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Diagnostic and prognostic performance of urinary neutrophil gelatinase-associated lipocalin in patients with cirrhosis and acute kidney injury

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Abstract

Background and Aims: Acute kidney injury (AKI) commonly occurs in patients with decompensated cirrhosis. Urinary neutrophil gelatinase–associated lipocalin (uNGAL) could help discriminate between different etiologies of AKI. The aim of this study was to investigate the use of uNGAL in (1) the differential diagnosis of AKI, (2) predicting the response to terlipressin and albumin in patients with hepatorenal syndrome-AKI (HRS-AKI), and (3) predicting in-hospital mortality in patients with AKI.

Approach and Results: One hundred sixty-two consecutive patients with cirrhosis and AKI were included from 2015 to 2020 and followed until transplant, death, or 90 days. Standard urinary markers and uNGAL were measured. Data on treatment, type, and resolution of AKI were collected. Thirty-five patients (21.6%) had prerenal AKI, 64 (39.5%) HRS-AKI, 27 (16.7%) acute tubular necrosis-AKI (ATN-AKI), and 36 (22.2%) a mixed form of AKI. Mean values of uNGAL were significantly higher in ATN-AKI than in other types of AKI (1162 ng/ml [95% CI 423–2105 ng/ml] vs. 109 ng/ml [95% CI 52–192 ng/ml]; $p < 0.001$). uNGAL showed a high discrimination ability in predicting ATN-AKI (area under the receiver operating characteristic curve, 0.854; 95% CI 0.767–0.941; $p < 0.001$). The best-performing threshold was found to be 220 ng/ml (sensitivity, 89%; specificity, 78%). The same threshold was independently associated with a higher risk of nonresponse (adjusted OR [aOR],

Abbreviations: ACLF, acute-on-chronic liver failure; AKI, acute kidney injury; aOR, adjusted OR; ATN-AKI, acute tubular necrosis-AKI; AUROC, area under the receiver operating characteristic curve; CKD, chronic kidney disease; HRS-AKI, hepatorenal syndrome-AKI; IQR, interquartile range; LT, liver transplantation; mAKI, Mixed form of AKI; MELD, Model for End-Stage Liver Disease; RRT, renal replacement therapy; sCr, serum creatinine; uNGAL, urinary neutrophil gelatinase–associated lipocalin;

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6.17; 95% CI 1.41–27.03; $p = 0.016$). In multivariable analysis (adjusted for age, Model for End-Stage Liver Disease, acute-on-chronic liver failure, leukocytes, and type of AKI), uNGAL was an independent predictor of in-hospital mortality (aOR, 1.74; 95% CI 1.26–2.38; $p = 0.001$).

Conclusions: uNGAL is an adequate biomarker for making a differential diagnosis of AKI in cirrhosis and predicting the response to terlipressin and albumin in patients with HRS-AKI. In addition, it is an independent predictor of in-hospital mortality.

INTRODUCTION

Acute kidney injury (AKI) is a common complication in decompensated cirrhosis, occurring in about 30%–50% of hospitalized patients.[1,2] Its negative impact on survival is well recognized.[2-4] The initial management of AKI requires the identification and treatment of precipitating factors, tapering diuretics, and discontinuing nephrotoxic drugs.[5,6] In patients who do not improve after the application of such measures, the differential diagnosis of AKI phenotypes is crucial, particularly between acute tubular necrosis (ATN)-AKI and hepatorenal syndrome (HRS)-AKI, mainly because a different treatment is needed.[5,6]

A correct diagnosis could be difficult even for the most experienced clinicians, and the classical urine markers, such as urine sediment, urinary protein, urinary sodium concentration, and fractional excretion of sodium, have limited accuracy in patients with cirrhosis.[7-9] Biomarkers of kidney injury could be useful in the differential diagnosis. Among these, urinary neutrophil gelatinase-associated lipocalin (uNGAL) and IL-18 are the most extensively studied and have been shown to be accurate in the differential diagnosis between AKI phenotyphes.[10-17] However, in most studies those biomarkers were measured in all patients with AKI, and their accuracy in the most interesting group (i.e., patients not improving after initial management) has not been extensively investigated. The aim of this study was to investigate the use of uNGAL in (1) the differential diagnosis of AKI in hospitalized patients with cirrhosis, (2) predicting the response to terlipressin and albumin in patients with HRS-AKI, and (3) predicting in-hospital mortality.

MATERIALS AND METHODS

Study design

According to the algorithm used in our hospital, uNGAL has been employed for the management of AKI in patients not resolved after the first 48 h of treatment since 2015. We analyzed retrospectively patients with cirrhosis consecutively admitted at the University Hospital of Padova from 2015 to 2020 with AKI lasting > 48 h and with uNGAL measured at 48–72 h from admission. Diagnosis of cirrhosis was based on clinical, biochemical, ultrasonographic, and endoscopic findings. AKI was defined as an increase in serum creatinine (sCr) ≥ 0.3 mg/dl (≥ 26.5 μmol/L) within 48 h or an increase in sCr $\geq 50\%$ from baseline. We excluded patients with age < 18 years; HCC with neoplastic portal vein thrombosis; extrahepatic malignancies; severe comorbidities, such as congestive heart failure in New York Hearth Association class ≥ 3, chronic obstructive pulmonary disease in Global Initiative for Lung Obstructive Disease class ≥ 3, chronic kidney disease (CKD) on renal replacement therapy (RRT); or prior solid organ transplantation. Clinical, demographic, and laboratory variables, including urine examination with proteinuria, urinary sodium, urine sediment, as well as treatments administered were collected. The following precipitating factors were identified: fluid loss (severe diarrhea, gastrointestinal bleeding, excessive diuresis due to diuretic therapy with loss of body weight > 500 g/day or 1000 g/day in patients without and with edema), infections, use of nephrotoxic drugs/vasodilators, and iodine contrast media. uNGAL was analyzed with a standardized method in our lab (chemiluminescent nanoparticle immunoassay, ARCHITECT Urine NGAL assay; Abbott). sCr values were collected daily until the resolution of AKI. Data on treatment, type, and evolution of AKI were also collected (see definitions below). Patients were followed up regularly until liver transplantation (LT), death, or 90 days. Permission for retrospective data analyses was obtained from the local ethics committee. Written informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki, seventh revision.

