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Improved prediction of mortality by combinations of inflammatory markers and standard clinical scores in patients with acute-on-chronic liver failure and acute decompensation

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Abbreviations: ACLF (Acute-on-Chronic Liver Failure), AD (Acute Decompensation), MR (Mannose receptor), NGAL (neutrophil gelatinase associated lipocalin), DAMPs (Damage associated molecular patterns), PAPMs (Pathogen associated molecular patterns), HNA (redox state of circulating albumin)

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Abstract

Background and aim: Acute-on-chronic liver failure (ACLF)
as a sinister prognosis and there is a need for accurate biomarkers and scoring systems to better characterize ACLF patients and predict prognosis. Systemic inflammation and renal failure are hallmarks in ACLF disease development and progression. We hypothesized that the combination of specific inflammatory markers in combination with clinical scores are better predictors of survival than the originally developed CLIF-C acute decompensation (AD) and CLIF-C ACLF scores.

Methods: We re-evaluated all previously measured inflammatory markers in 522 patients from the CANONIC study, 342 *without* and 180 *with* ACLF. We used the Harrell’s C-index to determine the best marker alone or in combination with the original scores and calculated new scores for prediction of mortality in the original CANONIC cohort.

Results: The best markers to predict 90-day mortality in patients *without* ACLF were the plasma macrophage activation markers soluble (s)CD163 and mannose receptor (sMR).

Urinary neutrophil gelatinase associated lipocalin (UNGAL) and sCD163 were predictors for 28-day mortality in patients *with* ACLF. The new developed CLIF-C AD+sMR score in patients *without* ACLF improved 90-days mortality prediction compared to the original CLIF-C AD score (C-index 0.82(0.78-0.86) vs. 0.74(0.70-0.78, P=0.004). Further, the new CLIF-C ACLF+sCD163+UNGAL improved the original CLIF-C ACLF score for 28-days mortality (0.85(0.79-0.91) vs. 0.75(0.70-0.80), P=0.039).

Conclusions: The capability of these inflammatory markers to improve the original prognostic scores in cirrhosis patients *without* and *with* ACLF points to a key role of macrophage activation and inflammation in the development and progression of AD and ACLF.

Key words:

Hepatic inflammation, Kupffer cell, ACLF, Cirrhosis

Acute decompensation, CD163, Mannose receptor, Neutrophil gelatinase associated lipocalin (NGAL)

Introduction:

Acute-on-chronic liver failure (ACLF) is a disease entity that may develop in patients with chronic liver disease. The patients present with increasing number of organ failures and therefore hold a sinister prognosis. Acute decompensation (AD) represents another spectrum of liver disease progression with development of complications to liver cirrhosis and an increased risk for the development of ACLF. The CLIF CANONIC study [1] aimed to investigate primarily ACLF but also AD in patients with liver cirrhosis. From the CANONIC study prognostic clinical scores have been developed. The CLIF-C ACLF score is calculated by the CLIF-C organ failure score (CLIF-C OF score [1]), combined with age and the white blood cell (WBC) count [2]. The CLIF-C AD score is based on age, S-sodium, WBC, creatinine and INR [3].

A hallmark of AD and especially ACLF development and progression is local and systemic inflammation. In cirrhotic patients *without* ACLF AD may develop as a consequence of bacterial translocation from the intestines and associated pathogen associated molecular patterns (PAMPs) with subsequent initially local intestinal inflammation followed by systemic inflammation. Further propagation may occur due to production of damage associated molecular patterns (DAMPs) in the liver and other organs [4]. ACLF is an acute incident developing in cirrhosis patients and most often caused by a precipitating event e.g. sepsis, alcoholic, viral or ischemic hepatitis, TIPS or surgical procedures accompanied by acute

systemic inflammation [1].

Both local and especially systemic inflammation are very important for both AD and ACLF, and the WBC is a key component of the prognostic CLIF-C AD and CLIF-C ACLF scores. During the last years a number of other markers of inflammation and liver disease severity has been investigated in ACLF and AD. Among these are macrophage activation markers soluble (s)CD163 and mannose receptor (sMR) [5] and Neutrophil Gelatinase-Associated Lipocalin (NGAL) [6, 7]. Plasma sCD163 is associated with inflammation and fibrosis in chronic viral hepatitis and non-alcoholic fatty liver disease (NAFLD) [8, 9]; and both sCD163 and sMR levels are associated with portal hypertension and prognosis in patients with liver cirrhosis [10-12]. Further, significantly elevated sCD163 levels predict prognosis in patients with acute liver failure and severe alcoholic hepatitis [13, 14], which suggest macrophages to play a key role in liver disease severity, progression and prognosis. Recently we also demonstrated reduced sCD163 and sMR levels following successful intervention in viral hepatitis and NAFLD [15-17]. NGAL is produced in a number of organs and cell types and especially demonstrated in the granules of neutrophil leucocytes. NGAL can be measured in plasma and urine with elevated levels in acute and chronic kidney diseases [18]. Recently, NGAL has been suggested as a marker for inflammation in experimental liver injury models [19, 20] and a few studies have investigated NGAL in patients with liver diseases [21-23].

