-FABP.

In the multivariate analysis, the best model that predicted 90-day mortality included uL-FABP levels together with MELD-Na score (C-statistic 0.810; 0.767–0.852) (Table 3). uL-FABP was an independent predictive factor after adjustment for variables that could influence uL-FABP levels, such as presence of AKI or bacterial infections, leukocyte count or CRP levels. In contrast to uL-FABP, u-NGAL levels were not associated with survival on multivariate analysis.

We next investigated the relationship between the probability of death and uL-FABP levels according to MELD-Na score values. uL-FABP levels modulated the 90-day prognostic value of MELD-Na. When patients were stratified according to median values of MELD-Na and uL-FABP in the cohort, 90-day mortality was significantly different between groups, in such a way that for the same MELD-Na group, patients with higher uL-FABP levels had significantly higher probability of mortality than those within the same MELD-Na group but with lower uL-FABP levels (Fig. 1).

### Validation cohort

To validate the role of uL-FABP we analyzed an independent cohort of 139 patients prospectively recruited within a subsequent 2-year period. The inclusion and exclusion criteria for the

**Table 1. Baseline demographic and clinical characteristics of patients included in the validation cohort compared to the study cohort.**

Variable	Study cohort (n = 305)	Validation cohort (n = 139)	p value
Age (years)	59 (52–68)	60 (52–65)	0.586
Female sex	108 (35)	44 (32)	0.439
Etiology			<0.001
Alcohol related	131 (43)	86 (62)	
Alcohol related + HCV	36 (12)	12 (9)	
HCV	95 (31)	19 (14)	
Other	43 (14)	22 (15)	
Presence of ascites	204 (67)	91 (66)	0.769
Presence of hepatic encephalopathy	101 (33)	51 (37)	0.462
Albumin (g/L)	28 (25–32)	29 (25–34)	0.169
Bilirubin (mg/dl)	2.4 (1.3–5.2)	2.5 (1.2–7.6)	0.796
INR	1.55 (1.32–1.88)	1.54 (1.30–1.95)	0.944
Serum creatinine (mg/dl)	1.2 (0.8–1.9)	1.1 (0.6–1.9)	0.063
Serum sodium (mEq/L)	135 (131–138)	136 (133–139)	0.015
Leucocyte count (x10 <sup>9</sup> /mm <sup>3</sup> )	5.7 (3.9–8.8)	6.4 (4.3–9.8)	0.136
C-reactive protein (mg/dl)	2.2 (0.9–5.1)	2.6 (0.9–5.1)	0.0504
MELD sodium score	21 (16–28)	21 (15–28)	0.579
AKI	143 (47)	74 (53)	0.214
Bacterial infections	117 (38)	78 (56)	0.001
ACLF	111 (36)	58 (42)	0.225
Grade 1	53 (48)	31 (54)	
Grade 2	34 (30)	17 (29)	
Grade 3	24 (22)	10 (17)	
u-NGAL (µg/g creatinine)	46 (23–125)	NA	–
Plasma L-FABP (ng/ml)	25 (17–39)	NA	–
uL-FABP (µg/g creatinine)	30 (15–69)	30 (13–66)	0.812

NA, not available; plasma L-FABP and u-NGAL were not available in patients from the validation cohort.

Values are presented as numbers (%) or median (IQR). Fisher exact test was used for categorical variables and the Mann-Whitney test for continuous variables.

ACLF, acute-on-chronic liver failure; AKI, acute kidney injury; INR, international normalized ratio; MELD, model for end-stage liver disease; u-NGAL, urinary neutrophil gelatinase-associated lipocalin; uL-FABP, urinary liver fatty acid-binding protein.

