**Plasma Cystatin C is a predictor of renal dysfunction, ACLF and mortality in patients
with acutely decompensated liver cirrhosis**

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Abbreviations: ACLF, Acute-on-Chronic Liver Failure; AD, Acute Decompensation of liver cirrhosis; AKI, Acute Kidney Injury; AKIN, Acute Kidney Injury Network; AUCROC, Area Under the Curve of the Receiver-Operating Characteristics; CANONIC study, EASL-CLIF Acute on chrONIC liver failure study; Crea, Serum Creatinine; CysC, Cystatin C; EASL-CLIF, European Association for the Study of the Liver – Chronic Liver Failure; eGFR, estimated Glomerular Filtration Rate; HR, Hazard Ratio; HRS, Hepatorenal Syndrome; MELD, Model of End Stage Liver Disease; NGAL, Neutrophil Gelatinase-Associated Lipocalin; OR, Odds Ratio;

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Abstract:

Background: The development of acute-on-chronic liver failure (ACLF) in patients with liver cirrhosis is associated with high mortality rates. Renal failure is the most significant organ dysfunction that occurs in ACLF. So far there are no biomarkers predicting ACLF. **Aim:** To investigate whether Cystatin C (CysC) and neutrophil gelatinase-associated lipocalin (NGAL) can predict development of renal dysfunction (RD), hepatorenal syndrome (HRS), ACLF and mortality, respectively. **Methods:** We determined the plasma levels of CysC and NGAL of 429 patients hospitalized for acute decompensation of cirrhosis in the CANONIC study. The patients were followed for 90 days. **Results:** Patients without RD or ACLF at inclusion, but with development of either, had significantly higher baseline concentrations of CysC and NGAL compared to patients without. CysC, but not NGAL was found to be predictive of RD (OR 9.4 [1.8; 49.7]), HRS (OR 4.2 [1.2; 14.8]) and ACLF (OR 5.9 [1.3; 25.9]). CysC at day3 was not found to be a better predictor than baseline CysC. CysC and NGAL were both predictive of 90 days mortality with hazard ratios for CysC of 3.1 (2.1; 4.7), and for NGAL of 1.9 (1.5; 2.4), respectively. **Conclusions:** Baseline CysC is a biomarker of renal dysfunction, HRS and ACLF and an independent predictor of mortality in patients with acutely decompensated liver cirrhosis. Determining CysC at day 3 did not provide any benefit. While NGAL is also associated with short-term mortality, it fails to predict development of RD, HRS and ACLF. Baseline CysC may add to identify patients at risk earlier and might improve clinical management.

Background:

Patients with liver cirrhosis often present with an acute decompensation (AD) such as ascites, hepatic encephalopathy or bacterial infections. Moreau et al. showed that patients with AD have a rather good short-term prognosis with a 28d mortality probability of 1.9% (1). Presence or development of acute-on-chronic liver failure (ACLF), as defined by Moreau et al., increases the 28d mortality rate to around 30%. Among patients with ACLF, renal failure is the most frequent organ failure, occurring in more than 50%. Moreover, subgroup analysis showed that renal failure is associated with a worse prognosis than any other single organ failure. Renal failure in patients with cirrhosis has a devastating impact on prognosis with a 1-month mortality rate around 50% (2-5). The most severe form of renal dysfunction is the hepatorenal syndrome type 1 (HRS) reducing the 3-months survival probability to less than 15% (6, 7).

In order to improve management of AD in cirrhosis, identifying patients with a high risk of exhibiting renal dysfunction and ACLF, respectively, within the next days or weeks could be very useful. A reliable predictor would offer the possibility of making important therapeutic decisions early on, such as listing for transplantation, and provide a window to focus on optimized treatment in order to prevent the deterioration of renal function.

Serum Creatinine (Crea), the most commonly used marker of renal function has several limitations in patients with liver cirrhosis. Synthesis of Crea is typically reduced in these patients, due to muscle wasting and decreased protein intake (8, 9). Thus serum Crea tends to overestimate the actual renal function. Moreover, in view of the limited sensitivity of Crea for detecting small changes of the glomerular filtration rate (GFR), Crea is not a promising predictor of deterioration of renal function in patients with liver cirrhosis.

