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**BIOMARKERS FOR ACUTE KIDNEY INJURY IN DECOMPENSATED
CIRRHOSIS: A PROSPECTIVE STUDY**

Running title: Biomarkers for AKI in cirrhosis.

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MAIN TEXT

Declarations:

Ethics approval and consent to participate: This study was approved by the ethical committee for human studies of Geneva University Hospitals and performed according to the Declaration of Helsinki. It was registered in clinicaltrials.gov (NCT01217983).

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author's contribution: DAJ analyzed the data and wrote the manuscript. LS designed the study and reviewed the manuscript. GB and VP collected the data. PL conducted biochemical analyses. EG and NG designed the study and reviewed the manuscript. PYM designed and supervised the study, and reviewed the manuscript. BP designed and supervised the study, analyzed the data and wrote the manuscript.

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List of abbreviations in the order of appearance:

AKI: Acute kidney injury

HRS: Hepatorenal syndrome

ATN: Acute tubular necrosis

AKIN: Acute kidney injury network

SCr: serum creatinine

CystC: Cystatin C

NGAL: Neutrophil Gelatinase-Associated Lipocalin

KIM-1: Kidney Injury Molecule 1

GFR: Glomerular Filtration Rate

RRI: Renal resistive index

CKD: Chronic kidney disease

ICU: Intensive care unit

NaFE: Sodium fractional excretion

ACR: Urinary albumin over creatinine ratio

CV : Coefficient variation

SD: Standard deviation

CI: Confidence Interval

AUC: Area under the curve

Coef: coefficient

Abstract

Background: Acute kidney injury (AKI) is a frequent complication in cirrhotic patients. As serum creatinine is a poor marker of renal function in this population, we aimed to study the utility of several biomarkers in this context.

Methods: A prospective study was conducted in hospitalized patients with decompensated cirrhosis. Serum creatinine (SCr), Cystatin C (CystC), NGAL and urinary NGAL, KIM-1, protein, albumin and sodium were measured on three separate occasions. Renal resistive index (RRI) was obtained. We analyzed the value of these biomarkers to determine the presence of AKI, its etiology [prerenal, acute tubular necrosis (ATN), or hepatorenal (HRS)], its severity and a composite clinical outcome at 30 days (death, dialysis and intensive care admission).

Results: We included 105 patients, of which 55 had AKI. SCr, CystC, NGAL (plasma and urinary), urinary sodium and RRI at inclusion were independently associated with the presence of AKI. SCr, CystC and plasma NGAL were able to predict the subsequent development of AKI. Pre-renal state showed lower levels of SCr, NGAL (plasma and urinary) and RRI. ATN patients had high levels of NGAL (plasma and urinary) as well as urinary protein and sodium. HRS patients presented an intermediate pattern. All biomarkers paralleled the severity of AKI. SCr, CystC and

plasma NGAL predicted the development of the composite clinical outcome with the same performance as the MELD score.

Conclusions: In patients with decompensated cirrhosis, early measurement of renal biomarkers provides valuable information on AKI etiology. It could also improve AKI diagnosis and prognosis.

Keywords: Acute kidney injury, biomarkers, cirrhosis, cystatin C, NGAL.

INTRODUCTION

Acute kidney injury (AKI) is a frequent complication in patients with cirrhosis admitted to the hospital (20%) and a major determinant of outcome (1-3). In most cases, the cause is pre-renal and AKI is volume-responsive. Hepatorenal syndrome (HRS) requiring vasoactive drugs may complicate its course. Acute tubular necrosis (ATN) is diagnosed in one third of cases (4). Those different etiologies of kidney injury may require specific therapies and are associated with different mortality rate (1, 5, 6). Current serum and urinary parameters can be useful for diagnosis, but they lack sensitivity for early detection and cannot discriminate between the different etiologies of AKI.

Recently, expert committees have recommended the use of the Acute Kidney Injury Network (AKIN) classification to be used for cirrhotic patients, as it seems to predict hospital mortality in those with or without ascites (7-11). Although serum creatinine (Scr) is the most widely used marker for renal function, its value may be affected by non-renal factors such as sex, race, age, body mass index, and drugs. In cirrhosis, the diagnostic value is even lower due to decreased hepatic synthesis, decreased skeletal muscle mass, low protein intake, increased distribution volume and increased tubular secretion (12, 13). Finally, the delay between renal function decrease and rise in Scr is a major limitation for its use as an early marker.

New biomarkers including Cystatin C (CystC), Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Kidney Injury Molecule 1 (KIM-1) have been proposed to overcome these limitations, and have shown benefits regarding diagnosis and prognosis in different populations (14). **CystC** is freely filtered by the glomerulus, reabsorbed then catabolized in the proximal tubule and not secreted. The increase in serum values precedes the elevation of SCr by 24 to 48 hours and, predicts the need of renal replacement therapy in intensive care setting (15). In cirrhotic patients it has

been successfully used to estimate glomerular filtration rate (GFR) (16, 17) and to predict mortality and development of HRS, as well as acute on chronic liver failure (18-20). **NGAL** is expressed in several human tissues (kidney, lung, stomach, and colon) and induced by injured epithelia. Serum and urinary NGAL have been tested after cardiac surgery and seem to be specific of tubular damage and predict prognosis (21). Some data in cirrhosis suggest that urinary NGAL predicts AKI and mortality and discriminates ATN from other forms of kidney injuries (3, 20, 22-26). **KIM-1**, an apical transmembrane glycoprotein located in proximal tubules, is increased during ischemia while soluble KIM-1 is released into urine (27, 28). In cirrhotic patients, a multicentric study showed higher urinary levels in patients with ATN and its association with AKI progression and death (3, 24).

Ultrasonography coupled with renal artery resistive indexes (RRI) measurement, can detect arterial vasoconstriction and predict HRS (29, 30). Combining RRI with new renal biomarkers might improve diagnostic performance of AKI in patients with cirrhosis.