**Table 2. Baseline characteristics of patients from study cohort according to 3-month survival.**

Variable	Alive <sup>#</sup> (n = 221)	Dead (n = 84)	HR (95% CI)	p value
Age (years)	58 (51–66)	56 (49–65)	0.998 (0.979–1.017)	0.8038
Sex (female)	82 (37)	26 (31)	0.773 (0.486–1.227)	0.2744
Etiology				0.9655
Alcohol	93 (42)	39 (45)	Ref	
Alcohol + HCV	27 (12)	9 (11)	0.849 (0.411–1.756)	
HCV	70 (32)	25 (30)	0.906 (0.547–1.500)	
Other	31 (14)	12 (14)	0.939 (0.490–1.796)	
Presence of ascites	134 (61)	70 (83)	2.834 (1.596–5.032)	0.0004
Presence of hepatic encephalopathy	54 (24)	47 (56)	3.331 (2.163–5.129)	<0.0001
Albumin (g/L)	28 (25–31)	28 (25–32)	0.988 (0.948–1.029)	0.5613
Bilirubin (mg/dl)	2.2 (1.2–3.9)	5.5 (2.3–22.9)	1.078 (1.061–1.096)	<0.0001
INR	1.5 (1.4–1.8)	1.9 (1.6–2.8)	2.450 (2.015–2.978)	<0.0001
Serum creatinine (mg/dl)	1.1 (0.8–1.8)	2.0 (1.1–2.9)	1.663 (1.448–1.909)	<0.0001
Serum sodium (mEq/L)	136 (132–138)	131 (126–136)	0.901 (0.872–0.930)	<0.0001
Leucocyte count (x10 <sup>9</sup> /mm <sup>3</sup> )	5.2 (3.7–7.8)	7.5 (5.4–12.0)	1.128 (1.083–1.175)	<0.0001
C-reactive protein (mg/dl)	2.2 (0.8–5.0)	2.4 (1.6–5.9)	1.013 (0.966–1.062)	0.5861
MELD score	17 (13–22)	29 (22–35)	1.118 (1.094–1.142)	<0.0001
MELD sodium score	21 (15–26)	31 (25–36)	1.140 (1.111–1.170)	<0.0001
AKI	83 (38)	60 (71)	3.727 (2.318–5.993)	<0.0001
Bacterial infections	81 (37)	36 (43)	1.280 (0.831–1.972)	0.281
ACLF at inclusion	57 (26)	54 (64)	4.445 (2.836–6.967)	<0.0001
Grade 1	36 (16)	17 (20)		
Grade 2	15 (7)	19 (23)		
Grade 3	6 (3)	18 (21)		
u-NGAL (µg/g creatinine)	39 (18–106)	84 (37–263)	1.001 (1.000–1.001)	<0.0001
Plasma L-FABP (ng/ml)	24 (16–37)	26 (18–46)	1.001 (0.997–1.005)	0.6018
uL-FABP (µg/g creatinine)	27 (12–64)	52 (23–117)	1.010 (1.003–1.016) <sup>##</sup>	0.006

Values are presented as numbers (%) or median (IQR). Fisher exact test was used for categorical variables and the Mann-Whitney test for continuous variables. ACLF, acute-on-chronic liver failure; AKI, acute kidney injury; HR, hazard ratio; INR, international normalized ratio; MELD, model for end-stage liver disease; uL-FABP, urinary liver fatty acid-binding protein; u-NGAL, urinary neutrophil gelatinase-associated lipocalin.  
<sup>#</sup>Transplanted patients were censored at the time of intervention.  
<sup>##</sup>HR per 10 unit increase.

validation cohort were the same as those used for the study cohort. Comparison of baseline demographic, clinical, and laboratory characteristics of the study cohort and validation cohort are shown in Table 1. Patients from the validation cohort had similar baseline characteristics to those from the study cohort. There were only differences in etiology of cirrhosis, with a lower prevalence of HCV infection and higher prevalence of infections at admission in the validation vs. study cohort. In the validation

cohort, fifty-eight (42%) patients had ACLF at admission and baseline MELD-Na score was 21 (15–28). Baseline median uL-FABP levels were similar between both cohorts.

Thirty-six (26%) patients in the validation cohort died during the 3-month follow-up period. Univariate analysis of survival is shown in Table S1. Results from the multivariate analysis validated those obtained in the study cohort, showing that uL-FABP and MELD-Na score were independent predictive factors of 3-month mortality, and the model had a good discrimination performance as shown graphically in Fig. 2 (log-rank, *p* <0.0001) and confirmed by the C-statistic for the study (0.810 [0.768–0.852]) and the validation (0.819 [0.886–0.752]) cohort. The calibration plot showed a good fit between the observed and the predicted survival probabilities, and no statistical differences from a perfect fit (*i.e.* intercept = 0 and slope = 1) were found for both the study and the validation cohorts (Fig. 3).

**Table 3. Independent predictive factors associated with 3-month mortality and ACLF development.**

Variable	Units	HR (LL-UL)	p value
<b>3-month mortality<sup>*</sup></b>			
MELD-Na	1	1.135 (1.106–1.165)	0.0001
uL-FABP	10	1.026 (1.011–1.041)	0.0006
<b>ACLF development<sup>†</sup></b>			
MELD-Na	1	1.321 (1.194–1.462)	<0.0001
uL-FABP	10	1.044 (1.017–1.072)	0.0014

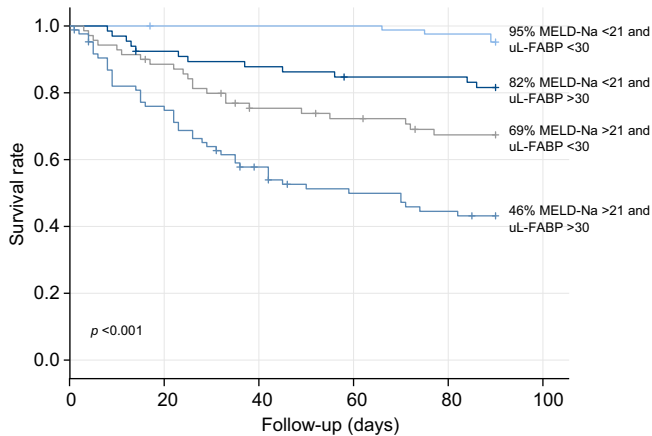
ACLF, acute-on-chronic liver failure; HR, hazard ratio; LL, lower limit; MELD-Na, model for end-stage liver disease-sodium; uL-FABP, urinary liver fatty acid-binding protein; UL, upper limit.