Two recent renal function markers currently under investigation are Cystatin C (CysC) and neutrophil gelatinase-associated lipocalin (NGAL), respectively. There is persuasive evidence that plasma levels of either marker can function as biomarkers of early renal dysfunction in several clinical situations (10-14). After renal injury an increase of either marker in plasma can be detected earlier than of Crea and estimations of GFR based on CysC are more accurate than creatinine based formulas (15-18). Moreover, both NGAL and CysC are less affected than Crea by factors which are common in patients with liver cirrhosis, like muscle wasting, reduced protein diet and high bilirubin levels (9, 19-22). Hence, both might be useful markers for the prognosis and care of patients with liver cirrhosis.

There are some studies investigating CysC and NGAL as markers of GFR in liver cirrhosis but few data are available regarding the prediction of acute deterioration of patients with cirrhosis. For example, Souza et al. evaluated over 200 patients awaiting liver transplantation and found that CysC based equations more precisely determined the glomerular filtration rate (GFR) than Crea based equations (23). This was confirmed in various groups of patients with liver cirrhosis (24, 25). Furthermore, previous small studies suggested CysC as a predictor for HRS and mortality (26, 27). In a small cohort of 49 patients CysC based estimations of GFR were a predictive marker for the overall survival (28). As with CysC, there are several studies which indicate NGAL as a valuable marker of the GFR in cirrhosis (11, 29). In a study of 34 patients plasma NGAL levels as well as CysC based GFR estimations were predictive for development of renal dysfunction within the next 48h (30). Moreover, plasma levels of NGAL were significantly higher in patients with ACLF than in patients without (31).

Altogether those studies indicate that CysC and NGAL could be useful biomarkers in patients with liver cirrhosis. However, neither marker has been investigated as predictor of clinical

deterioration in a large and well characterized study population yet. Thus, the purpose of the present study was to evaluate the potential role of CysC and NGAL, respectively in predicting development of the newly defined ACLF, of renal dysfunction and HRS and in predicting the probability of survival in patients with acute decompensation of cirrhosis from the CANONIC study population.

Methods:

Study population

The study population comprised patients who were included in the CANONIC study, as detailed elsewhere (1). Informed consent in writing was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. The CANONIC study had 29 participating liver units of eight European countries. It was initiated by the EASL-CLIF Consortium with the purpose of developing a reliable definition of the syndrome of “acute-on-chronic liver failure”, in order to identify patients with a high risk of short-term mortality. The CANONIC study enrolled patients who were hospitalized for an acute decompensation of liver cirrhosis.

For the present investigation we selected all patients who had continuous study surveillance and blood sampling after inclusion (study visits on d1, d2, d3-7, d8-14, d15-21 and d22-28).

This resulted in a total number of 429 patients with plasma samples available for determination of CysC, NGAL and Crea, respectively. Each study visit comprised of blood sampling, physical examination and taking patient's history of clinical events or changes in medication since last study visit. On d28 (if already discharged) and d90 the patient was contacted by the investigator to evaluate the vital and transplant status.

Data management and laboratory parameters

The clinical data of all patients were collected by the CANONIC study team, as described. Creatinine, Cystatin C, and NGAL were determined using reagents from Beckman Coulter, Biomed, and Bioporto, respectively. All assays were run according to the manufacturers' protocols on automated clinical chemistry analyzers from Beckman Coulter (AU 680 and AU 5800). The intra- and inter-assay variabilities were as follows: Crea: (intra) 1.2%, (inter) 2.0%; NGAL: (intra) 1.2%, (inter) 1.5%; CysC: (intra) 1.8%, (inter) 1.4%.

The estimated GFR was calculated by two different equations: Cyst-eGFR (32) and CKD-EPI-Creatinine-Cystatin (33), respectively. ACLF was diagnosed by using the established definition criteria published by Moreau et al. (1). Renal dysfunction was defined as Crea ≥ 1.5 mg/dl, according to the criteria of ACLF and the CLIF SOFA Score. The presence or absence of HRS was stated by the study investigator.