From the published data on biomarkers and prediction of prognosis in patients with AD and ACLF the macrophage activation markers sCD163 and sMR along with NGAL seem to be the most promising [5, 6]. We aimed to further investigate these biomarkers to provide insights into prognosis and further mechanistic information on the pathogenesis of AD and ACLF. We hypothesized that sCD163, sMR and NGAL can serve as single markers for prognosis in cirrhotic patients with AD and ACLF, and that new developed scores improve the prognostic capability compared to the original CLIF-C AD and CLIF-C ACLF scores.

Methods:

We included 522 patients from the CANONIC study where biomarkers were investigated in all patients and published during the past years. The biomarkers included macrophage activation markers, sCD163 and sMR [5], plasma and urine NGAL [6], 29 cytokines focused on plasma TNF, interleukin 6 (IL-6), IL-8, IL-10 [24], the redox state of circulating albumin (HNA2), a marker of systemic oxidative stress, and plasma renin and copeptin [24], as markers of systemic circulatory function, along with the plasma IL-1 receptor (IL-1R) [25].

The CANONIC study was a multicenter study aimed at evaluating the frequency,

characteristics, and outcome of ACLF in patients admitted to hospital for acute decompensation of cirrhosis in 29 liver units from 8 European countries. In the current study, ACLF was defined according to the criteria of the CANONIC study [1], which are based on presence of organ failure(s) as defined according to CLIF-C SOFA score. Briefly, patients with ACLF were those with either: 1) single kidney failure; 2) single liver, coagulation, circulatory or respiratory failure associated with serum creatinine levels between 1.5 and <2 mg/dl and/or hepatic encephalopathy grades I or II; 3) single cerebral failure (hepatic encephalopathy grades III or IV) associated with serum creatinine ranging from 1.5 and <2 mg/dl; or 4) two or more organ failures.

Out of the 1343 patients enrolled in the CANONIC study, 684 had both urine and plasma samples at the time of inclusion, and NGAL was measured as previously described [6]. Patients with urinary tract infection at the time of urine collection were excluded. Macrophage activation markers, sCD163 and sMR, were measured in 853 out of 1343 included patients in the CANONIC study as previously described [5]. Proinflammatory cytokines were measured in 522 patients, 237 with ACLF, as previously described [24]. We planned to investigate and compare all previously analyzed biomarkers in the same analysis and therefore restricted the present dataset to the 522 patients from the CANONIC study where all variables were present (342 patients with AD and 180 with ACLF).

For final evaluation and prediction we investigated the newly developed scores to the highest number of subjects included in the CANONIC study and where we had all available data (n=853 for sMR and n=129 for sCD163 and UNGAL) for AD and ACLF prediction, respectively.

Informed consent in writing was obtained from each patient and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in approval by the institutional review committee of participating centers.

Statistics:

From the above manuscripts we focused on inflammatory markers and cytokines showing significant associations with ACLF and AD prognosis and with data on all biomarkers in a cohort of 522 patients. Discrete variables are shown as counts (percentage) and continuous variables as mean (SD). Non-normally distributed variables are summarized by the median (interquartile range; IQR) and were log-transformed for some statistical analyses and for graphical comparisons. In univariate statistical comparisons, the chi-square test was used for categorical variables, whereas the Student t-test was used for normally distributed continuous

variables and the Wilcoxon signed rank test for continuous variables not normally distributed. Harrell's concordance index (C-index) was used to estimate the discrimination ability of all markers and the new scores [26]. As a proportional hazard competing risk (PH-CR) model was used, C-index values and the corresponding 95% confidence intervals (CIs) were estimated treating the transplanted patients as censored at the end of the follow-up, assuming that none of them could die before [27]. Statistical comparisons of the C-index were carried out for the main study time-points using the integrated discriminating improvement statistics [26]. To corroborate the results observed, a confirmatory analysis was carried out by estimating the Area Under the ROC curve (AUROC).