<sup>\*</sup>C-statistic: 0.810 (0.767–0.852); AUROC by time: 0.902 at 7 days, 0.846 at 14 days, 0.859 at 28 days, 0.854 at 45 days, 0.853 at 60 days and 0.825 at 90 days; Variables included in multivariate analysis were: AKI, ascites, hepatic encephalopathy, ACLF, circulatory failure, renal failure, liver failure, coagulation failure, brain failure, ACLF grade, number of organ failures, serum creatinine, bilirubin, INR, leucocyte count, serum sodium, mean arterial pressure, uL-FABP, u-NGAL, MELD, MELD-Na, Child Pugh score.

<sup>†</sup>C-statistic: 0.878 (0.808–0.948); AUROC by time: 0.868 at 7 days, 0.885 at 14 days, 0.896 at 28 days, and 0.902 after 45 days; Variables included in multivariate analysis were: AKI, ascites, hepatic encephalopathy, serum creatinine, bilirubin, INR, leucocyte count, serum sodium, mean arterial pressure, uL-FABP, u-NGAL, MELD, MELD-Na, Child Pugh score.

**Relationship between urine L-FABP levels and ACLF**

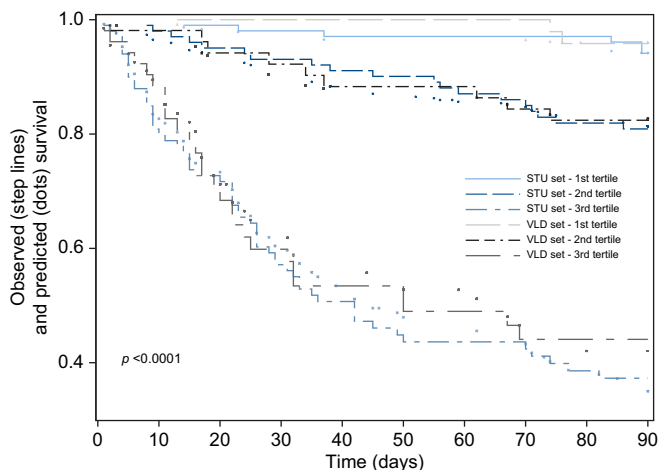
We next sought to determine the relationship between uL-FABP and the presence or development of ACLF. In the study cohort, 111 (36%) patients had ACLF at admission (Table 1). Patients with ACLF had significantly higher baseline uL-FABP levels compared to those of patients without ACLF (45 [18–89] vs. 25 [14–60] µg/g of creatinine, *p* = 0.005; respectively). Moreover, uL-FABP levels increased in parallel with ACLF severity. By contrast, there were no significant differences in plasma L-FABP levels according to the presence and severity of ACLF (Fig. 4). Similar findings were observed in the validation cohort: patients with ACLF also had higher levels of uL-FABP compared to those of patients who did



**Fig. 1. 90-day survival according to median MELD-Na and median uL-FABP.** Kaplan-Meier survival curves grouped per median MELD-Na and median uL-FABP levels in the study cohort. Units of uL-FABP are  $\mu\text{g/g}$  of creatinine. Level of significance:  $p < 0.001$  (Log-rank test). MELD, model for end-stage liver disease; uL-FABP, urinary liver fatty acid-binding protein.

not have ACLF at admission (54 [23–187] vs. 21 [9–39]  $\mu\text{g/g}$  of creatinine,  $p < 0.001$ , respectively). Moreover, uL-FABP levels also increased with disease severity (Fig. S1). Interestingly, uL-FABP levels also correlated with the type of acute decompensation as defined by the classification of the PREDICT study,<sup>29</sup> i.e. patients with pre-ACLF had significantly higher uL-FABP levels compared to patients with stable or unstable DC (58 [35–106] vs. 23 [12–56] and 19 [14–41]  $\mu\text{g/g}$  of creatinine in stable and unstable DC, respectively;  $p = 0.027$ ).

We also investigated the relationship between uL-FABP levels and the types of organ failures in patients with ACLF. Renal failure was the most common organ failure (77 patients, 25%), followed by liver failure (43 patients, 14%) and circulatory failure (41 patients, 13%). Notably, patients with liver failure, coagulation failure, and circulatory failure had significantly higher uL-FABP levels than patients without these organ failures. By



**Fig. 2. Survival plot with the observed (step lines) and predicted (marks) probabilities of death stratified per tertiles from the Kaplan-Meier and Cox model for the study (STU) and validation (VLD) cohorts.** Level of significance:  $p < 0.0001$  (Log-rank test).