Statistical analysis

Data were collected by the CANONIC investigator team using an electronic case report form. Values of NGAL, CysC and Crea were directly entered into a data sheet. Quantitative variables were expressed as mean and SD or median and interquartile range, where appropriate. They were compared by using the students T test or the U-Mann Whitney test, respectively. Categorical variables were expressed as proportions and compared by the Chi-Square test / Fisher Exact Test.

Parameters found to be significantly associated with development of renal dysfunction, HRS or ACLF, were tested in a logistic regression model (previously log-transformed, if needed) for their potential to predict the corresponding outcome. In order to compare the predictive value of parameters at baseline versus day3, for these analyses we selected patients who did not develop ACLF, RD nor HRS, within the first three days after inclusion. Multivariate logistic regression analyses included independent variables with statistically significant

results in the univariate analyses. The variables were log-transformed before they were entered in logistic models. This could not be done for deltas of day3 minus baseline values, since they possibly resulted negative. The accuracy of prediction was compared by estimating the area under the curve of the receiver-operating characteristics (AUCROC).

Regarding the prediction of 90 day mortality we used a competing risk approach under the subdistribution hazard model with transplantation regarded as a competing event.

Comparison of the prediction accuracy in these models was performed by assuming normality of C-indexes. ROC analyses were performed to determine the best mathematical cut-off value of CysC for the prediction of ACLF, RD and HRS development as well as 90 day mortality. Since each best cut-off value was close to CysC = 1.5 mg/l, sensitivities and specificities were calculated with this predefined, best collective cut-off value.

Tests were considered statistically significant at a two-sided $p < 0.05$. In all figures significant differences are indicated by (*), while differences with a high significance ($p < 0.001$) are indicated by (**).

Results

Description of study population at enrolment

The 429 patients included in the current study had a mean age of 56.3 (± 11.8) years and a proportion of 63.4% were male (table 1). At enrolment 176 patients (41.0%) had ACLF and 147 (34.3%) had renal dysfunction defined as serum creatinine ≥ 1.5 mg/dl. HRS was present in 34 of 428 patients (7.9%; 1 patient without information for HRS at enrolment).

As expected, patients with ACLF at inclusion had higher MELD and Child Pugh scores than patients without ACLF. All tested markers of renal function, Crea, CysC and NGAL, were lower in the non-ACLF group (table 2). Similar results were found regarding patients with or without renal dysfunction at inclusion. The levels of CysC and NGAL were increased in case of renal dysfunction with a high significance, proving their reliability in patients with

decompensated cirrhosis (table 2). When the ACLF group was separated into the three different grades of ACLF (table 3), we found an increase of several parameters for each higher grade of ACLF, for example in MELD score, CRP level and proportion of patients with infections at baseline. Regarding renal markers all grades of ACLF had higher levels than the non-ACLF group.

Cystatin C, NGAL and Creatinine as predictors of clinical deterioration

Development of ACLF

To investigate the potential role of renal markers in predicting ACLF we focussed on the 209 patients without ACLF at time of inclusion and with appropriate follow up. 69 patients developed ACLF during follow up, 140 did not. There were no significant differences between the groups regarding age and sex (table 4). Baseline levels of Crea, CysC and NGAL were higher in the group with development of ACLF. Besides we found further baseline parameters that were significantly different between patients with later development of ACLF and without (supplement 1; online publication only). For example the eGFR equations Cyst-eGFR and CKD-EPI-Creatinine-Cystatin showed a decreased baseline eGFR in patients with ACLF development ($p < 0.01$ for both). Contrarily, the proportion of patients presenting with AKI at time of inclusion was not significantly different between the groups. Analysing the deltas of day3-baseline of Crea, CysC and NGAL, respectively, we found that deltas of CysC and of Crea were not significantly higher in the group of patients with ACLF development.

We then determined the potential of each renal marker at baseline and of CysC at day3 to predict development of ACLF by using a logistic regression model. The Odds ratios at baseline for Crea, CysC and NGAL were 3.5 (0.9; 13.5), 5.9 (1.3; 26.0) and 1.5 (0.8; 2.8) respectively, suggesting only CysC as a significantly predictive factor (figure 1). The corresponding AUCROC of baseline CysC was 0.68 (0.55, 0.82). Multivariate logistic regression demonstrated baseline CysC as an independent predictor. With the predefined

cut-off value of 1.5 mg/l the development of ACLF was predicted by CysC with 58.0% sensitivity and 66.4% specificity, respectively. The day3 value of CysC was a significant predictor of ACLF development, too (OR 4.6 (1.1; 19.9) AUCROC 0.63 (0.48; 0.79)).