Patients with acute decompensation of cirrhosis have a state of sodium avidity with fluid overload, and are at increased risk of AKI. Creatinine can be even less accurate in such patients with ascites. Therefore, we prospectively explored, in patients with cirrhosis and ascites admitted to the hospital, the diagnostic performance of new biomarkers and renal resistive index in the development of AKI and clinical outcome.

MATERIAL AND METHODS

Study design

In 2012, we conducted over 2 years a prospective observational cohort study in patients admitted to the internal medicine department of Geneva University Hospitals for decompensated cirrhosis (i.e. with ascites). All consecutive patients were

screened and approached for enrolment early after hospital admission. Inclusion criteria were an age ≥ 18 years and known or suspected cirrhosis with ascites confirmed by ultrasonography. Exclusion criteria were proven multifocal hepatocellular carcinoma, known CKD stage V (estimated GFR $< 15\text{ml/min/1.73m}^2$) or dialysis before admission, prior kidney or liver transplantation, recent upper gastrointestinal bleeding (< 2 weeks), or more than 24 hours delay between the admission and inclusion. Informed consent was sought from all eligible patients, or from a surrogate decision maker (relative or physician in charge) if the patient was unable to provide consent.

Patients were followed from admission for 30 days or until discharge from the hospital. Those who were discharged prior to day 30 were contacted by phone to assess clinical outcome. All patients with cirrhosis and ascites were treated based on standard of care. Withdrawal of diuretics, volume replacement, therapeutic paracentesis with IV albumin substitution, as well as terlipressin administration were performed in the setting of HRS. This study was approved by the ethical committee for human studies of Geneva University Hospitals and performed according to the Declaration of Helsinki. It was registered in clinicaltrials.gov (NCT01217983).

Objectives

The primary objective was the diagnosis and early detection of AKI in patients with decompensated cirrhosis using a panel of serum (SCr, CysC, NGAL) and urinary (sodium, protein, NGAL, KIM-1) biomarkers as well as RRI.

Secondary objectives were to grade the severity of AKI, to differentiate between various etiologies (pre-renal, HRS, ATN) and to predict clinical outcome (death, transfer to intensive care or necessity for renal replacement therapy) at 30 days.

Sample collection and laboratory measurement

Blood and urine samples were serially collected at inclusion (T1), 2-3 days (T2) and 4-7 days (T3) after hospital admission. Plasma and urine creatinine were measured using the IDMS-traceable Jaffe kinetic compensated method (Synchro Creatinine, Beckman Coulter, Brea, CA) on a UniCel DxC 800 clinical system (Beckman Coulter). Electrolytes were measured by indirect potentiometry (Unicel DxC 800 Synchro Clinical System). Sodium fractional excretion (NaFe) was computed as: $(\text{urine Na} \times \text{serum creatinine}) / (\text{urine creatinine} \times \text{serum Na})$. Urinary total protein was measured and protein / creatinine ratio calculated in g/mg. Urine albumin was measured only by immunonephelometry on a BN Prospec automated analyzer (Siemens Healthcare Diagnostics, Marburg, Germany) and Urinary albumin / creatinine ratio (ACR) computed in mg/mmol. CystC was measured by automated latex-enhanced immunonephelometric method (Cystatin C immunoparticules, Dako, Denmark) on an Image immunochemistry analyzer (Beckman Coulter, USA). The assay's range was 0.2-10.0 mg/l. The intra-assay coefficient variation (CV) was 0.98 % for a mean concentration of 3.95 mg/l and 1.35 % for a mean concentration of 0.96 mg/l. The inter-assay CV was 3.21 % for a mean concentration of 4.42 mg/l and 7.11 % for a mean concentration of 1.16 mg/l.

Additional samples were collected on EDTA-plasma, serum and urine and immediately centrifuged at 3000g for 10', aliquoted in 1ml tubes and frozen at -80°C. There was no freeze/thaw cycle. No protease inhibitors were added to the samples. The laboratory staff was blinded to clinical data.

NGAL was measured in duplicate and batch on frozen plasma and urine using an ELISA assay (NGAL Rapid ELISA Kit, Bioporto, Hellerup, Denmark). The limit of detection was 0.1ng/mL and the assay range 0.2-20ng/mL. The intra-assay CV for plasma and urine were 1.9-2.9% and 3.4-4.3%, respectively. The inter-assay CV was

11.4-12.4% and 4.7-22.7%, respectively. KIM-1 was only measured at inclusion in duplicate and batch on frozen urine using an ELISA assay (Human KIM-1 ELISA kit, ICL, Portland, OR 97224 USA). The limit of detection was 0.02ng/mL and the assay range 0.03-0.197ng/mL. The intra and inter-assay CV were both <10%.

Ultrasound of the kidney was done within 24h of inclusion by the same operator (BP). Gray-scale B mode sonography was first be performed, followed by color Doppler with a 3.5 MHz phased-array transducer. RRI [= (peak systolic velocity – end diastolic velocity)/peak systolic velocity] were reported. The methodology of ultrasound has been previously described in details (31).

Definitions

The diagnosis of cirrhosis was based either on a combination of clinical, biological and radiological data, or histology.

Diagnosis of AKI was defined as a rise in creatinine of $\geq 26.4 \mu\text{mol/l}$ (0.3mg/dl) or 50% from baseline according to AKIN (11). Severity of AKI was classified through AKIN stages 1 to 3, using the maximal creatinine value during the stay (11).

The causes of AKI were distributed as pre-renal, HRS, ATN and other cause of parenchymal kidney disease according to available clinical and para-clinical data. HRS diagnosis was based on the standardized International Ascites Club (IAC) criteria (11, 32). There is a lack of standardized criteria in cirrhotic patients for pre-renal and ATN diagnosis. Therefore, we defined pre-renal AKI as intravascular volume depletion with reversibility of kidney impairment after fluid resuscitation in the appropriate clinical context (22, 26). On the contrary, ATN was considered when there was no response to the volume repletion, in presence of septic shock and/or nephrotoxic agents, increased urinary sodium concentration and no HRS criteria. The presence of tubular cells on urine microscopic analysis was also accounted for ATN

diagnosis. The definite cause of AKI was adjudicated after patient's discharge by a panel of experts made of internists, hepatologists and nephrologists who reviewed the patient's data while blinded to biomarkers values. Final diagnosis was based on the agreement of at least 2 experts.