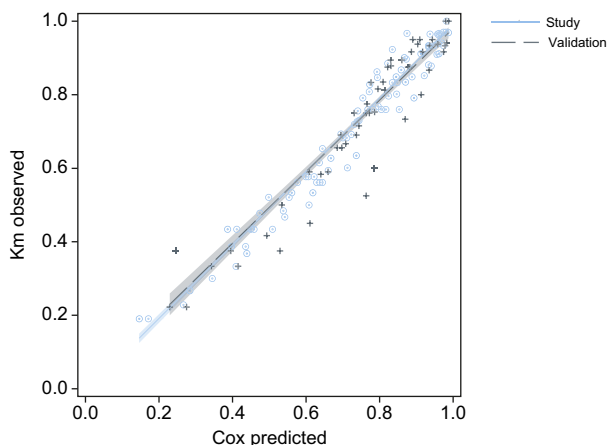
contrast, uL-FABP levels did not correlate with brain, renal, and respiratory failure (Table 4). Because L-FABP can be overexpressed in the kidneys in the setting of acute tubular injury and increased uL-FABP levels have been reported in patients with cirrhosis and AKI,<sup>30,31</sup> we further investigated the potential relationship between uL-FABP and kidney failure. Interestingly, patients meeting the criteria of AKI ( $n = 143$ ) had significantly higher levels of uL-FABP compared to patients without AKI ( $n = 162$ ) (39 [15–87] vs. 25 [13–60]  $\mu\text{g/g}$  of creatinine, respectively;  $p = 0.01$ ). Moreover, when the etiology of AKI was considered, patients with acute tubular necrosis (ATN) had significantly higher levels compared to patients with hepatorenal syndrome (89 [30–149] vs. 39 [14–66]  $\mu\text{g/g}$  of creatinine, respectively;  $p < 0.001$ ). Taken together, these findings suggest that uL-FABP levels are increased in the setting of AKI, particularly in the presence of ATN.

To further investigate the role of uL-FABP as a biomarker for ACLF, we assessed whether uL-FABP levels were able to predict the development of ACLF. Eighteen out of the 194 patients without ACLF at admission (9%) in the study cohort developed it during hospitalization or during the 3-month follow-up period (median time 7 [2–13] days): 3 patients (17%) ACLF 1, 6 patients (33%) ACLF 2 and 9 patients (50%) ACLF 3. Patients who developed ACLF during follow-up had significantly worse liver and kidney function tests at admission compared to patients who did not develop ACLF, as assessed by higher MELD-Na score, bilirubin or international normalized ratio levels, and with the presence of AKI at admission (Table S2). However, the presence of bacterial infections at admission was not different between patients who developed or did not develop ACLF. Patients who developed ACLF during follow-up had significantly higher uL-FABP levels at admission compared to patients who did not develop ACLF. In the multivariate analysis, again MELD-Na score and uL-FABP levels were the only independent factors associated with the development of ACLF during follow-up (C-statistic 0.878 [0.808–0.948]) (Table 3). u-NGAL levels showed a moderate correlation with uL-FABP levels ( $r = 0.335$ ,  $p < 0.001$ ) and were associated with development of ACLF in the univariate analysis but not in the multivariate analysis.

## Discussion

In the present study we investigated the role of uL-FABP as a prognostic biomarker in patients with DC, with and without ACLF. The study has 3 major findings. First, uL-FABP levels are independently associated with 3-month mortality in hospitalized patients with DC, together with MELD-Na score. Second, uL-FABP levels are associated with liver-related organ failures and with circulatory failure in patients with ACLF. Finally, uL-FABP levels are associated with the risk of developing ACLF during follow-up.

MELD-Na has been shown to improve the prognostic accuracy of the MELD score and is able to reclassify patients who are at higher risk of mortality despite lower MELD scores.<sup>32,33</sup> In this regard, MELD-Na is the most widely used method for liver allocation in patients awaiting liver transplantation because it provides a good prognostic stratification of patients with DC. Nonetheless, there are still limitations when using MELD-Na. In fact, data from a recent study in a large cohort of patients with cirrhosis show that patients with persistently low MELD-Na score values still have significantly higher rates of liver-related mortality.<sup>32</sup> Therefore, there is a need to improve prognosis



**Fig. 3. Calibration plot comparing the observed and predicted probabilities of death from the Kaplan-Meier and Cox model for the study and validation cohorts.** The estimated intercept (95% CI) and slope (95% CI) for the study set were -0.01 (-0.025 to 0.006) and 1.001 (0.981 to 1.02), respectively, and for the validation set 0.006 (-0.035 to 0.047) and 0.974 (0.922 to 1.026), respectively. No statistical significance was found when testing whether the intercept was different from 0 ( $p = 0.212$  and  $p = 0.765$  for the study and validation sets, respectively), and when testing whether the slope was different from 1 ( $p = 0.936$  and  $p = 0.326$  for the study and validation sets, respectively). The Brier score (95% CI) for the study and validation sets were 0.216 (0.182-0.250) and 0.206 (0.159-0.252), respectively.

