Early predictors of acute kidney injury in patients with cirrhosis and bacterial infection: urinary neutrophil gelatinase-associated lipocalin and cardiac output as reliable tools

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ABSTRACT

Background: Hemodynamic abnormalities and acute kidney injury (AKI) are often present in infected cirrhotic patients. Hence, an early diagnosis of AKI is necessary, which might require the validation of new predictors as the determinations of urinary neutrophil gelatinase-associated lipocalin (uNGAL) and cardiac output.

Methods: We evaluated 18 infected cirrhotic patients subdivided into two groups at admission (0 hours). In Group I, we collected urine samples at 0 hours, 6 hours, 24 hours, and 48 hours for uNGAL and fractional excretion of sodium determinations. In Group II, we measured cardiac output using echocardiography.

Results: The age of patients was 55.0 ± 1.9 years, and 11 patients were males. The Model for End-Stage Liver Disease score was 21 ± 1, whereas the Child–Pugh score was C in 11 patients and B in 7 patients. Both patients in Group I and Group II showed similar baseline characteristics. In Group I, we diagnosed AKI in 5 of 9 patients, and the mean time to this diagnosis by measuring serum creatinine was 5.4 days. Patients with AKI showed higher uNGAL levels than those without AKI from 6 hours to 48 hours. The best accuracy using the cutoff values of 68 ng uNGAL/mg creatinine was achieved at 48 hours when we distinguished patients with and without AKI in all cases. In Group II, we diagnosed AKI in 4 of 9 patients, and cardiac output was significantly higher in patients who developed AKI at 0 hours.

Conclusion: Both uNGAL and cardiac output determinations allow the prediction of AKI in infected cirrhotic patients earlier than increments in serum creatinine.

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Introduction

Bacterial infections are responsible for 30–50% of hospital admissions among patients with cirrhosis [1]. Infection sites include the peritoneum, urinary tract, lungs, and dermis [2]. Patients with cirrhosis and bacterial infection have a high incidence of acute kidney injury (AKI), which occurs in about one-third of these patients. The mortality rate by bacterial-induced infection AKI ranges from 15% to 78% depending on the site of infection and stage of cirrhosis [1]. Early recognition and treatment of such a severe complication in cirrhotic patients is correlated with better clinical outcomes [3]. In the past three decades, treatment with suitable antibiotics and large-volume albumin infusion in the...
first 6 hours from diagnosis of infections has reduced mortality rates from 80% to 20%. In addition, AKI development also reduced by almost 70% in these patients [3,4]. However, the benefit of such a treatment may be restricted to high-risk patients with serum creatinine (SCr) > 1 mg/dL and/or serum bilirubin > 4 mg/dL [5–7].

Although SCr and fractional excretion of sodium (FENa) have been used to identify patients with AKI, there is great concern about their limitations. Besides being a marker of renal function rather than kidney injury, SCr may be underestimated in cirrhotic patients because of their hypervolemic state, low musculor mass, and decreased hepatic production of creatinine [8]. Furthermore, SCr may take up to 2 days to increase after kidney injury. For these reasons, using SCr to identify high-risk patients among those with cirrhosis and bacterial infection may overlook a significant number of patients.

Urinary neutrophil gelatinase-associated lipocalin (uNGAL) has been used as a better predictor of AKI than SCr and FENa [9]. NGAL is a 22-kDa peptide expressed by activated neutrophils and kidney tubular injured cells. It is eliminated in urine, and its dosages may rise 6–8 hours before SCr in patients with AKI.

Another potential marker of AKI development in patients with cirrhosis is cardiac output. A previous study demonstrated that low cardiac output in cirrhotic individuals in the outpatient setting predicted AKI development in the subsequent year [10]. In addition, Ruiz-del-Arbol et al [11] also reported similar findings in patients admitted to hospital with tense ascites without bacterial infections. However, no studies to date have correlated cardiac output at admission and AKI development during bacterial infection in cirrhosis.

Thus, we hypothesized that uNGAL and cardiac output may be early predictors of AKI in patients with cirrhosis and bacterial infection.