GFR was estimated by CKD-EPI equation. CKD was diagnosed in case of known CKD or $eGFR < 60 \text{ ml/min/1.73m}^2$.

The baseline creatinine was defined as the most recent steady value available within the year before admission.

Statistical analyses

Analyses were conducted using Stata version 13.0 (StataCorp, College Station, TX), and statistical significance defined as a p-value < 0.05 .

Continuous variables were reported as mean \pm standard deviation or median and interquartile range and compared by t-test or Wilcoxon rank-sum test according to their distribution. Categorical variables were presented as numbers (n) and proportions (%) and compared by Chi-square test. We built mixed linear models taking into account the day and the repeated measures to obtain the significance of the changes of biomarkers over time.

Logistic regression was applied to explore the association of each renal biomarker separately at inclusion and the outcome of interest. All the biomarkers except RRI were log-transformed. The main outcome was AKI. Death, ICU admission, and dialysis were combined in one single composite secondary outcome. Results were reported as coefficient with 95% confidence intervals (CI) and corresponding p value. Only biomarkers having a significant association with the outcome of interest ($p < 0.05$) were introduced in the multivariate model. For the main outcome, covariates used for multivariate adjustment were chosen according to the literature or if

significantly different in the univariate comparisons between AKI or non-AKI patients. For the secondary composite outcome, we aimed to test if the biomarkers were able to predict the outcome independently of the presence of AKI and infection. ROC analyses were conducted to compute the area under the curve (AUC) for each biomarker.

For AKI causes, differences were assessed through non parametric Kruskal-Wallis test. For AKIN stages, the non-parametric p test for trend was used to assess differences in biomarkers' levels between groups.

As sensitivity analyses, we studied also the subgroup of patients who had normal renal function at inclusion and developed later AKI (=delayed AKI group) and compared them to those without AKI.

RESULTS

We assessed 8'897 hospitalized patients from which 4.9% had a principal or secondary diagnosis of cirrhosis and 2.2% had confirmed ascites (**supplementary Figure 1**). From the 193 patients with decompensated cirrhosis, 88 (45.6%) did not meet the inclusion criteria, mainly for delayed inclusion, denial of consent, and multifocal hepatocellular carcinoma. Other reasons for exclusion were: already included (n=6), many exclusion criteria (n=2), previous liver transplant (n=1), dialysis (n=1), palliative care (n=1), language (n=1) and secondary refusal (n=1). Excluded patients were predominantly males (67%) with mean age of 60.6±6.7 years. Finally, 105 patients constituted the study population. AKI occurred in 52.4% (n=55). The causes of AKI were pre-renal (n=41, 74.5%), ATN (n=8, 14.5%) and HRS (n=6, 11%). No other cause of parenchymal kidney disease was observed. Regarding AKI severity, 52.7% (n=29) presented with AKIN stage 1, 32.7% stage 2 (n=18) and 14.5 (n=8) stage 3. Only 1 patient needed renal replacement therapy. None had received

any nephrotoxic drug during the last 2 weeks before admission. The etiology of cirrhosis was mainly alcohol alone (n=84, 80%), or in association with viral hepatitis (n=20, 19%).

Baseline characteristics

Table 1 shows participants' characteristics and renal biomarkers on inclusion. The mean age was 58.0 ± 10.2 years and 71.4% were males. AKI patients had a higher prevalence of hypertension (34.5% vs 6%, $p < 0.001$) and use of diuretics on admission (70.4% vs 38.9%, $p = 0.001$) than non-AKI patients. As compared with patients without AKI, those with AKI were older, had lower baseline eGFR, lower mean blood pressure and higher MELD score. Compared to non-AKI patients, the AKI group showed higher serum creatinine, CystC and NGAL, higher urinary NGAL as well as higher RRI but lower urinary sodium. There was no difference in urinary KIM-1 levels, NaFe, proteinuria or ACR.

Complications during follow-up

Complications during the follow-up are provided in **Table 2**. Patients with AKI had longer hospital stays, presented more complications (death, ICU admissions, infections or spontaneous bacterial peritonitis), received more frequently vasoactive drugs and had higher volumes of ascites evacuated than non-AKI patients. Dialysis rate was similar between groups.

Diagnostic performance of biomarkers to detect AKI

We analyzed the association of each individual biomarker at T1 (inclusion) with the occurrence of AKI during hospital stay. Variables used for adjustment were mean blood pressure, age, infection, diuretics and hypertension, which all were found to be

different in AKI compared to non-AKI. SCr [coef 2.71 (95%CI 1.19; 4.23), $p < 0.001$], CystC [coef 3.59 (95%CI 1.61; 5.57), $p < 0.001$], plasma NGAL [coef 1.42 (95%CI 0.58; 2.25), $p = 0.001$], urinary NGAL [coef 0.34 (95%CI 0.03; 0.65), $p = 0.031$] and RRI [coef 9.36 (95%CI 1.15; 17.5), $p = 0.025$], were positively associated with AKI. Urinary sodium was the only biomarker inversely associated with AKI [coef -0.58 (95%CI -1.02; -0.13), $p = 0.010$]. ROC curves and AUC for individual biomarker performances are shown in **Figure 1A**. CystC showed the highest AUC: While difference with RRI and urinary NGAL AUC was significant ($p = 0.039$ and $p = 0.006$ respectively), difference with serum creatinine and NGAL AUC were not.