AUCROC comparison between day3 CysC and baseline CysC showed no significant difference (supplement 2; online publication only). Moreover, the tested eGFR equations were found to be significant predictors of ACLF development. However, prediction accuracy of baseline CysC was not inferior to those of the two tested eGFR equations.

Development of renal dysfunction

Out of the study population 197 patients without renal dysfunction at baseline (RD), defined as Creatinine < 1.5 mg/dl, had samples available for follow up analysis. Approximately 14% of them (27 of 197) developed RD during follow up. There were no significant differences in age, sex nor MELD score compared to patients who did not develop RD (table 5). However, the baseline markers of renal function showed significantly higher values in the group of patients with development of RD. Moreover, significant differences between the groups were found regarding the proportion of patients with AKI at enrolment, regarding the estimated GFR and regarding deltas of day3-baseline of Crea, CysC and NGAL, respectively (supplement 1).

Regarding the baseline values of NGAL and CysC at enrolment only the latter turned out to be predictive of RD development (NGAL OR 1.6 (0.9; 3.1); Cystatin C OR 9.4 (1.8; 49.6)) (figure 1). Creatinine was not used in a logistic regression model since renal dysfunction is defined by creatinine values. With 1.5 mg/l as cut-off value, development of renal dysfunction was predicted by CysC with 63.0% sensitivity and 73.5% specificity, respectively. The Odds ratios of the eGFR equations using CysC were significantly lower than 1, so both equations were predictors of renal dysfunction development. By AUCROC analyses we found no significant difference in prediction accuracy compared to baseline CysC (supplement 2).

Development of HRS

To investigate which parameters are associated with the development of HRS we focussed on the 394 patients who did not have HRS at inclusion. 42 of them exhibited HRS during follow up whereas 350 did not (data of 2 patients missing). Baseline characteristics of both groups are shown in “table 6”. Baseline values of Crea, CysC as well as NGAL were significantly higher in patients with HRS development ($p < 0.0001$ for all). Moreover, values of eGFR equations were significantly lower in patients with HRS development ($p < 0.0001$ for all) (supplement 1). The deltas day3-baseline of Crea, CysC and NGAL were not significantly different between patients with and without HRS development (table 6).

CysC baseline values were predictive of HRS development (OR 4.2 [1.2; 12.8]; AUCROC 0.71 [0.60; 0.83]), whereas NGAL and Creatinine were not (figure 1). In a multivariate analysis after adjusting for other clinical parameters, CysC was shown to be an independent predictor of HRS development. The eGFR equations as well as CysC of day3 were significant predictors, but not superior compared to baseline CysC (supplement 2). CysC at a cut-off at 1.5 mg/l yielded a sensitivity and specificity of 83.3% and 51.7%, respectively.

Cystatin C, NGAL and Creatinine as predictors of mortality

The overall 90 day mortality rate was 27.6% (116/421). A total number of 49 patients underwent transplantation, hence transplant-free survival rate was 61.0% (1 patient died after transplantation during follow up).

Baseline characteristics at inclusion showed that many variables, such as MELD score and presence of ACLF were significantly associated with 90 day mortality. Regarding markers of renal function baseline values of all three, Crea, CysC and NGAL, were remarkably higher in

patients who died within 90 days ($p < 0.0001$ for all markers) (table 7). Similarly all eGFR equations and the presence of AKI were significantly associated with 90 day mortality as well. In contrast, deltas of day3-baseline of Crea, CysC and NGAL were not significantly higher in the non-survivor group.

By competing risk approach all renal markers at baseline were shown to be predictors of 90 day mortality. The hazard ratios of Crea, CysC and NGAL were 2.2 (1.6; 3.0), 3.1 (2.1; 4.7) and 1.9 (1.5; 2.3), respectively. In a multivariate analysis after adjusting for other clinical parameters, each of them was found to be an independent predictor. The prediction accuracies of Crea, CysC and NGAL were not significantly different. Furthermore, we compared the C-indexes of the eGFR equations, also predictors of 90 day mortality, to CysC and found no significant difference in the prediction accuracy (supplement 3; online publication only). A CysC cut-off value of 1.5 mg/l was found to predict 90 day mortality with 73.3% sensitivity and 52.5% specificity, respectively.