Methods

Study patients

We conducted this study at the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, which is a tertiary care hospital in São Paulo, Brazil, from November 2011 to June 2012. We evaluated 18 consecutive patients in whom cirrhosis and bacterial infection were present at admission. We diagnosed cirrhosis by liver biopsy or a combination of clinical, laboratory, radiological, and endoscopic data, and we defined bacterial infection according to the International Sepsis Definitions Conference 2001 [12]. We excluded patients with severe comorbidities, septic shock, chronic kidney disease, use of nephrotoxic drugs, hemodialysis treatment, or pre-existing liver transplantation.

All patients had clinical and laboratory data collected at admission (0 hours) to evaluate hepatic and renal functions at that time. Moreover, we measured SCr daily during hospital stay to AKI diagnosis. We defined AKI as the conventional criteria used in cirrhotic patients (i.e., a rise in SCr of at least 50% from baseline to a final value above 1.5 mg/dL) [13].

In our institution, the treatments of patients with cirrhosis and AKI are as follows: (1) patients with suspected prerenal azotemia receive resuscitation volume with crystalloids or albumin according to the physician’s decision and (2) patients with suspected hepatorenal syndrome receive albumin 1 g/kg/d for 2 days and, if renal function does not improve, we prescribe terlipressin in combination with albumin infusion.

We divided the patients into two groups: those for uNGAL measurements (Group I) and those for cardiac output study (Group II).

In Group I, we collected urine samples at 0 hours, 6 hours, and 48 hours for NGAL and FENa determinations. We measured uNGAL in duplicate using an enzyme-linked immunosorbent assay kit (NGAL ELISA human kit 036, BioPorto Diagnostics, Gentoft, Denmark), and we used the cutoff values of uNGAL of 68 ng and 130 ng/NGAL/mg creatinine in agreement with previous studies [14,15]. We also quantified urinary and plasma sodium for FENa determinations using flame photometry (model FC 280, CELM, São Paulo, SP, Brazil).

In Group II, we measured outflow tract area of left ventricle, velocity-time integral, and heart rate by echocardiography at admission, and we calculated cardiac output using the formula: cardiac output = left ventricle outflow tract area × velocity time integral × heart rate

Statistical analysis

We expressed data as mean ± standard error of the mean. We used unpaired Student t test to compare baseline characteristics of patients in Group I with those of Group II and between parameters of patients who developed AKI or not. Values of P < 0.05 were considered significant.

Ethical statement

The Research Ethics Committee of our institution (Comissão de Ética para Análise de Projetos de Pesquisa—CAPPesq, da Diretoria Clínica do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo) approved this study, which follows ethical standards established by the Declaration of Helsinki. All patients wrote and signed an informed consent before enrollment in the study.

Results

Baseline clinical characteristics

Table 1 shows the baseline clinical characteristics of infected cirrhotic patients in Group I and Group II at hospital admission. The mean age of patients was 55.0 ± 19 years. There were 11 (61%) males and 7 (39%) females. The etiology of cirrhosis was alcohol in 6 patients (33%), cryptogenic in 4 patients (22%), alcohol associated with hepatitis C in 3 patients (17%), hepatitis C in 2 patients (11%), and other etiologies in 3 (17%) patients. The Child–Pugh score was C in 11 (61%) patients and B in 7 (39%) patients, and Model for End-Stage Liver Disease (MELD) score was 21 ± 1. Furthermore, the baseline clinical characteristics of patients in Group I were similar to those of patients in Group II.

Although we found similar MELD score in both groups, MELD score was higher in patients who developed AKI than those patients who did not (26 ± 1 vs. 16 ± 2, P = 0.001), at hospital admission. However, both groups showed similar values of C-reactive protein that were 61 ± 19 mg/L vs. 40 ± 12 mg/L in patients with and without AKI, respectively.

As expected, SCr did not allow predicting AKI at 0 hours. SCr values were 1.95 ± 0.37 mg/dL and 1.22 ± 0.29 mg/dL, P = 0.14
in patients who developed AKI or not, respectively. In patients who developed AKI, six of nine patients fulfilled hepatorenal syndrome criteria, one had AKI secondary to nephrotoxic drug (contrast + polymyxin) whose prescription was done after hospital admission, one probably had acute tubular necrosis secondary to hemorrhagic shock, and one was thought to have transitory AKI related to infection.