When combining together several biomarkers to predict AKI at T1, adding CystC to SCr increased the AUC from 0.81 to 0.85 but failed to reach statistical significance ($p = 0.055$). Adding all relevant markers to SCr (CystC, plasma NGAL, urinary NGAL and RRI) further improved the AUC to 0.87 but still fell short of significance ($p = 0.054$).

Amongst 55 patients with AKI, 29 (52.7%) had normal baseline renal function at T1 but developed AKI later (=delayed AKI group). Compared to the non-AKI group, those patients had higher SCr (69 vs 59.5, $p = 0.003$), CystC (1.28 vs 1.02, $p < 0.001$), serum NGAL (118.8 vs 65.6, $p = 0.007$) and RRI (0.75 vs 0.72, $p = 0.034$) at T1. We performed backward stepwise multivariate logistic regression adjusting for diuretics and hypertension: SCr [coef 1.80 (95%CI 0.08; 3.51), $p = 0.039$], CystC [coef 2.44 (95%CI 0.49; 4.38), $p = 0.014$] and serum NGAL [coef 0.92 (95%CI 0.01; 1.85), $p = 0.049$] at T1 remained positively associated with later AKI occurrence. CystC showed the highest AUC on ROC analysis (0.73) although the difference with other biomarkers was not statistically different.

Evolution of biomarkers

The evolution of the biomarkers measured over the first week (T1-T2-T3) is depicted in **Figure 2** for all patients. In patients without AKI, SCr and urinary NGAL did not vary over time whereas CystC, plasma NGAL, urinary sodium and NaFe increased over time. On the contrary, in patients with AKI, we observed a decrease of creatinine and urinary NGAL, and no change in CystC levels. When we compared AKI vs non AKI patients biomarkers levels, taking into account time-revolution, only NaFE was not statistically different between the 2 groups.

We also looked at the biomarkers evolution in patients without AKI at admission but developing it later (“delayed AKI”): CystC and plasma NGAL increased whereas SCr, urinary sodium and NaFe did not vary over time (**supplementary Figure 2**). Levels of SCr, CysC, plasma NGAL, urinary sodium and NaFe were different between non AKI and delayed AKI groups whereas urinary NGAL was not.

Difference in renal biomarkers according to AKI cause

In three-group comparison between pre-renal, ATN and HRS (table 3), SCr, maximal SCr during stay as well as plasma and urinary NGAL at baseline (T1) were lower in patients with pre-renal injury than in patients with ATN or HRS. Urinary protein excretion and ACR were higher in ATN than in pre-renal injury or HRS. RRI was the lowest in the pre-renal group and showed a significant trend across the three groups ($p=0.027$).

In two-group comparison between ATN and pre-renal/HRS, plasma NGAL ($p=0.009$), urinary NGAL ($p=0.015$), urinary protein excretion ($p=0.004$), ACR ($p<0.001$) and NaFE ($p=0.032$) were higher in ATN than in pre-renal injury and HRS.

In two-group comparison between ATN and HRS, urinary protein excretion ($p=0.045$), ACR ($p=0.006$), urinary sodium ($p=0.018$) and NaFE ($p=0.028$) were higher in ATN than in HRS.

Renal biomarkers and AKI severity

CystC, plasma and urinary NGAL and RRI at T1 increased progressively with the severity of the renal injury (**Figure 3**).

Prognostic performance of renal biomarkers

Finally, we examined the relationship between biomarkers and the secondary composite clinical outcome (**supplementary Table 1**). Only SCr, CystC, plasma NGAL and RRI were significantly associated with outcome in univariate analyses and were entered into a multivariate model adjusted for the presence of AKI and infection. Serum Cr, CystC and serum NGAL remained associated with the outcome, but not RRI.

Based on the AUC ROC values, the diagnostic performance to predict a poor outcome was similar between biomarkers and the MELD score at inclusion (**Figure 1B**).

DISCUSSION

In this prospective single center study, we explored the value of both new and traditional biomarkers at several time points for the diagnosis of AKI and their ability to early detect AKI, classify its etiology, grade its severity and predict clinical outcome in patients with decompensated cirrhosis and ascites.

First, we measured an incidence of AKI in this population of 52.4%, which is higher than what has been previously described in cirrhotic patients without ascites. SCr,

CystC, NGAL (plasma and urinary) and RRI at inclusion were independently associated in multivariate analysis with the presence of AKI. CystC showed the best performance for the detection of AKI and performed significantly better than urinary NGAL and RRI. A combined model including SCr, CystC, plasma and urinary NGAL as well as RRI provided the best diagnostic performance to detect AKI but just failed to significantly outperform the use of SCr alone. Interestingly, NaFE was <1% irrespective of the presence of AKI and similar between AKI and non-AKI patients. This finding could reflect cirrhotic hemodynamic alterations leading to low NaFE irrespective of the presence of AKI (33). Amongst all tested biomarkers, only SCr, CystC and plasma NGAL were able to predict the subsequent development of AKI during hospital stay in patients with normal renal function at inclusion.

Second, several biomarkers were able to reliably differentiate between the three main etiologies of AKI in cirrhotic patients. Pre-renal state showed lower levels of SCr and NGAL (plasma and urinary) reflecting the functional nature of the process sparing tubular structure. On the opposite, ATN patients specifically showed high levels of NGAL (plasma and urinary) as well as urinary protein and sodium likely reflecting tubular damage in this setting. HRS patients presented an intermediate pattern of biomarkers that could be interpreted as reflecting the mixed pathophysiological nature of the disease. The role of RRI in differentiating various cause of AKI in this setting was less clear but seemed to show lower values in functional state compared to structural disease. NaFE was <1% irrespective of the cause of AKI. ATN however showed significantly higher NaFE compared to pre-renal/HRS and HRS respectively. These findings potentially highlight the structural nature of ATN. They are also in accordance with recent evidence showing that 83% of cirrhotic patients with histological evidence of ATN had NaFE <1% (34).

Third, the level of all tested biomarkers increased with each stage of AKI severity.

Finally, Cr, CystC and plasma NGAL predicted the development of complications such as death, intensive care admission or dialysis with the same performance as the MELD score.