Discussion

The current study was performed to investigate the prognostic role of CysC and NGAL, two novel markers of renal function, in the setting of acutely decompensated liver cirrhosis. We analysed the data of 429 patients who were hospitalised due to acute decompensation of liver cirrhosis and included in the CANONIC trial. The main findings are that both, CysC and NGAL, were significant predictors of 90 day mortality. However, only CysC and not NGAL was found to independently predict renal dysfunction, HRS and ACLF development.

It is well known that Crea as a marker of renal function has several relevant limitations in patients with liver cirrhosis (34). Still, it is generally used because there are no established alternatives so far. Over the last years CysC and NGAL were suggested as valuable novel plasma biomarkers.

Besides markers in blood, several proteins have been evaluated in urine as potential biomarkers in cirrhosis, for example KIM-1, IL-18 and NGAL (19). Urinary NGAL, an indicator of tubular damage, has been proposed as a biomarker of renal dysfunction in liver cirrhosis (35). Recent data suggested NGAL in urine as a predictor of ACLF as well as of mortality (31, 36). However, there are some issues regarding urinary NGAL, the most relevant being that urinary NGAL concentrations increase in patients with urinary tract infections (37).

Therefore, the current study investigated NGAL and Cystatin C in blood plasma.

We analysed a large cohort of patients admitted due to acute decompensation of cirrhosis. Hence, our study provides a realistic image of the clinical situation. A proportion of 34% of all patients already had renal dysfunction at the time of inclusion, 41% had ACLF. In both groups we found a significant elevation of plasma levels of CysC as well as of NGAL.

Development of ACLF dramatically increases probability of short-term mortality in acutely decompensated cirrhosis. Therefore it could be very helpful to identify patients who do not exhibit ACLF, but are at high risk to develop ACLF. This could influence treatment decisions, such as admission to ICU or evaluation for liver transplantation. We analysed whether CysC or NGAL could predict development of renal dysfunction, HRS, ACLF and mortality, respectively.

Comparing baseline parameters of patients with and without development of RD revealed some remarkable differences. Paradoxically we found that patients with development of RD had less often infections at the time of inclusion (11.1% vs 31.2%; $p=0.03$); accordingly, an early infection seems to protect patients from renal deterioration, maybe due to better adaptation of immune response. Significant differences between the groups were shown with respect to the plasma levels of Crea, CysC and NGAL, which were markedly lower in

patients without development of RD. However, only CysC, but not NGAL, was found to be predictive for RD development.

Similar results were obtained with respect to the development of HRS. Baseline levels of all three, Crea, CysC and NGAL, were significantly higher in the group of patients who were about to develop HRS during follow up ($p < 0.0001$ for all). Again, CysC was the only independent predictor.

Regarding ACLF development interestingly neither CRP, leukocyte count nor the proportion of patients with an infection at inclusion did differ among the groups. Thus, based on our data, an infection or inflammation seems not to predict development of ACLF. In terms of renal markers, we found all three significantly increased in the group with ACLF development. Logistic regression revealed only CysC as an independent predictive factor for ACLF. In accordance with Ariza et al. (31) plasma NGAL did not predict ACLF development.

At 90 days after inclusion 116 of 421 patients were deceased. Comparing the baseline parameters of both groups revealed significantly higher concentrations of Crea, CysC and NGAL in the non-survivor group ($p < 0.0001$ for all). Furthermore, the baseline values of all three markers were predictive for 90 day transplant-free mortality. Remarkably, Ariza et al. found plasma NGAL not to be predictive of short-time mortality (31). However, in contrast to Ariza et al., who selected patients according to the availability of urine samples, we analysed only patients of the CANONIC population, who had a continuous follow-up surveillance during hospitalisation. Hence, even though patients of both studies were recruited from the same population, they are different, which may explain some diversity in results.