The role of uNGAL in predicting AKI

In Group I, 5 of 9 patients (56%) developed AKI in mean time of 5.4 days that was associated with 100% in-hospital mortality rate. As shown in Table 2 and illustrated in Fig. 1, uNGAL was significantly higher in patients with AKI than in those without AKI at 6 hours, 24 hours, and 48 hours. We also observed that the cutoff values of 68 ng NGAL/mg creatinine stratified better than 130 ng NGAL/mg creatinine with an accuracy of 77.8% to predict AKI (sensitivity, 80%; specificity, 75%; positive predictive value, 80%; and negative predictive value, 75%) at 0 hours. The best accuracy using uNGAL was achieved at 48 hours, which distinguished patients with and without AKI in 100% of cases. However, increments in SCr only occurred after 72 hours, as illustrated in Fig. 2.

With regard to FENa, we did not find any statistical significance between patients with and without AKI, as shown in Table 2.

The role of cardiac output in predicting AKI

In Group II, 4 of 9 patients (44%) developed AKI, and these patients showed hyponatremia and higher cardiac output, heart rate, as well as MELD and Child–Pugh scores than those patients who did not develop AKI at hospital admission, as shown in Table 3. The fact that patients who developed AKI showed high MELD and Child–Pugh scores allowed us to diagnose worse liver function in these patients at hospital admission. In addition, only cardiac output levels showed significant increments to be considered a reliable predictor of AKI at hospital admission (Fig. 3). With regard to mean arterial blood pressure, SCr, serum albumin, and serum bilirubin, we did not find any statistical difference between patients who developed AKI or not (Table 3).

Discussion

Our study shows that both uNGAL and cardiac output determinations allow the prediction of AKI in infected cirrhotic patients earlier than increments in SCr. It is noteworthy that uNGAL measured as soon as 6 hours from hospital admission was already accurate to predict AKI development, as opposed to SCr. This biomarker allowed AKI diagnosis in a mean time of

### Table 1. Baseline clinical characteristics of infected cirrhotic patients in Group I and Group II at admission

| Group | Pt | Sex | Age (y) | Infection site | CRP (mg/L) | SCr (mg/dL) | MELD score | uNGAL (ngNGAL/mg creatinine) | CO AKI |
|-------|----|-----|---------|---------------|------------|-------------|-------------|--------------------------------|--------|
| I     | 1  | F   | 66      | SBP           | 33.1       | 0.74        | 15          | 19.2                           | –      |
| I     | 2  | F   | 48      | SBP+ skin     | 21.7       | 0.74        | 23          | 42.8                           | –      |
| I     | 3  | M   | 51      | SBP           | 104.7      | 0.58        | 9           | 54.8                           | –      |
| I     | 4  | M   | 44      | SBP           | 22.6       | 1.04        | 26          | 26.8                           | –      |
| I     | 5  | M   | 63      | SBP           | 155.5      | 3.77        | 29          | 152.6                          | –      |
| I     | 6  | F   | 64      | SeBP          | 94.1       | 1.22        | 20          | 89.7                           | –      |
| I     | 7  | M   | 51      | SBP           | 47.8       | 3.35        | 25          | 100.5                          | –      |
| I     | 8  | M   | 53      | SBP           | 84.4       | 3.21        | 32          | 296.0                          | –      |
| I     | 9  | M   | 50      | UTI+ skin     | 4.0        | 2.28        | 23          | 264.0                          | –      |
| II    | 10 | M   | 63      | Skin          | 26.3       | 1.11        | 24          | –                              | 9.7    |
| II    | 11 | M   | 39      | Cholecystitis | –          | 0.74        | 13          | –                              | 4.4    |
| II    | 12 | M   | 63      | Cholangitis   | 97.2       | 1.75        | 30          | –                              | 9.9    |
| II    | 13 | F   | 63      | SBP           | 75.3       | 0.82        | 13          | –                              | 3.7    |
| II    | 14 | F   | 53      | UTI           | 2.7        | 1.02        | 17          | –                              | 4.1    |
| II    | 15 | M   | 49      | SBP           | 27.4       | 1.86        | 18          | –                              | 7.7    |
| II    | 16 | F   | 62      | Skin          | 3.7        | 0.83        | 9           | –                              | 8.5    |
| II    | 17 | F   | 56      | UTI           | 3.1        | 0.76        | 22          | –                              | 8.1    |
| II    | 18 | M   | 61      | UTI           | –          | 2.75        | 29          | –                              | 8.4    |
| Mean  |    |     | 55 ± 2  |               | 50 ± 11    | 1.59 ± 0.25 | 21 ± 1     |                               |        |