Overall, we confirm that the use of several plasma and urinary biomarkers has meaningful implications in cirrhotic patients presenting with AKI as it improves etiological classification thus potentially enhancing early and specific therapeutic decisions. Moreover, CystC and serum NGAL showed promising performance in early diagnosis of AKI and prognostic assessment of these patients.

Compared to other publications, our study comprises several differences. First, we included exclusively patients with proven presence of ascites to focus specifically on this sub-population of decompensated patients. Second, we repeatedly measured biomarkers at days 3 and 7 after inclusion. This allowed us to explore the short time dynamic of these biomarkers in AKI and see how different the evolution can be according to the presence and timing of AKI. Only one other study made repeated measurement in this setting (33). However, authors focused only on urinary NGAL in a sub-population of infected patients. Third, no other study included systematical measurement of RRI in this setting in addition to several biomarkers.

Recently, Belcher et al. from the TRIBE-AKI consortium reported their results on the utility of biomarkers for the differential diagnosis of AKI in patients with cirrhosis (3). Using a combination of urinary biomarkers (NGAL, IL-18, KIM-1, L-FABP, albumin), they demonstrated that it was possible to better discriminate ATN from HRS. Their study differs from ours as they separated patients in “progressors” and “non-progressors” based on creatinine trend over time. Moreover, only a fraction of included patients had ascites while plasma biomarkers as well as RRI were not

obtained. Main results however are consistent between both studies as ATN was easily separated from functional disease based on NGAL and urinary protein. ATN was more difficult to distinguish from HRS but we confirmed higher urinary albumin and NaFE in the first group. Our findings are also consistent with the data of Ariza et al. who found that urinary NGAL and ACR had good performance in separating ATN from pre-renal state and HRS (26).

In a recent publication, Markwardt et al. also specifically focused on patients with decompensated cirrhosis with a follow-up of 90 days (20). In this study, CystC, but not plasma NGAL, could predict subsequent AKI development. Yap et al. also assessed the predictive capacity of several biomarkers for subsequent HRS over a 12-week follow-up in 43 Child B/C cirrhotic patients: urinary NGAL and KIM-1 were associated with this outcome whereas CystC and plasma NGAL were not (35). Compared to these two studies, our results differ slightly as we found plasma NGAL as well as CystC to be predictive of subsequent AKI.

Several studies explored predictive capacity of biomarkers on clinical outcomes and showed that urinary NGAL was an independent predictor of short-term mortality (20, 33). Belcher et al. demonstrated that urinary biomarkers were associated with progression of AKI and mortality (24). More recently, Puthumana et al. (34) confirmed in a meta-analysis comprising 1129 patients that urinary NGAL and IL-18 could identify ATN and predict short-term mortality with satisfactory performances. A recent study however failed to confirm an independent relationship at 30 days between urinary NGAL levels and mortality (25). In our study, we found that plasma NGAL levels were associated with a composite outcome of death, ICU admission or renal

replacement therapy independent of infection or AKI. This finding adds relevant information, as urinary samples can be difficult to obtain.

None of those previous reports described the evolution of biomarkers over time. We observed significant changes in some biomarkers during the first week after inclusion and differences between AKI and non-AKI patients, even in patients with delayed AKI. Unfortunately, definite conclusions are difficult to draw as the real onset of the injury is unknown and some changes could be due related to eGFR and not kidney damage itself.

We acknowledge some limitations in our study. First, although adequately powered to characterize our main outcome, we cannot exclude that an insufficient sample size prevented us to detect meaningful differences in secondary outcomes and subgroup analyses. Likewise, the limited number of patients also prevented us from adjusting for other potential confounders. Second, in the absence of gold standard, we used variations of SCr to define AKI, which could have contributed to an underestimation of renal injury. This limitation is however shared by every study in this field. Third, etiological diagnosis of AKI was left to the adjudication of an expert committee. Although there are standardized criteria for HRS diagnosis, there is no strict consensus to define ATN or pre-renal in cirrhotic patients. In our study, pre-renal AKI was diagnosed according to volume-responsiveness and ATN according to the combination of various criteria. Although previous publications on this field have used similar strategies (22, 26), there is still a risk of miss-classification. Histological confirmation, although rarely done in this setting, would have represented a suitable gold standard. Likewise, pre-renal, HRS and ATN represent a continuum of diseases and distinguishing between pure etiological patterns might prove difficult. Finally, the

design of the study itself did not allow us to fully characterize the temporality of AKI in cirrhosis as half of the patients already suffered from AKI at inclusion. We tried to reduce this limitation by performing subgroup time analysis on the other half who developed AKI later during hospital stay.

CONCLUSION

In conclusion, this prospective study confirms that patients with cirrhosis and ascites are at very high risk of AKI. While a majority suffers from pre-renal injury, we show that a combination of plasma and urinary biomarkers might allow etiological diagnosis of functional state, HRS and ATN thereby potentially allowing early therapeutic decisions. These biomarkers might also have a role in early detection of AKI as well as overall prognostic assessment.

Such a combination of biomarkers could be used to design future interventional studies aiming at improving the outcome of these patients.