Results:

Baseline characteristics for the two groups of patients investigated are presented in Table 1. The ACLF patients were more likely to have alcoholic liver cirrhosis but otherwise there was no difference in ethiology, age or gender between the two groups. Decompensation with ascites or subrogates, hepatic encephalopathy and bacterial infections were more frequent in ACLF patients. Similarly, more ACLF patients displayed organ failures from liver kidney, brain, coagulation, heart and lung than patients without ACLF. This also included kidney dysfunction and mild to moderate hepatic encephalopathy. Further, we observed the expected differences in laboratory values (bilirubin, INR, albumin, creatinine and sodium) between patients with and without ACLF.

The patients *with* ACLF had a higher MELD score than patients without ACLF and a CLIF-C ACLF score of 49 while patients *without* ACLF had a CLIF-C AD score of 53. Further, there were significant differences in 28 day and 90 days mortality between the groups.

In addition, we observed significant differences in all cytokines and biomarkers between patients *without* and *with* ACLF.

CLIF-C AD score and biomarkers for the prediction of 90-day prognosis in patients *without* ACLF.

We calculated the 90-day Harrell's concordance index of MELD, CLIF-C-AD score and individual biomarkers alone and combined (Table 2). Both the MELD (0.70) and CLIF-C AD (0.73) scores performed well for the prediction of 90-day mortality. Only the macrophage activation markers sCD163 (0.70) and sMR (0.74) performed similarly to the CLIF-C AD score. Further, when sCD163 (0.77) or sMR (0.79) were added to the CLIF-C AD score there

was a strong trend towards improvement of the CLIF-C AD score. A similar trend in improvement was observed for the CRP (0.76).

Since sMR showed the strongest prediction for 90-day prognosis in patients *without* ACLF we developed a new AD score based on age, creatinine, INR, WBC, sodium, and sMR (Table 3). This new CLIF-C AD+sMR (0.82) score was significantly better than the original CLIF-C AD (0.74) score in the prediction of the 90-day mortality in patients *without* ACLF. Further, sCD163 did not improve the prognostic accuracy of the CLIF-C AD+sMR score and was therefore not included.

In Figure 1A we present AUROC for MELD, CLIF-C AD score and the new developed CLIF-C-AD+sMR score in all patients *without* ACLF from the CANONIC study, and the new score (0.82) was a better predictor than the original CLIF-C AD (0.76) score, P=0.005.

Interestingly, the CLIF-C AD+sMR score (0.72(0.65-0.79) was also a good predictor for ACLF development (n=342) and performed better than the CLIF-C AD (0.69(0.62-0.77) and MELD 0.63(0.54-0.71) scores.

CLIF-C ACLF score and biomarkers for the prediction of 28-days prognosis in patients *with* ACLF.

We calculated the 28-day Harrell's concordance index of MELD, CLIF-C ACLF score and individual biomarkers alone and combined (Table 4). Both the MELD (0.69) and CLIF-C ACLF (0.76) scores performed well for the prediction of 28-days mortality. The best single biomarkers for prediction of mortality were UNGAL (0.76) and sCD163 (0.70) and added to the CLIF-C-ACLF score there was a trend for improvement in prediction especially for UNGAL (0.83).

These two parameters were included in the development of a new CLIF-C ACLF score for the prediction of 28-days mortality (Table 5). First, we calculated a CLIF-C ACLF+UNGAL score (0.83) based on the CLIF-C OF score, age, WBC, and U-NGAL with a trend towards improvement compared to the original CLIF-C ACLF (0.75) score (P=0.068). Next, we added sCD163 to the scores and the CLIF-C ACLF+sCD163+UNGAL (0.85) significantly improved the 28-day prediction of mortality compared to the original CLIF-C ACLF (0.75) score. Thus, the addition of sCD163 improved the prognostic accuracy of CLIF-C ACLF+UNGAL score.

In Figure 1B we present AUROC for MELD, CLIF-C ACLF score and the new developed CLIF-C- ACLF+sCD163+UNGAL score in all patients with ACLF from the CANONIC study (n=129), and the new score (0.87) was a better predictor than the original CLIF-C AD (0.79) score (P=0.018).

Of importance for 28-day mortality the novel CLIF-C ACLF+UNGAL+sCD163 score performed equally well in patients *with* (0.86(0.76-0.95) and *without* 0.85(0.78-0.92) bacterial infections; and better than the CLIF-C ACLF (0.70(0.56-0.84) vs. 0.77(0.69-0.86)), CLIF-C OF (0.70(0.57-0.83) vs. 0.70(0.60-0.80)) and MELD (0.67(0.55-0.78) vs. 0.69(0.60-0.78)) for patients *with* and *without* bacterial infections, respectively. Similarly, the new score performed similarly well in patients *with* (0.85(0.78-0.92) and *without* (0.85(0.77-0.96) kidney dysfunction; and again better than the CLIF-C ACLF (0.77(0.68-0.86) vs. 0.73(0.60-0.85)), CLIF-C OF (0.72(0.63-0.81) vs. 0.73(0.60-0.87)) and MELD (0.71(0.62-0.80)) vs. 0.67(0.53-0.80)) for patients *with* and *without* kidney dysfunction, respectively.