AKI, acute kidney injury; CRP, C-reactive protein; CO, cardiac output; F, female; M, male; MELD, Model for End-Stage Liver Disease; Pt, patients; SBP, spontaneous bacterial peritonitis; SCr, serum creatinine; SeBP, secondary bacterial peritonitis; uNGAL, urinary neutrophil gelatinase-associated lipocalin; UTI, urinary tract infection.

### Table 2. Urinary NGAL and FENa of infected cirrhotic patients who developed AKI or not at different evaluation times

| Time (h) | AKI (n = 5) | No AKI (n = 4) | P      |
|----------|-------------|----------------|--------|
| uNGAL (ng NGAL/mg Cr) | 169 ± 49 | 50 ± 18 | 0.078 |
| 6        | 143 ± 33   | 48 ± 13 | 0.047 |
| 24       | 141 ± 33   | 30 ± 10 | 0.025 |
| 48       | 188 ± 29   | 33 ± 24 | 0.026 |

FENa (%) | 0.80 ± 0.36 | 0.12 ± 0.06 | 0.140 |
| 6        | 1.24 ± 0.58 | 0.32 ± 0.13 | 0.211 |
| 24       | 1.23 ± 0.68 | 0.29 ± 0.15 | 0.413 |
| 48       | 0.61 ± 0.27 | 0.32 ± 0.27 | 0.589 |

Data are expressed as mean ± standard error of the mean; AKI, acute kidney injury; FENa, fractional excretion of sodium; uNGAL, urinary neutrophil gelatinase-associated lipocalin.
5.4 days. This fact reinforces the utility of uNGAL as an early predictor of AKI. Furthermore, other studies show the importance of uNGAL to distinguish the patients with transient AKI from those with intrinsic AKI during sepsis [16]. Moreover, in a recent experimental study, we demonstrated that only uNGAL was able to predict AKI [17]. We treated normal and hypercholesterolemic rats with acute infusion of angiotensin II, and we observed a reduction in glomerular filtration rate and increments in diuresis and natriuresis in both groups. However, we found renal damage with a histologic examination only in the kidneys of hypercholesterolemic rats, and in the same group, we have also observed increments in uNGAL values.

A high cardiac output measured at admission also predicted AKI development during hospital stay in our patients. Krag et al [18] proposed a cardiorenal link in advanced cirrhosis, especially in those cirrhotic patients with bacterial infection in a recent publication. However, our findings concerning this parameter differ from other data from cirrhotic individuals in the outpatient setting and from patients without bacterial infection [10,11]. We would like to point out that our cirrhotic patients evaluated in the present study showed serious infections. Thus, they were more likely to develop AKI associated with a hyperdynamic circulation rather than a cause–effect relationship. Furthermore, we also confirmed that patients with high scores of end-stage chronic liver disease were more susceptible to developing AKI.

Cardiac output measurement using echocardiography is a readily available method that may be performed as a point-of-care test by any appropriately trained clinician. It may become a good option to identify patients at high risk of AKI development, especially in low-income institutions, where AKI biomarkers such as uNGAL are less likely to be available in the near future.