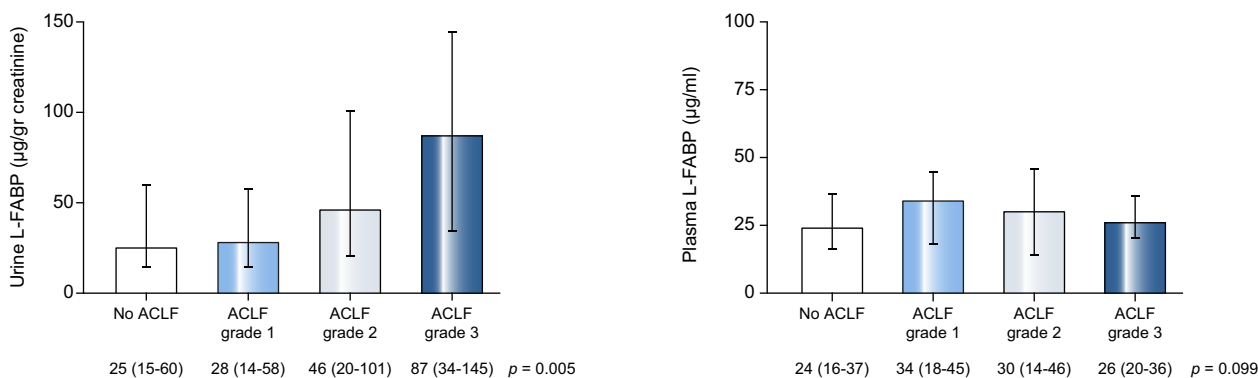
stratification in patients with DC. Findings from the present study clearly show that uL-FABP levels represent an independent predictor of 3-month mortality together with MELD-Na score.

L-FABP has been reported to be increased in the context of liver injury in different conditions.<sup>5-10</sup> In addition, L-FABP is involved in inflammatory processes and lipid metabolism.<sup>34,35</sup> It is well known that DC is associated with chronic systemic inflammation that correlates with disease severity and clinical outcomes.<sup>36-38</sup> Therefore, we hypothesize that there are a couple of major reasons explaining why uL-FABP could act as a prognostic biomarker in cirrhosis. First, uL-FABP levels may reflect not only liver injury, but also multiorgan dysfunction of patients with DC. Second, uL-FABP levels may reflect the systemic inflammatory milieu occurring in DC, which is not captured by MELD-Na score variables.

To date, several scores have been reported to predict mortality in patients with ACLF.<sup>39-42</sup> However, there is no widely

validated method or biomarker to predict the risk of developing ACLF. Considering its dismal short-term prognosis, identifying tools that help to predict the development of ACLF is an unmet need. In the present study we showed that uL-FABP is significantly higher in patients with ACLF and that it correlates with some organ failures, particularly liver, circulation and coagulation. Moreover, uL-FABP levels also correlated with the existence of renal failure, specifically with the presence of ATN. The discrepancy between differences in uL-FABP levels according to the definition of renal failure used, either the ACLF definition or the AKI definition, is likely because of the different diagnostic criteria used (>2 mg/dl of serum creatinine in ACLF and AKI standard criteria, respectively).<sup>19,20</sup> In addition, interestingly, our results showed that uL-FABP levels are higher in patients without ACLF who will develop ACLF during follow-up. Therefore, uL-FABP could be a useful biomarker to identify patients at risk of ACLF. Nevertheless, this finding should be interpreted with caution because of the reduced number of patients developing ACLF during follow-up in the current series.

L-FABP plays a role in fatty acid trafficking and metabolism, and in the conversion of fatty acids to eicosanoid intermediates and in the stabilization of leukotrienes.<sup>2</sup> Recent data suggest that patients with ACLF have impairment in fatty acid beta oxidation<sup>43</sup> similar to that of inflammatory conditions such as sepsis. In addition, it has been reported that patients with ACLF display a specific lipid profile that correlates with the stage of the disease and prognosis.<sup>13</sup> High levels of leukotriene 4 have been associated with the presence of ACLF and its severity. Moreover, differences in lipid profile were found in patients with liver, coagulation or circulatory failure, despite the lack of differences in lipid profile in patients with renal, brain or respiratory failure.<sup>13</sup> Our findings are consistent with these results and taken together we can hypothesize that increased uL-FABP levels may reflect the activation of inflammatory pathways through lipid mediators occurring in ACLF, where FABPs, and particularly L-FABP may play an important role. The reason why urine but not plasma levels of L-FABP are predictive of prognosis is intriguing and could not be unraveled from the results of the current study. It is possible that an increased renal production of the peptide could explain the relationship between uL-FABP levels and prognosis in some patients, particularly in those with associated ATN. However, further studies should be performed to investigate this issue.