Percent improvement in prediction of the new scores throughout 1-year follow-up with respect to MELD, CLIF-C ACLF and CLIF-C AD scores

The newly developed scores markedly improved the percentage of prediction at 28-, 90-, 180-, and 365 days mortality compared to MELD and CLIF-C ACLF scores for patients *with* ACLF and for patients *without* ACLF compared to MELD and CLIF-C AD scores (Figure 2).

Discussion:

In the present study we re-investigated all previous investigated biomarkers predicting mortality in patients with cirrhosis from the CANONIC study. The main finding is the capability of the macrophage activation markers and UNGAL to improve the original prognostic scores in cirrhosis patients *without* (sMR) and *with* (sCD163 and UNGAL) ACLF, respectively. This points to a key role of macrophage activation and inflammation in the development and progression of AD and ACLF, which may be a potential future target strategy.

In the present study we investigated patients included in the CANONIC study comprising both patients with cirrhosis and AD and ACLF, respectively. A number of predictors for morbidity and mortality has been described for patients with liver cirrhosis in general and includes the CP- and MELD scores, clinical information on previous decompensation episodes, as well as measures of portal hypertension and liver stiffness. Recently, an AD score derived from the CANONIC study for the prognosis of acute decompensation in patients with cirrhosis was proposed [3]. For ACLF patients the CANONIC study revealed the CLIF-C ACLF score [2]. These scores improved prognostication beyond CP and MELD scores; however, there are still need for better scoring systems for selecting patients at highest risk for increased morbidity and mortality, and selection for specific treatments including liver transplantation. Importantly, we

investigated central pathogenic biomarkers in these patient groups and were able to further improve the originally derived scoring systems.

Systemic inflammation is a hallmark in the development and progression of ACLF where an over activated immune response is accompanied by an inappropriate systemic inflammatory response with subsequent organ failures [4, 28]. It is well known that cirrhotic patients have elevated levels of proinflammatory cytokines (e.g. TNF, Il-6), and immune cells from cirrhotic patients have more pronounced *in vivo* cytokine production after LPS stimulation compared to controls [29]. Especially, the innate immune system with monocytes and macrophages are involved in this cytokine production; and we showed that the macrophage activation markers sCD163 and sMR are elevated in association with liver diseases severity and portal hypertension [10, 11, 30]. This may suggest that with increasing liver disease severity there is increased macrophage activation, and when these macrophages are further activated by infection or inflammation they initiate and/or participate in an exaggerated immune response and cytokine storm leading to systemic inflammation, organ failure and ACLF. A recent study supported this with increased inflammatory marker levels in AD and where ACLF patients showed the largest number of abnormal markers suggesting "full-blown" systemic inflammation. Further, among AD-patients IL-8, IL-6, IL-1ra, HNA2 were independent predictors for 28-day progression defined as ACLF or death [31]. To further understand how inflammation is associated with metabolism and organ failure Moreau et al. investigated the blood metabolome in patients with cirrhosis, with and without ACLF. In ACLF patients the intensity of the blood fingerprint metabolite increased with ACLF severity and interestingly was significantly associated with macrophage activation and the elevated sCD163 and sMR levels. The metabolite represents different metabolic pathways including proteolysis and lipolysis; aminoacid catabolism; extra-mitochondrial glucose metabolism through glycolysis, pentose phosphate, and D-glucuronate pathways; depressed mitochondrial ATP-producing fatty acid beta-oxidation; and extramitochondrial amino acid metabolism [32].

Triggers for this immune response may be PAMPs that react with pattern-recognition receptors including Toll-like receptors. Bacterial infections are very frequent in ACLF patients with spontaneous bacterial peritonitis and pneumonia accounting for approximately 30% of precipitating events [1] while viral hepatitis is more frequent in Asian ACLF [33]. Another mechanism may be DAMPS during sterile inflammation caused by injured or dying hepatocytes [4]. The consequence of this over-activated immune response and production of inflammatory cytokines is impairment of the microcirculation in affected organs accompanied by impaired cell function and subsequent cell death [4]. This may further stimulate both local

and liver macrophages in a viscous cycle but also lead to increased liver NGAL expression with increased plasma and urinary NGAL levels [6].