Our study had some limitations. First, we studied a small number of patients. Nevertheless, we could find a result with statistical significance. However, our number of patients did not allow a multivariate analysis, which might show that increases in urine NGAL or increases in cardiac output could independently predict AKI. Further studies with a higher number of patients are warranted to confirm our findings with regard to the best cutoffs of uNGAL, especially, and test

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**Figure 1. Time course of urinary NGAL.** The values of urinary NGAL measured at 0 hours, 6 hours, 24 hours, and 48 hours in infected cirrhotic patients who developed acute kidney injury (◆) or not (■). *P < 0.05 vs. patients without acute kidney injury. NGAL, neutrophil gelatinase-associated lipocalin.

**Figure 2. Urinary NGAL and SCr.** The levels of urinary NGAL (◆) and SCr (■) in infected cirrhotic patients who developed AKI during hospitalization. AKI, acute kidney injury; NGAL, neutrophil gelatinase-associated lipocalin; SCr, serum creatinine.

**Table 3. Comparison of clinical characteristics from infected cirrhotic patients (Group II) at admission**

|                       | Patients with AKI (n=5) | Patients without AKI (n=4) | P    |
|-----------------------|-------------------------|---------------------------|------|
| SCr (mg/dL)           | 1.59 ± 0.44             | 1.05 ± 0.21               | 0.270|
| Plasma Na⁺ (mEq/L)    | 132.5 ± 2.2             | 141.0 ± 1.3               | 0.010|
| Serum albumin (g/dL)  | 2.20 ± 0.27             | 2.72 ± 0.28               | 0.231|
| Serum bilirubin (mg/dL)| 10.06 ± 5.42             | 1.81 ± 0.34               | 0.128|
| INR (sec)             | 2.22 ± 0.26             | 1.42 ± 0.11               | 0.016|
| MELD score            | 26.2 ± 1.9              | 14.0 ± 1.6                | 0.002|
| Child–Pugh score      | 13.50 ± 0.64            | 7.80 ± 0.58               | 0.001|
| Heart rate (beats/min)| 97.0 ± 3.3              | 77.0 ± 6.2                | 0.034|
| Cardiac output (L/min)| 9.02 ± 0.45             | 5.68 ± 1.00               | 0.027|
| Mean blood pressure (mmHg)| 93.0 ± 4.1            | 89.4 ± 9.6                | 0.763|

Data are expressed as mean ± standard error of the mean. *P < 0.05, †P < 0.01, ‡P < 0.001 versus patients without acute kidney injury. AKI, acute kidney injury; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; SCr, serum creatinine.
these results by multivariate analysis. It would be very interesting to test if uNGAL can predict AKI regardless of severity of infection in cirrhotic patients, as some studies suggest that its levels are influenced by the severity of inflammation in other scenarios [19]. Second, we used an AKI definition that differs from new definitions such as Acute Kidney Injury Network (AKIN) and Kidney Disease: Improving Global Outcomes (KDIGO) criteria. Although we recognize the importance of these new definitions, we decided to use the conventional definition of AKI in cirrhosis because AKIN and KDIGO had not been adequately validated in cirrhotic patients when the study was designed.

In a recent study, Santos et al [20] showed acute renal dysfunction in sugarcane harvesting workers. These individuals were healthy men and might have a diagnosis of AKI if we use KDIGO criteria, but if we use AKIN criteria, we might not diagnose AKI. Furthermore, Sutherland et al [21] have also concluded that a single and universal AKI definition is required in pediatric population as well because pediatric version of Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease criteria did not show the same incidence of AKI when applied. These findings may reinforce the need for a special validation with cirrhotic patients.

We have also found high MELD and Child-Pugh scores at hospital admission in patients who developed AKI, but C-reactive protein values were similar in patients with and without AKI. A high MELD and Child-Pugh scores mean severe liver dysfunction in these patients. However, MELD score includes Scr in its formula, which has many limitations as described previously. Taken together, these results may reinforce the need to find an easy tool to predict AKI in infected cirrhotic patients as cardiac output measurement. Thus, a special treatment may be prescribed as soon as possible to reduce mortality rate in these cases.

In conclusion, both uNGAL and cardiac output determinations allow the prediction of AKI in patients with cirrhosis and bacterial infection earlier than increments in Scr.

**Conflicts of interest**

The authors have no conflicts of interest to declare.

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