**Fig. 4. Urinary and plasma L-FABP levels according to the presence and severity of ACLF.** Level of significance:  $p = 0.005$  and  $p = 0.099$ , respectively. (Kruskal-Wallis test). ACLF, acute-on-chronic liver failure; L-FABP, liver fatty acid-binding protein; uL-FABP, urinary liver fatty acid-binding protein.

**Table 4. uL-FABP levels according to the presence of organ failures in patients with ACLF.**

	Yes	No	p value
Liver failure	79 (36–136)	25 (13–60)	<0.0001
Coagulation failure	65 (25–136)	28 (15–65)	0.0034
Circulatory failure	81 (39–148)	25 (14–60)	<0.0001
Brain failure	41 (19–97)	29 (15–67)	0.1198
Renal failure	36 (15–84)	29 (15–65)	0.2661
Respiratory failure	55 (22–110)	29 (15–68)	0.1371

Values are presented as median (IQR). Units are µg/g of creatinine. Fisher exact test was used for categorical variables.

ACLF, acute-on-chronic liver failure; uL-FABP, urinary liver fatty acid-binding protein.

The current study has some strengths and limitations that should be mentioned. First, the study includes a large prospectively collected cohort of patients with DC. In addition, results were validated in an independent cohort of patients. However, this is a single-center study and, therefore, results should be validated in multicenter studies. Finally, the design of our study does not allow us to draw mechanistic conclusions about the role of L-FABP in the pathophysiology of ACLF. However, our results suggest a mechanistic hypothesis that will need to be investigated in future studies.

In conclusion, our study shows that urinary L-FABP is a promising biomarker to predict mortality in patients with DC. In addition, L-FABP is a good biomarker of ACLF and may reflect that inflammation and impaired lipid metabolism are involved in the pathophysiology of the syndrome.

#### Abbreviations

AKI, acute kidney injury; ACLF, acute-on-chronic liver failure; ATN, acute tubular necrosis; AUROC, area under the receiver operating characteristic curve; CRP, C-reactive protein; DC, decompensated cirrhosis; FABPs, fatty acid-binding proteins; L-FABP, liver fatty acid-binding protein; MELD, model for end-stage liver disease; uL-FABP, urinary liver fatty acid-binding protein; u-NGAL, urinary neutrophil gelatinase-associated lipocalin.

#### Financial support

AJ is funded by Contratos Río Hortega (CM19/00044) granted by Instituto de Salud Carlos III, by the Award 'Emili Letang' granted by Hospital Clínic de Barcelona and by the "Ajut per a la Iniciació a la Recerca" granted by Societat Catalana de Digestologia. Supported by a grant awarded to P.G. (PI16/00043 and PI20/00579), integrated in the Plan Nacional Investigación científica, Desarrollo e Innovación Tecnológica (I+D+I) and cofunded by Instituto Carlos III (ISCIII)-Subdirección General de Evaluación and the European Regional Development Fund. E.S. and G.C. are funded by Plan Nacional Investigación científica, Desarrollo e Innovación Tecnológica (I+D+I) and cofunded by Instituto Carlos III (ISCIII)-Subdirección General de Evaluación and the European Regional Development Fund (PI18/00727) and IG is funded by Plan Nacional Investigación científica, Desarrollo e Innovación Tecnológica (I+D+I) and cofunded by Instituto Carlos III (ISCIII)-Subdirección General de Evaluación and the European Regional Development Fund (PI18/0862), European H2020 Program; LIV-ERHOPE PROJECT - Grant number 731875.

#### Conflicts of interest

PG declares that he has received research funding from Malinckrodt, Grifols and Gilead. He has participated on advisory boards for Novartis, Gilead and Martin Pharmaceuticals.

Please refer to the accompanying ICMJE disclosure forms for further details.

#### Authors' contributions

The authors listed above have all contributed to this manuscript and approve the version of this submission. AJ, IG, CE, SP, ES and PG contributed to the conception and design of the study, acquisition of data, the analysis and interpretation of the data and drafting the manuscript; CS, MC, MPG, OB, LE, ABR, MC, LN, EA, ATM, NF, EP, MMR, WJ, FR and GC participated in the generation and collection of data, assembly of data, analyses of the results, interpretation of data, and/or critical revision of the manuscript for important intellectual content.

#### Data availability statement

The datasets generated and analyzed during the current study are not publicly available due to confidentiality agreements.

#### Acknowledgements

We thank Nicki Van Berckel, Roser Poblet, and Beatriz Márquez for administrative support and help in the preparation of this manuscript. We also thank the faculty and nurses of the Liver Unit, and the patients who participated in the study and their families. We are indebted to the Biobank core facility of the IDIBAPS for the technical help provided.

#### Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2021.08.031>.