Plasma sCD163 is a marker of macrophage activation with CD163 cleaved by the TACE/ADAM17 enzyme also responsible for shedding of TNF-alpha, which explain the strong correlation between sCD163 and TNF levels [34]. The sCD163 may represent both local production and systemic monocyte/macrophage activation and increased levels have been described in patients with sepsis and pneumonia with the highest levels in patients with chronic liver diseases [35, 36]. As more than 80% of body macrophages resides in the liver sCD163 levels reflect to a high degree liver macrophage activation and we have previously demonstrated a gradient across the liver in patients with NAFLD and liver cirrhosis [17, 37]. Similar findings have been presented for sMR [30] including a gradient across the liver but without association to bacterial translocation [11]. However, the MR is in addition to macrophages also expressed on endothelial and dendritic cells, and the shedding is most likely caused by proteolytic cleavage [38], and different from sCD163, which is shed by the TACE/ADAM enzyme [34]. From previous studies the highest sCD163 and sMR levels are found in patients with increasing liver disease severity with the highest levels in ACLF and ALF patients [39]. Importantly, circulating sCD163 and sMR are easily obtainable, stable during freezing and thawing, and both in house and commercial ELISA assays are available, which makes them ideal biomarkers for use also in daily clinical practice.

NGAL is a novel biomarker for inflammation and in liver diseases NGAL is stimulated by cell injury and regeneration as shown in acute and chronic liver injury models [19, 20, 40]. Plasma NGAL was higher in cirrhotic patients with impaired kidney function and in chronic viral hepatitis C patients [22, 41]. Gungor et al. showed higher plasma NGAL in cirrhotic patients with hepatorenal syndrome Type 1 (HRS-1) compared to HRS-2, and higher than controls and patients with cirrhosis and normal kidney function. Further, plasma NGAL was an independent predictor for mortality in these patients with an AUC of 0.82 similar to the MELD (AUC=0.81) and the CP-score (AUC=0.80) [42]. Ariza et al. investigated UNGAL in patients with decompensated cirrhosis with acute kidney injury (AKI), HRS-1 and acute tubular necrosis (ATN) and found that UNGAL was best to predict ATN. Further, UNGAL was significantly elevated in ACLF patients and predicted ACLF with AUROC of 0.88 and UNGAL also predicted mortality in the whole group of patients investigated (AUROC=0.88) [23]. These data were confirmed in the CANONIC study investigating plasma and urinary NGAL; and with higher UNGAL/g creatinine levels in ACLF patients, and UNGAL was an independent predictor for ACLF. Further, UNGAL independently predicted 28-day mortality and further

improved the MELD score for mortality prediction while plasma NGAL was only a predictor in univariate analysis [6]. Interestingly, in liver biopsies Lipocalin-2 gene expression was highest in ACLF patients signifying intrahepatic production. A recent study partly confirmed these data with increased plasma NGAL in HBV ACLF patients with a poor prognosis and adding NGAL to the MELD score further improved prognostication from AUROC 0.76 (MELD) to 0.90 (MELD+NGAL) [43]. In further support, a translational study demonstrated elevated plasma NGAL in relation to liver disease severity and renal function along with higher levels in non-survivors compared to survivors [44].

A limitation of UNGAL measurement is elevated levels in relation to urinary tract infections, which are frequent in cirrhotic and especially ACLF patients. Further, UNGAL may derive both from local production in the urinary tract or extraction from the circulation [21]. Further, in anuric patients UNGAL can obviously not be obtained, which makes the use of UNGAL as a biomarker less useful.

The main strength of the present study is the large number of well-characterized patients included in the CANONIC study at different European liver centers. This may also hold the risk of referral bias, as the hospitals involved in the study are primarily referral hospitals with highly specialized liver units. Another bias may be selection bias as all previous parameters were not investigated in the total number of patients from the original CANONIC cohort. However, sCD163 and sMR were investigated in 851 patients (185 with ACLF) and UNGAL in 716 patients (148 with ACLF) suggesting that the derived data and results are robust. Further, the study is in essence cross-sectional which may cause difficulties in interpretation of causality; however, in the original studies prospective examination of sCD163 and sMR showed that patients with stable or decreasing sCD163 levels had better prognosis compared to patients showing increased levels [5].

We have provided novel scores based on specific biomarkers that improve the prediction for risk of death in patients with cirrhosis with AD and ACLF. However, assays for the biomarkers sCD163 and sMR are not yet internationally standardized, and typically measured by non-certified ELISA kits. To implement these in daily clinical practice will require standardization and preferably establishment of assays on automated analyses-platforms.