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Table 1: Participants' general characteristics on inclusion (n=105)

Variables	Total (n=105)	Non AKI (n=50)	AKI (n=55)	p
Male gender, n (%)	75 (71.4)	32 (64)	43 (78.2)	0.108
Age years	58.0±10.2	55.0±10.0	60.7±9.7	0.004
Mean blood pressure mmHg	77.7±12.6	81.0±12.0	74.5±12.5	0.005
Biological tests				
*Baseline SCr µmol/l	62 (49-84)	54 (44-55)	72 (57-88)	<0.001
*Baseline CKD-EPI ml/min/1.73m ²	101.2 (85.9-113.3)	110.2 (98.4-120.4)	90.4 (78.5-106.5)	<0.001
MELD score	14.8 (10.0-19.8)	11.7 (9.2-15.8)	18.0 (13.8-23.2)	0.002
Plasma sodium mmol/l	134.2±4.8	134.7±4.1	133.8±5.4	0.37
Bilirubin µmol/l	77 (37-140)	74.5 (34-144)	78 (37-139)	0.79
INR	1.5 (1.2-1.7)	1.5 (1.2-1.7)	1.5 (1.2-1.7)	0.74
Renal Biomarkers at inclusion				
SCr µmol/l	69 (56-112)	59.5 (50-71)	100 (67-162)	<0.001
Serum CystC mg/l	1.27 (1.02-1.83)	1.03 (0.91-1.24)	1.54 (1.27-2.16)	<0.001
Plasma NGAL ng/ml	94.0 (59.6-173.6)	65.6 (49.2-102.6)	150.4 (75.8-224.4)	<0.001
Urinary NGAL ng/ml	30.6 (14.3-77.3)	24.7 (10.0-46.2)	41.6 (24.3-92.0)	0.004
Urinary NGAL/creat ng/mmol	2.9 (1.5-6.7)	2.22 (1.35-5.20)	4.12 (2.04-7.68)	0.009
Urinary KIM-1 ng/ml	0.16 (0.03-0.42)	0.14 (0.01-0.43)	0.22 (0.06-0.42)	0.35
Urinary KIM-1/creat ng/mmol	0.018 (0.004-0.048)	0.014 (0.006-0.048)	0.023 (0.003-0.048)	0.56
Urinary sodium mmol/l	29 (6-86)	64 (19-99)	19.5 (5-67)	0.009
NaFE %	0.2 (0.1-0.6)	0.3 (0.1-0.6)	0.2 (0.1-0.6)	0.48
Urinary protein/creat g/mg	0.11 (0.09-0.23)	0.11 (0.08-0.23)	0.12 (0.09-0.23)	0.34
ACR mg/mmol	0.9 (0.5-3.2)	0.9 (0.4-2.2)	1.1 (0.5-4.3)	0.18

RRI	0.75±0.07	0.72±0.01	0.78±0.01	<0.001
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Continuous variables are expressed as mean ± sd or median and interquartile (IQR).

*Baseline SCr and eGFR are the values before admission.

ACR: Urinary albumin over creatinine ratio. NaFE: sodium fractional excretion calculated as $100 \times (\text{Na u} \times \text{creat pl} / \text{Na pl} \times \text{creat u} \times 1000)$. SCr: serum creatinine. CystC: Cystatin C. RRI: renal resistive index

Table 2: Clinical evolution during the follow-up (n=105)

Variables	Total (n=105)	Non AKI (n=50)	AKI (n=55)	p
Hospital stay (days)	14 (10-19.5)	13 (9-16)	15 (11-24)	0.022
ICU admission, n(%)	18 (17.1)	3 (6.0)	15 (27.3)	0.004
Death, n(%)	7 (6.7)	1 (2.0)	6 (10.9)	0.07
Dialysis, n(%)	1 (0.95)	0 (0.0)	1 (1.82)	0.338
Vasoactive agents, n(%)	10 (9.5)	0 (0.0)	10(18.2)	0.002
Infection, n(%)	47 (44.8)	17 (34)	30 (54.5)	0.034
SBP, n(%)	12 (11.4)	1(2.0)	11 (20.0)	0.004
GI bleeding, n(%)	3(2.9)	1(2.0)	2 (3.6)	0.62
Paracentesis, n(%)	92 (87.6)	45 (90.0)	47 (85.5)	0.48
Total volume, ml	4385 (1475-9425)	3000 (700-7550)	6400 (2400-12400)	0.028

Continuous variables are expressed as median and (interquartile range)

SBP: spontaneous bacterial peritonitis. GI: gastrointestinal

Table 3: Renal function: glomerular and tubular markers at inclusion according to the cause of AKI (n=55)

Biomarkers	Pre-renal (n=41)	ATN (n=8)	HRS (n=6)	P
SCr $\mu\text{mol/l}$	81 (66-133)	173.5 (97.5-239.0)	194.5 (124-210)	0.004
Maximal SCr during stay $\mu\text{mol/l}$	116 (94-151)	213.5 (157.5-268.5)	221 (175-272.0)	<0.001
Serum CystC mg/l	1.50 (1.24-1.93)	2.12 (1.28-2.47)	2.19 (1.83-2.40)	0.050
Plasma NGAL ng/ml	110.5 (71.6-191.2)	221.3 (188.9-609.2)	234.4 (198.3-331.4)	0.001
Urinary NGAL ng/ml	30.6 (21.4-54.6)	113.7 (71.0-227.3)	95.2 (72.3-118.0)	0.003
Urinary NGAL/creat ng/mmol	2.8 (1.8-6.0)	11.8 (6.3-38.2)	7.0 (6.9-7.5)	0.003
Urinary KIM-1 ng/ml	0.16 (0.08-0.35)	0.30 (0.01-0.38)	0.54 (0.46-0.69)	0.120
Urinary KIM-1 /creat ng/mmol	0.02 (0.01-0.04)	0.03 (0.01-0.08)	0.05 (0.02-0.06)	0.490
Urinary sodium mmol/l	22.0 (5-70.5)	34.5 (11.0-69.0)	5.0 (5.0-16.0)	0.063
Urinary NaFE %	0.11 (0.04-0.6)	0.70 (0.18-1.43)	0.05 (0.04-0.20)	0.065
Urinary protein g/mg	0.11 (0.08-0.18)	0.52 (0.21-1.16)	0.13 (0.11-0.22)	0.015
ACR mg/mmol	0.9 (0.5-2.6)	31.0 (5.9-78.4)	1.1 (0.6-1.8)	0.001
RRI	0.77 \pm 0.06	0.79 \pm 0.07	0.82 \pm 0.05	0.085*

NaFE: sodium fractional excretion calculated as $100 \times (\text{Na u} \times \text{creat pl} / \text{Na pl} \times \text{creat u} \times 1000)$. ACR: Urinary albumin over creatinine ratio RRI: renal resistive index

*p for trend = 0.027

Figure 1

ROC curves comparing individual biomarkers measured at inclusion (T1) for (A) AKI diagnosis during hospital stay and (B) composite outcome (death+ intensive care+ dialysis)

Creat: serum creatinine. KIM1u: urinary KIM1. NGAL: plasmatic NGAL. NGALu: urinary NGAL. RRI: renal resistive index. AUC: Area under the curve.