#### References

*Author names in bold designate shared co-first authorship*

- [1] Hotamisligil GS, Bernlohr DA. Metabolic functions of FABPs—mechanisms and therapeutic implications. *Nat Rev Endocrinol* 2015;11:592–605.
- [2] Furuhashi M, Hotamisligil GS. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. *Nat Rev Drug Discov* 2008;7:489.
- [3] Storch J, Thumser AE. Tissue-specific functions in the fatty acid-binding protein family. *J Biol Chem* 2010;285:32679–32683.
- [4] Wang G, Bonkovsky HL, de Lemos A, Burczynski FJ. Recent insights into the biological functions of liver fatty acid binding protein 1. *J Lipid Res* 2015;56:2238–2247.
- [5] **van den Broek MAJ, Bloemen JG**, Dello SAWG, van de Poll MCG, Olde Damink SWM, Dejong CHC. Randomized controlled trial analyzing the effect of 15 or 30 min intermittent Pringle maneuver on hepatocellular damage during liver surgery. *J Hepatol* 2011;55:337–345.
- [6] **van de Poll MCG, Derikx JPM**, Buurman WA, Peters WHM, Roelofs HMJ, Wigmore SJ, et al. Liver manipulation causes hepatocyte injury and precedes systemic inflammation in patients undergoing liver resection. *World J Surg* 2007;31:2033–2038.
- [7] Karvellas CJ, Speiser JL, Tremblay M, Lee WM, Rose CF, Group USALFS. Elevated FABP1 serum levels are associated with poorer survival in acetaminophen-induced acute liver failure. *Hepatology* 2017;65:938–949.
- [8] Pelsers MMAL, Morovat A, Alexander GJM, Hermens WT, Trull AK, Glatz JFC. Liver fatty acid-binding protein as a sensitive serum marker of acute hepatocellular damage in liver transplant recipients. *Clin Chem* 2002;48:2055–2057.
- [9] Charlton M, Viker K, Krishnan A, Sanderson S, Veldt B, Kaalsbeek AJ, et al. Differential expression of lumican and fatty acid binding protein-1: new insights into the histologic spectrum of nonalcoholic fatty liver disease. *Hepatology* 2009;49:1375–1384.
- [10] Akbal E, Köklü S, Koçak E, Çakal B, Güneş F, Başar Ö, et al. Liver fatty acid-binding protein is a diagnostic marker to detect liver injury due to chronic hepatitis C infection. *Arch Med Res* 2013;44:34–38.