In conclusion, we demonstrated that specific markers of macrophage activation improved the original prognostic scores in cirrhosis patients *without* and *with* ACLF, which points to a key role of macrophage activation in the development and progression of AD and ACLF. In addition, UNGAL was a marker for ACLF mortality and the combination of NGAL and sCD163 improved the prognostic capability beyond the original CLIF-C ACLF score.

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Figure 1A: AUROC for the new developed score in all patients *without* ACLF

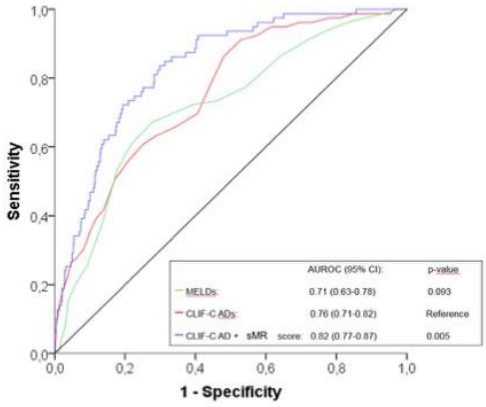

Figure 1B: AUROC for the new developed score in all patients *with* ACLF

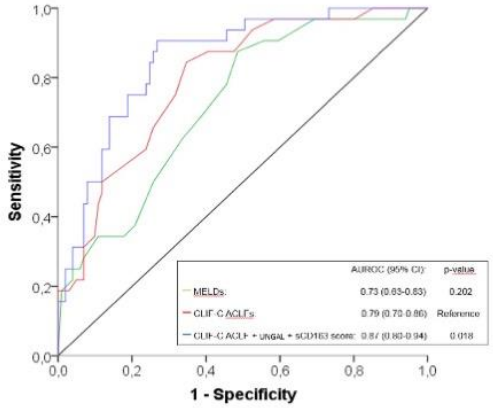

Figure 3. Percent improvement in prediction for 28, 90, 180 and 365 days mortality.

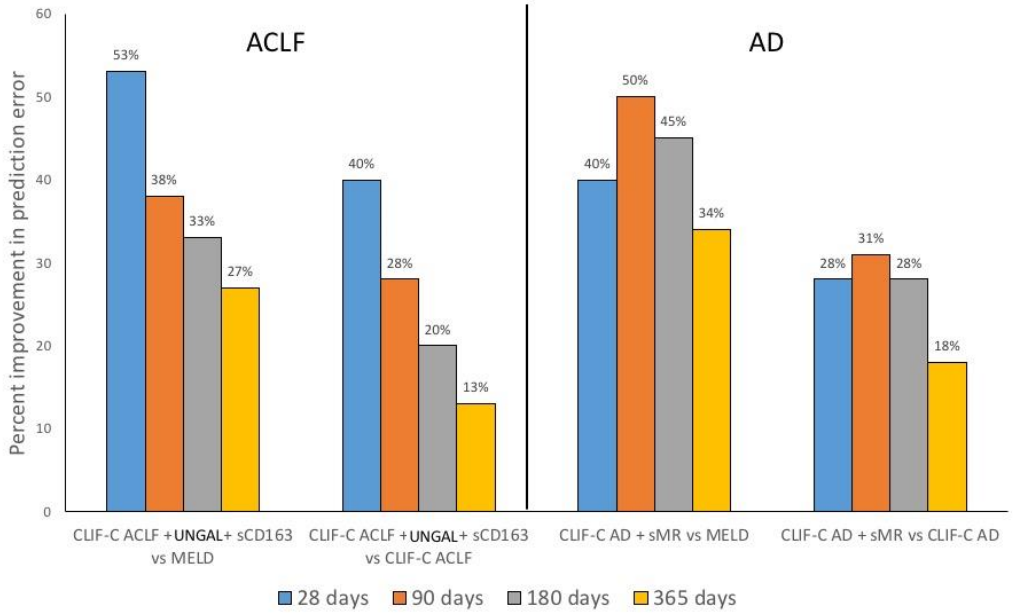

Table 1. Baseline characteristics of patients *without* ACLF (n=342) and *with* ACLF (n=180).