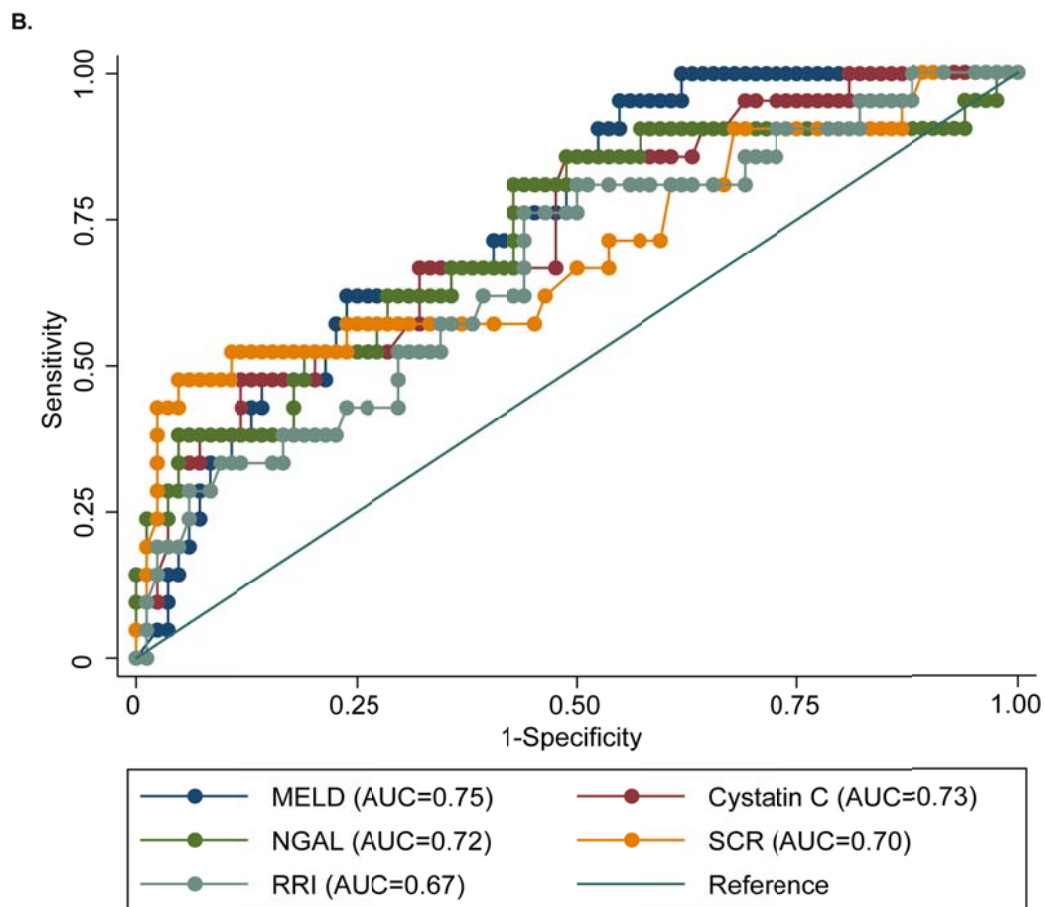
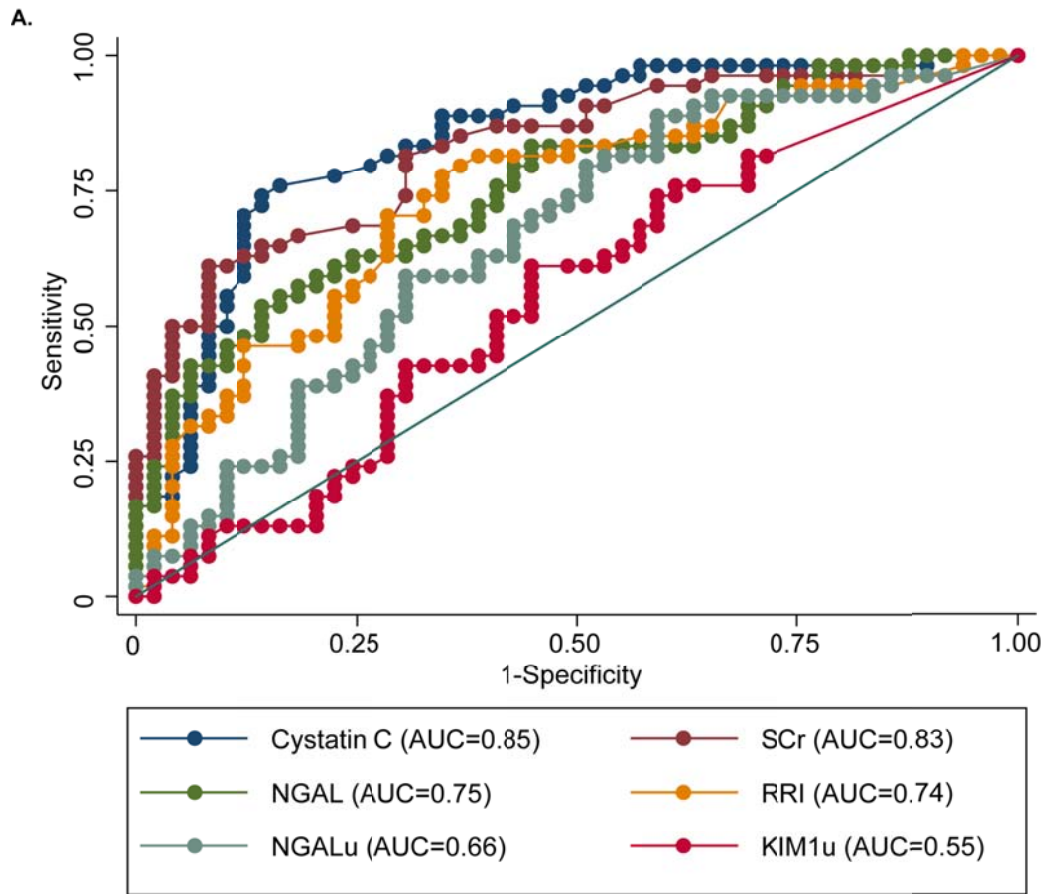