- [11] Graupera I, Coll M, Pose E, Elia C, Piano S, Solà E, et al. Adipocyte fatty-acid binding protein is overexpressed in cirrhosis and correlates with clinical outcomes. *Sci Rep* 2017;7:1829.
- [12] Belcher JM, Sanyal AJ, Peixoto AJ, Perazella MA, Josepg L, Thiessen-Philbrook H, et al. Kidney biomarkers and differential diagnosis of patients with cirrhosis and acute kidney injury. *Hepatology* 2013;60:622–632.
- [13] López-Vicario C, Checa A, Urdangarin A, Aguilar F, Alcaraz-Quiles J, Caraceni P, et al. Targeted lipidomics reveals extensive changes in circulating lipid mediators in patients with acutely decompensated cirrhosis. *J Hepatol* 2020;73:817–828.
- [14] Solà E, Kerbert AJC, Verspaget HW, Moreira R, Pose E, Ruiz P, et al. Plasma copeptin as biomarker of disease progression and prognosis in cirrhosis. *J Hepatol* 2016;65:914–920.
- [15] **Ariza X, Graupera I**, Coll M, Solà E, Barreto R, García E, et al. Neutrophil gelatinase-associated lipocalin is a biomarker of acute-on-chronic liver failure and prognosis in cirrhosis. *J Hepatol* 2016;65:57–65.
- [16] Grønbaek H, Rødgaard-Hansen S, Aagaard NK, Arroyo V, Moestrup SK, García E, et al. Macrophage activation markers predict mortality in patients with liver cirrhosis without or with acute-on-chronic liver failure (ACLF). *J Hepatol* 2016;64:813–822.
- [17] Macdonald S, Andreola F, Bachtiger P, Amoros A, Pavesi M, Mookerjee R, et al. Cell death markers in patients with cirrhosis and acute decompensation. *Hepatology* 2018;67:989–1002.
- [18] European Association for the Study of the Liver. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J Hepatol* 2018;69:406–460.
- [19] Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-Chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013;144:1426–1437.e9.
- [20] Angeli P, Ginès P, Wong F, Bernardi M, Boyer TD, Gerbes A, et al. Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. *J Hepatol* 2015;62:968–974.
- [21] Huelin P, Solà E, Elia C, Solé C, Risso A, Moreira R, et al. Neutrophil gelatinase-associated lipocalin for assessment of acute kidney injury in cirrhosis. A prospective study. *Hepatology* 2019;70:319–333.
- [22] Dempster AP, Laird NM, Rubin DB. Maximum likelihood from incomplete data via the EM algorithm. *J R Stat Soc Ser B* 1977;39:1–38.
- [23] D'Agostino Sr RB, Grundy S, Sullivan LM, Wilson P. Group for the CHDRP. Validation of the framingham coronary heart disease prediction scores: results of a multiple ethnic groups investigation. *JAMA* 2001;286:180–187.
- [24] Gerds TA, Cai T, Schumacher M. The performance of risk prediction models. *Biometrical J* 2008;50:457–479.
- [25] Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, et al. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001;33:464–470.
- [26] Harrell Jr FE, Califf RM, Pryor DB, Lee KL, Rosati RA. Evaluating the yield of medical tests. *JAMA* 1982;247:2543–2546.
- [27] Heagerty PJ, Zheng Y. Survival model predictive accuracy and ROC curves. *Biometrics* 2005;61:92–105.
- [28] Collins GS, Reitsma JB, Altman DG, Moons KGM. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD). *Ann Intern Med* 2015;162:735–736.
- [29] Trebicka J, Fernandez J, Papp M, Caraceni P, Laleman W, Gambino C, et al. The PREDICT study uncovers three clinical courses of acutely decompensated cirrhosis that have distinct pathophysiology. *J Hepatol* 2020;73:842–854.
- [30] Yamamoto T, Noiri E, Ono Y, Doi K, Negishi K, Kamijo A, et al. Renal L-type fatty acid-binding protein in acute ischemic injury. *J Am Soc Nephrol* 2007;18:2894–2902.
- [31] Belcher JM, Garcia-Tsao G, Sanyal AJ, Thiessen-Philbrook H, Peixoto AJ, Perazella MA, et al. Urinary biomarkers and progression of AKI in patients with cirrhosis. *Clin J Am Soc Nephrol* 2014;9:1857–1867.
- [32] Mazumder NR, Atiemo K, Daud A, Kho A, Abecassis M, Levitsky J, et al. Patients with persistently low MELD-Na scores continue to be at risk of liver-related death. *Transplantation* 2020;104:1413–1418.
- [33] Kim WR, Biggins SW, Kremers WK, Wiesner RH, Kamath PS, Benson JT, et al. Hyponatremia and mortality among patients on the liver-transplant waiting list. *N Engl J Med* 2008;359:1018–1026.
- [34] Nakamura T, Sugaya T, Koide H. Urinary Liver-Type Fatty Acid-Binding protein in septic shock: effect of Polymyxin B-immobilized fiber hemoperfusion. *Shock* 2009;31.
- [35] Derikx JPM, Poeze M, van Bijnen AA, Buurman WA, Heineman E. Evidence for intestinal and liver epithelial cell injury in the early phase of sepsis. *Shock* 2007;28:544–548.
- [36] Clària J, Stauber RE, Coenraad MJ, Moreau R, Jalan R, Pavesi M, et al. Systemic inflammation in decompensated cirrhosis: characterization and role in acute-on-chronic liver failure. *Hepatology* 2016;64:1249–1264.
- [37] Sole C, Solà E, Huelin P, Carol M, Moreira R, Cereijo U, et al. Characterization of inflammatory response in hepatorenal syndrome: relationship with kidney outcome and survival. *Liver Int* 2019;39:1246–1255.
- [38] Solé C, Solà E, Morales-Ruiz M, Fernández G, Huelin P, Graupera I, et al. Characterization of inflammatory response in acute-on-chronic liver failure and relationship with prognosis. *Sci Rep* 2016;6:32341.
- [39] **Jalan R, Saliba F, Pavesi M**, Amoros A, Moreau R, Ginès P, et al. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. *J Hepatol* 2014;61:1038–1047.
- [40] O'Leary JG, Reddy KR, Garcia-Tsao G, Biggins SW, Wong F, Fallon MB, et al. NACSELD acute-on-chronic liver failure (NACSELD-ACLF) score predicts 30-day survival in hospitalized patients with cirrhosis. *Hepatology* 2018;67:2367–2374.
- [41] Hernaez R, Liu Y, Kramer JR, Rana A, El-Serag HB, Kanwal F. Model for end-stage liver disease-sodium underestimates 90-day mortality risk in patients with acute-on-chronic liver failure. *J Hepatol* 2020.
- [42] Grønbaek H, Møller HJ, Saliba F, Zeuzem S, Albillos A, Ariza X, et al. Improved prediction of mortality by combinations of inflammatory markers and standard clinical scores in patients with acute-on-chronic liver failure and acute decompensation. *J Gastroenterol Hepatol* 2021;36:240–248.
- [43] **Moreau R, Clària J, Aguilar F, Fenaille F**, Lozano JJ, Junot C, et al. Blood metabolomics uncovers inflammation-associated mitochondrial dysfunction as a potential mechanism underlying ACLF. *J Hepatol* 2020;72:688–701.