Baseline characteristics	**No ACLF (N=342)**	**ACLF (N=180)**	**P value**
Age (years)	56±12	56±11	0.814
Male	227(66.4)	117(65.0)	0.753
Etiology			
Alcohol (n, %)	157(48.9)	99(58.2)	0.049
HCV (n, %)	78(24.3)	29(17.1)	0.065
Alcohol + HCV (n, %)	29(9.0)	20(11.8)	0.337
Other (n, %)	57(17.8)	22(12.9)	0.167
Ascites with subrogates	267(78.1)	175(97.2)	<0.001
HE (n, %)	90(26.3)	105(58.3)	<0.001
GI bleeding (n, %)	65(19.0)	20(11.1)	0.020
Bacterial infection (n, %)	78(22.9)	63(35.4)	0.002
Organ Failures			
Liver (n, %)	38(11.1)	72(40.0)	<0.001
Kidney (n, %)	-	104(57.8)	-
Brain (n, %)	8(2.3)	35(19.4)	<0.001
Coagulation (n, %)	12(3.5)	44(24.4)	<0.001
Cardiac (n, %)	5(1.5)	29(16.1)	<0.001
Respiratory (n, %)	3(0.9)	14(7.8)	<0.001
Kidney dysfunction (n, %)	61(17.8)	20(11.1)	0.044
Mild to moderate HE (n, %)	81(23.8)	74(41.1)	<0.001
Laboratory values			
Bilirubin (mg/dL)	3.1(1.6-7.0)	6.9(2.1-17.1)	<0.001
INR	1.5(1.3-1.8)	1.8(1.4-2.5)	<0.001
Albumin (g/dL)	2.9(2.5-3.2)	3.0(2.4-3.4)	0.626
Creatinine (mg/dL)	0.9(0.7-1.4)	2.2(1.0-3.2)	<0.001
Sodium (mmol/L)	136±6	134±7	0.010
Scores			
MELDs	17±6	27±7	<0.001
CLIF-C ADs	53±9	-	-
CLIF-C ACLFs	-	49±9	-
Mortality			
28 day	22(6.4)	48(26.7)	<0.001
3 month	55(16.1)	72(40.0)	<0.001
Biomarkers			
Leucocyte count (10^9/L)	6.3(4.4-9.4)	8.4(5.3-12.4)	<0.001
C-reactive protein (g/L)	18(7-41)	27(11-52)	0.004
TNFa (pg/mL)	20(14-29)	29(17-43)	<0.001
IL6 (pg/mL)	24(12-48)	40(16-118)	<0.001
IL8 (pg/mL)	42(22-84)	89(41-177)	<0.001
IL10 (pg/mL)	3.8(1.2-10.8)	8.2(2.1-32.0)	<0.001
IL1Ra (pg/mL)	11(5-28)	23(9-63)	<0.001
Urine NGAL (ng/ml)	29(12-83)	86(28-299)	<0.001
Plasma NGAL (ng/ml)	139(102-213)	235(147-448)	<0.001
sCD163 (mg/L)	8.8(5.2-12.6)	13.8(7.9-19.0)	<0.001
sMR (mg/L)	0.8(0.6-1.1)	1.0(0.7-1.5)	<0.001
HNA2 (%)	5.2(2.7-9.2)	11.0(7.0-15.3)	<0.001
Renin (microIU/mL)	72(20-274)	121(34-353)	<0.001
PCC (pmol/L)	11(4-28)	33(14-61)	<0.001

Table 2. Ninety-day Harrell's concordance index (C-index) of MELD, CLIF-C AD score and individual biomarkers (first column), and each biomarker added to CLIF-C ADs (second column) in patients without ACLF*.

	Mortality at 90 days		
Variables	**Individual C-index(95% IC)**	**C-index(95% IC) after adding biomarkers to CLIF-C ADs**	**P-value between CLIF-C ADs + biomarkers vs CLIF-C ADs**
MELD	**0.70(0.63-0.76)**		
CLIF-C AD	**0.73(0.66-0.79)**		
Log(CRP)	0.68(0.61-0.75)	0.76(0.69-0.82)	0.076
Log(TNFa)	0.60(0.53-0.68)	0.73(0.67-0.79)	0.126
Log(IL6)	0.65(0.55-0.70)	0.72(0.66-0.78)	0.901
Log(IL8)	0.66(0.59-0.72)	0.75(0.70-0.81)	0.175
Log(IL10)	0.57(0.49-0.65)	0.71(0.65-0.78)	0.470
Log(IL1Ra)	0.57(0.49-0.64)	0.73(0.66-0.79)	0.374
Log(UNGAL)	0.62(0.54-0.70)	0.74(0.68-0.81)	0.346
Log(PNGAL)	0.63(0.55-0.71)	0.73(0.67-0.80)	0.157
Log(sCD163)*	**0.70(0.64-0.77)**	**0.77(0.72-0.82)**	**0.082**
Log(sMR)*	**0.74(0.67-0.80)**	**0.79(0.74-0.84)**	**0.072**
Log(HNA2)	0.66(0.59-0.73)	0.73(0.67-0.79)	0.251
Log(Renin)	0.60(0.52-0.67)	0.72(0.66-0.79)	0.642
Log(Copeptin)	0.64(0.57-0.72)	0.73(0.66-0.80)	0.860

* Variable coefficients used for CLIF-C ADs were those originally described. sCD163 and sMR, which were the best markers predicting prognosis and increasing the accuracy of CLIF-C ADs, were included in the assessment of a new score for patients with AD.