In 2012 a new eGFR equation, CKD-EPI-Creatinine-Cystatin, based on Crea and CysC, was published and subsequently validated in patients with liver cirrhosis (23, 33, 38). It was shown to reflect renal function more precisely than previous eGFR equations. Therefore we investigated the predictive potential of this equation in our study population. We additionally tested an equation based on CysC only (Cyst-eGFR) (32). In the present study, both eGFR equations were significant predictors of ACLF, RD and HRS development, as well as of 90 day mortality. However, none of them had better prediction accuracy than CysC alone.

Recently the concept of acute kidney injury (AKI) was introduced in cirrhosis. The definition of AKI was adapted from the AKIN (acute kidney injury network) criteria. Rather than the absolute Crea concentrations it takes into account its relative changes. AKI was described as predictor of mortality in patients with liver cirrhosis (39, 40). The reason for primarily focusing on RD defined by a Crea cut-off of 1.5 mg/dl, instead of AKI in the CANONIC protocol and in our study was that RD is part of the definition of ACLF as well as part of the CLIF-SOFA-Score. However, we also determined the presence of AKI in our study population. AKI was a significant predictor of 90 day mortality. Since AKI is of dichotomic nature, an AUCROC comparison to CysC regarding the predictive value was not possible. Remarkably, the presence of AKI was not significantly associated with the development of ACLF.

Recent studies on risk scores in patients with cirrhosis showed that calculations at day3 had better predictive values than using baseline data (41, 42). One could hypothesize that an increase of renal markers within the first three days after inclusion could be a strong predictor of clinical deterioration as well as mortality. Therefore we compared the prediction accuracy of baseline CysC to CysC of day3, but found no significant differences. Similarly, calculating the deltas of day3 minus baseline of Crea, CysC and NGAL, respectively, did not provide any benefit. Thus waiting two or more days seems of no benefit to improve CysC prediction of relevant deterioration.

In conclusion, the current study proves the potential of CysC as a valuable biomarker in patients with decompensated liver cirrhosis. We demonstrate that the baseline value of CysC is a predictor of the development of renal dysfunction, HRS and ACLF, as well as of 90 day transplant-free mortality. Moreover, the prediction accuracy of CysC was not inferior to Crea and/or CysC based eGFR equations. CysC $\geq$ 1.5 mg/l was found to be the best collective cut-off value with a good sensitivity for all events, e.g. over 80% sensitivity for HRS. Even though plasma NGAL was also associated with some above mentioned events, the results of the current study indicate a superiority of CysC in most aspects. Hence, CysC may help to early identify patients at risk of deterioration and might improve clinical management.

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Figure legend:

Figure 1: Renal markers at baseline as predictors for ACLF, renal dysfunction and HRS development. The figure shows the Odds ratios (with 95% CI), assessed by logistic regression of each marker's baseline value.

Table 1

Baseline characteristics of study population	
Age (years)	56.3 ± 11.8
Age (≥ 60)	38.9%
Sex (Male)	63.4%
Bilirubin (mg/dl)	3.7 (1.7 – 8.9)
INR	1.6 (1.3 – 2.1)
Na (mEq/l)	134.4 ± 6.5
MELD	21.4 ± 7.7
CHILD-PUGH	10.2 ± 2.1
ACLF = yes	41.0%
ACLF I	21.0%
ACLF II	15.6%
ACLF III	4.4%
HRS type 1	7.9%
ICU at admission	17.6%
Prior acute decompensation	77.3%
Creatinine (mg/dl)	1.1 (0.8 - 1.7)
Crea < 0.8	15.2%
0.8 < Crea < 1.5	50.6%
Crea ≥ 1.5	34.3%
NGAL (ng/ml)	221 (135 - 377)
Cystatin C (mg/l)	1.6 (1.2 - 2.2)

Table 1

Markers of renal function at baseline. Description by presence of ACLF, renal dysfunction and HRS, respectively.							
	ACLF		Renal dysfunction		HRS		
	Yes (n=176)	No (n=253)	Yes (n=147)	No (n=282)	Yes (n=34)	No (n=394)	
Creatinine (mg/dl)	1.7 (1.1-2.5)	0.9 (0.8-1.3)	2.1 (1.7-2.9)	0.9 (0.8-1.1)	2.4 (1.6-3.6)	1.1 (0.8-1.6)	p<0.0001 (for each category)
NGAL (ng/ml)	305 (211-546)	170 (113-274)	377 (269-617)	160 (111-244)	455 (214-760)	212 (129-348)	p<0.0001 (for each category)
Cystatin C (mg/l)	2.1 (1.5-2.1)	1.4 (1.1-1.8)	2.5 (2.1-3.1)	1.3 (1.0-1.6)	2.81 (1.8-3.2)	1.5 (1.1-2.1)	p<0.0001 (for each category)