Figure 2

Box plots showing biomarkers' evolution over the first week after admission in blood (A) and urine (B) according to AKI development.

No AKI: Patients without AKI during hospital stay (n=50).

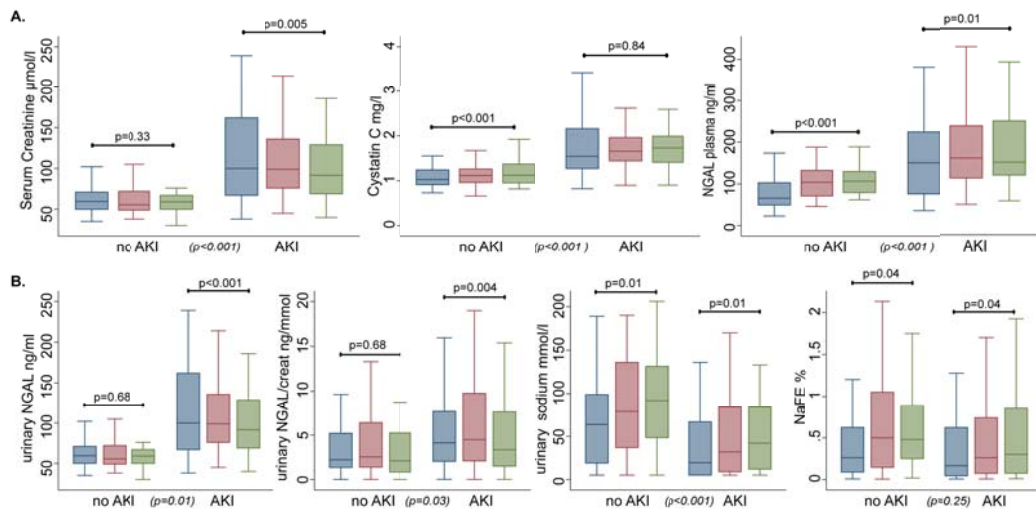
AKI: Patients who presented AKI at baseline (T1) or during hospital stay (n=55).

Admission time (T1) in blue, T2 in red and T3 in green.

AKI: Acute Kidney Injury. NaFE: sodium fractional excretion.

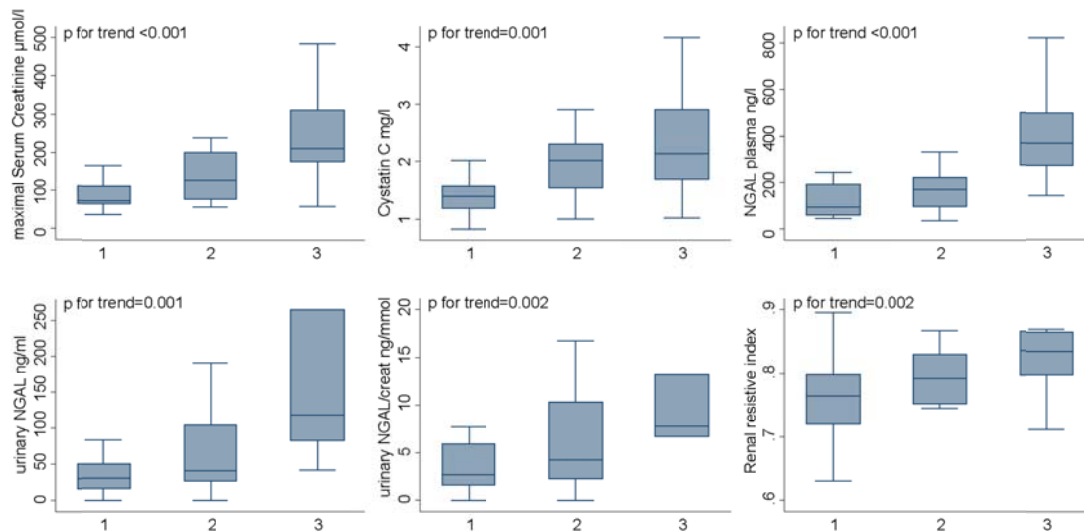
KIM-1, ACR and RRI are not reported as they were only measured at T1.

P-value comparing AKI vs non-AKI accounting for time-repeated measures are showed in italic in the X axis

**Figure 3**

Box plot showing the association of median biomarkers' levels according to AKIN stages (N=55).

N=29 for stage 1, n=18 for stage 2 and n=8 for stage 3.



Supplementary material:

Supplementary table 1

Supplementary Table 1.docx

Association with the composite outcome

Supplementary figure 1

Supplementary_Fig1.pdf

Study flowchart

Supplementary Figure 2:

Box plots showing biomarkers' evolution over the first week after admission in blood (A) and urine (B) according to delayed AKI development during hospitalization.

No AKI: Patients without AKI during hospital stay (n=50).

Delayed AKI: Patients admitted without AKI at baseline (T1) who subsequently developed AKI during hospital stay (n=29).

Admission time (T1) in blue, T2 in red and T3 in green.

AKI: Acute Kidney Injury. NaFE: sodium fractional excretion.

*P-value comparing AKI vs delayed AKI accounting for time-repeated measures are
showed in italic in the X axis*