Table 3. Coefficients and c-index of the new score in AD patients at 90 days*.

CLIF-C AD + sMR score = ***10*(0.02*age(years) + 0.11*logCreatinine(mg/dL) + 1.96*logINR + 0.75*logWBC(10^9/L) -0.02*Na(mmol/L) + 1.43*logsCD206(mg/L)*** **Mortality at 90 days**	
CLIF-C AD	C-index=0.74(0.70-0.78)
Variables	**Coefficients**
Constant	+4
Age	0.02
Log(Creatinine)	0.11
Log(INR)	1.96
Log(WBC)	0.75
Sodium	-0.02
Log(sMR)	1.43
Readjusting	*10
C-index	0.82(0.78-0.86)
P (CLIF-C AD)	0.004

* Variable coefficients of CLIF-C ADs + sMR were recalculated due to the addition of the new marker.

Table 4. Twenty-eight day Harrell's concordance index (C-index) of MELD, CLIF-C ACLF score and individual biomarkers (first column), and each biomarker added to CLIF-C ACLFs (second column) in patients with ACLF*.

	Mortality at 28 days		
Variables	**C-index(95% IC)**	**C-index(95% IC) after adding biomarkers to CLIF-C ACLFs**	**P-value between CLIF-C ACLFs + biomarkers vs CLIF-C ACLFs**
MELD	**0.69(0.62-0.76)**		
CLIF-C ACLF	**0.76(0.68-0.83)**	**0.76(0.68-0.83)**	
Log(CRP)	0.57(0.49-0.66)	0.75(0.67-0.83)	0.346
Log(TNFa)	0.55(0.47-0.63)	0.77(0.70-0.84)	0.496
Log(IL6)	0.61(0.53-0.70)	0.76(0.69-0.83)	0.729
Log(IL8)	0.65(0.58-0.72)	0.77(0.70-0.83)	0.416
Log(IL10)	0.60(0.52-0.68)	0.75(0.68-0.83)	0.305
Log(IL1Ra)	0.59(0.51-0.67)	0.76(0.68-0.83)	0.933
Log(UNGAL)	**0.76(0.69-0.83)**	**0.83(0.76-0.89)**	**0.076**
Log(PNGAL)	0.67(0.57-0.76)	0.78(0.71-0.86)	0.552
Log(sCD163)	**0.70(0.63-0.78)**	**0.79(0.72-0.86)**	**0.231**
Log(sMR)	0.66(0.59-0.74)	0.76(0.69-0.83)	0.370
Log(HNA2)	0.58(0.50-0.66)	0.78(0.72-0.85)	0.264
Log(Renin)	0.55(0.46-0.64)	0.75(0.68-0.83)	0.357
Log(Copeptin)	0.63(0.54-0.72)	0.77(0.69-0.85)	0.474

* Variable coefficients used for CLIF-C ACLFs were those originally described. Urine NGAL and sCD163, which were the best markers predicting prognosis and increasing the accuracy of CLIF-C ACLFs, were included in the assessment of a new score for patients with ACLF.

Table 5. Coefficients and C-index of the new score in ACLF patients at 28 days*.

CLIF-C ACLF + UNGAL score	
Mortality at 28 days	
CLIF-C ACLF	C-index=0.75(0.70-0.80)
Variables	**Coefficients**
CLIF-C OF	0.41
Age	0.05
Log(WBC)	0.45
Log(UNGAL)	0.33
C-index	0.83(0.77-0.89)
P (CLIF-C ACLF)	0.068
CLIF-C ACLF + UNGAL + sCD163 score = **10*(0.29*CLIF-C OF + 0.05*age(years) + 0.54*logWBC(10^9/L) + 0.32*logUNGAL(ng/ml) + 0.97*logsCD163(mg/L)**	
Mortality at 28 days	
Variables	**Coefficients**
Constant	-6
CLIF-C OF	0.29
Age	0.05
Log(WBC)	0.54
Log(UNGAL)	0.32
Log(sCD163)	0.97
Readjusting	*10
C-index	0.85(0.79-0.91)
P (CLIF-C ACLF)	0.039
P (CLIF-C ACLF) + NGAL	0.415

* Variable coefficients of CLIF-C ACLFs + UNGAL + sCD163 score were recalculated due to the addition of the new markers.