Table 1

Baseline characteristics of patients with different grades of ACLF.				
	No ACLF (n=253)	ACLF I (n=90)	ACLF II (n=67)	ACLF III (n=19)
Age	55.7 ± 2.0	59.7 ± 11.8	55.1 ± 10.9	52.6 ± 9.9
Sex (Male)	62.5%	67.8%	65.7%	47.4%
Infections at inclusion	25.8%	30.3%	33.3%	63.1%
Leukocytes (x10 ⁹ cells/l)	6.3 (4.3 – 9.7)	6.9 (4.6 – 9.9)	9.9 (6.1 – 13.8)	10.8 (5.5 – 18.0)
CRP (mg/l)	18.0 (6.5 – 41.4)	23.2 (10.5 – 50.7)	25.7 (12.4 – 48.0)	48.0 (32.6 – 76.7)
Bilirubin (mg/dl)	3.0 (1.7 – 6.4)	2.3 (1.1 – 6.9)	13.3 (5.0 – 22.3)	25.3 (6.5 – 35.0)
INR	1.6 (1.3 – 1.9)	1.4 (1.2 – 1.8)	2.2 (1.7 – 2.7)	2.6 (2.2 – 3.8)
Na (mEq/l)	134.7 ± 6.2	134.0 ± 6.9	133.7 ± 5.9	133.9 ± 9.0
MELD	17.6 ± 5.6	23.3 ± 5.2	28.9 ± 6.5	35.6 ± 5.8
Child-Pugh	9.8 ± 1.8	9.4 ± 2.0	11.7 ± 1.7	12.8 ± 1.7
Creatinine (mg/dl)	0.9 (0.8 – 1.3)	2.0 (1.3 – 2.8)	1.3 (0.9 – 1.9)	2.1 (1.3 – 3.5)
NGAL (ng/ml)	170.0 (113 – 274)	295 (203 – 496)	262 (189 – 446)	718 (346 – 1178)
Cystatin C (mg/l)	1.4 (1.1 – 1.8)	2.3 (1.7 – 2.9)	1.7 (1.3 – 2.5)	2.5 (1.5 – 3.4)

Table 1

Baseline characteristics. Description by development of ACLF.			
No ACLF at baseline. Development afterwards:			
	Yes (n=69)	No (n=140)	p
Age	57.7 ± 11.6	55.5 ± 11.5	0.194
Sex (male)	63.7%	62.1%	0.819
Creatinine (mg/dl)	1.2 (0.8-1.5)	0.9 (0.8-1.1)	0.001*
- Delta d3-basal	0.0 (-0.1 - 0.1)	0.0 (-0.1 - 0.1)	0.467
NGAL (ng/ml)	212 (118-320)	155 (110-247)	0.048*
- Delta d3-basal	31 (-8 - 67)	-3 (-39 - 24)	0.027*
Cystatin C (mg/l)	1.7 (1.2-2.2)	1.3 (1.0-1.7)	0.001*
- Delta d3-basal	-0.01 (-0.12 - 0.14)	0.03 (-0.09 - 0.18)	0.570
Bilirubin (mg/dl)	3.8 (2.2-8.7)	2.9 (1.7-6.1)	0.059
INR	1.7 (1.4-2.2)	1.5 (1.3-1.8)	0.004*
MELD	19.9 ± 6.0	16.8 ± 5.1	0.001*

Table 1

Baseline characteristics. Description by development of RD.			
No RD at baseline. Development afterwards:			
	Yes (n=27)	No (n=170)	p
Age	58.1 ± 8.9	53.4 ± 12.1	0.053
Sex (male)	74.1%	65.3%	0.369
Creatinine (mg/dl)	1.2 (0.9 - 1.3)	0.9 (0.7 - 1.1)	0.001*
- Delta d3-basal	0.4 (0.1 - 1.0)	0.0 (-0.1 - 0.1)	0.001*
NGAL (ng/ml)	221 (154 - 324)	166 (113 - 256)	0.022*
- Delta d3-basal	74 (-18 - 421)	-5 (-44 - 34)	0.003*
Cystatin C (mg/l)	1.6 (1.2 - 1.9)	1.3 (1.0 - 1.5)	0.005*
- Delta d3-basal	0.3 (0.1 - 0.9)	0.0 (-0.1 - 0.2)	0.001*
Bilirubin (mg/dl)	5.7 (2.2 - 16.8)	5.4 (2.2 - 10.0)	0.452
INR	1.7 (1.4 - 2.0)	1.7 (1.4 - 2.3)	0.428
MELD	21.4 ± 7.4	20.6 ± 7.1	0.549

Table 1

Baseline characteristics. Description by development of HRS.			
No HRS at baseline. Development afterwards:			
	Yes (n=42)	No (n=350)	p
Age	59.1 ± 11.6	56.2 ± 11.9	0.130
Sex (male)	59.5 %	64.6 %	0.520
Creatinine (mg/dl)	1.7 (1.2 - 2.5)	1.0 (0.8 - 1.5)	0.001*
- Delta d3-basal	0.0 (-0.5 - 0.4)	0.0 (-0.2 - 0.1)	0.964
NGAL (ng/ml)	318 (225 - 461)	198 (123 - 312)	0.001*
- Delta d3-basal	-2 (-61 - 107)	-3 (-48 - 40)	0.800
Cystatin C (mg/l)	2.1 (1.7 - 2.8)	1.5 (1.1 - 2.0)	0.001*
- Delta d3-basal	0.0 (-0.2 - 0.5)	0.0 (-0.2 - 0.2)	0.461
Bilirubin (mg/dl)	5.5 (2.3 - 13.7)	3.5 (1.7 - 8.4)	0.101
INR	1.7 (1.3 - 2.0)	1.6 (1.3 - 2.1)	0.994
MELD	24.8 ± 6.7	20.3 ± 7.4	0.001*

Table 1

Characteristics of study population at baseline. Description by 90-day mortality.			
	Alive (n=305)	Dead (n=116)	p
Age	56.0 ± 12.1	57.2 ± 10.8	0.367
Sex (male)	64.3 %	62.1 %	0.676
Bilirubin (mg/dl)	2.9 (1.5 – 7.2)	6.8 (2.8 – 18.9)	<.0001*
INR	1.5 (1.3 – 1.9)	2.0 (1.6 – 2.5)	<.0001*
AKI	13.9% (28/201)	44.0% (33/75)	<.0001*
CRP (mg/l)	20.0 (6.5 – 40.0)	34.6 (17.7 – 60.0)	<.0001*
Infections at Inclusion	27.0 %	37.4 %	0.039*
MELD	19.4 ± 6.8	26.5 ± 7.7	<.0001*
Child-Pugh	10.0 (8.0 – 11.0)	11.5 (10.0 – 13.0)	<.0001*
ACLF	33.4 %	61.2 %	<.0001*
Creatinine (mg/dl)	1.0 (0.8 – 1.6)	1.4 (1.0 – 2.3)	<.0001*
- Delta d3-basal	0.0 (-0.2 - 0.1)	0.0 (-0.3 - 0.2)	0.056
NGAL (ng/ml)	197 (123 – 309)	308 (187 – 571)	<.0001*
- Delta d3-basal	-5 (-55 - 33)	-2 (-40 - 76)	0.157
Cystatin C (mg/l)	1.5 (1.1 – 2.0)	1.9 (1.5 – 2.8)	<.0001*
- Delta d3-basal	0.0 (-0.2 - 0.2)	0.1 (-0.2 - 0.4)	0.262
Cyst-eGFR	51.6 ± 25.3	37.9 ± 19.4	<.0001*
CKD-EPI Creatinine-Cystatin	59.4 ± 28.9	43.6 ± 25.2	<.0001*

Figure 1: Renal markers at baseline as predictors for ACLF, renal dysfunction and HRS development. The figure shows the Odds ratios (with 95% CI), assessed by logistic regression of each marker's baseline value.

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