Definitions

Staging, resolution, and progression of AKI were defined according to the criteria of the International

Club of Ascites.[5] Stage 1 AKI was defined as an increase in sCr $\geq$ 0.3 mg/dl ($\geq$26.5 µmol/L) or an increase in sCr $\geq$ 1.5-fold to 2-fold from baseline, Stage 2 as an increase in sCr > 2-fold to 3-fold from baseline, and Stage 3 as an increase in sCr > 3-fold from baseline or sCr $\geq$ 4.0 mg/dl (353.6 µmol/L) with an acute increase $\geq$ 0.3 mg/dl (26.5 µmol/L) or initiation of RRT.[5] AKI staging was defined as the stage of AKI at the first fulfillment of diagnostic criteria for AKI, while peak AKI stage was defined as the maximum stage reached during hospitalization. Progression of AKI was defined as an increase in sCr that leads to fulfillment of the diagnostic criterion for a higher stage of AKI and/or need for RRT. Response to treatment was defined as a complete or partial resolution of AKI according to the International Club of Ascites criteria.[5] AKI phenotypes were classified as follows: prerenal AKI was diagnosed when there was a history of excessive fluid loss or bleeding and renal function improved after i.v. fluid administration; HRS-AKI was diagnosed in patients with ascites if there was a lack of response after 2 days of volume expansion with albumin and withdrawal of diuretics when there was no evidence of any of the following: (1) shock, (2) recent use of nephrotoxic drugs, (3) renal structural damage defined by proteinuria 500 mg/day and/or microhematuria with >50 red blood cells per high-power field, and (4) ultrasonographic abnormalities.[18] ATN-AKI was diagnosed if three of the following six criteria were met: (1) sodium excretion fraction > 2%, (2) urinary osmolarity < 400 mOsm/L, (3) urinary sodium > 40 mEq/L, (4) presence of shock or nephrotoxic drug use, (5) presence of granular/muddy-brown casts at urine sediment, and (6) presence of renal tubular epithelial cells at urinary sediment.[19,20] All forms of AKI which could not be classified according to these criteria were reported as a mixed form of AKI (mAKI). The final diagnosis of the phenotype of AKI was confirmed by two hepatologists well experienced in the management of patients with decompensated cirrhosis (S. P. and P. A.), who were blinded to uNGAL values. CKD was defined as abnormalities of kidney structure or function, present for >3 months, with a glomerular filtration rate < 60 ml/min/1.73 m^2, according to the Kidney Disease–Improving Global Outcomes guidelines.[21] AKI in patients with CKD as well as HRS-AKI in patients with CKD were defined according to what has been recently proposed by Angeli et al.[18]

Statistical analysis

Continuous variables with a normal distribution were reported as mean and SD and compared with the Student *t* test or analysis of variance. Non-normally distributed continuous variables were reported as median and interquartile range (IQR) and compared with the Mann-Whitney test or the Kruskall-Wallis test. Categorical variables were reported as proportions and compared with the chi-squared test and/or Fisher's test, when appropriate. The discrimination ability of uNGAL to identify patients with ATN-AKI was analyzed by evaluating the area under the receiver operating characteristic curve (AUROC) and the relative CI. The optimal threshold value for discriminating patients with and without ATN-AKI was calculated using the Youden Index. Univariable and multivariable logistic regression analyses were used to evaluate risk factors of in-hospital mortality. Results were expressed as OR and 95% CI. A *post hoc* analysis of 90-day mortality was performed. The proportional-hazards model for competing risks proposed by Fine and Gray was used to identify predictors of mortality.[22] LT was considered as an event "competing" with mortality. Results were expressed as subdistribution HR (sHR) with 95% CI. Multivariable models were adjusted for age, Model for End-Stage Liver Disease (MELD) score, acute-on-chronic liver failure (ACLF) grade, white blood cells, uNGAL, and HRS-AKI. Non-normally distributed variables were log-transformed before being included in logistic regression and proportional hazard models, to lessen the influence of extreme laboratory values. All analyses were two-tailed and considered statistically significant for p values < 0.05. The statistical analysis was performed using SPSS software, version 27.0.

RESULTS

Study population

We evaluated 370 patients, of whom 208 had at least one of the exclusion criteria. Therefore, 162 patients were included in the study (Figure S1). Table 1 shows all demographic, clinical, and laboratory characteristics of the included patients; the mean age was of 62 ± 10 years, and most were male (76.5%) with alcoholic cirrhosis (48.1%). Mean MELD and Child-Pugh scores were 25 ± 8 and 9 ± 2, respectively. Of 162 patients, 49 (30.2%) had HCC, 62 (38.3%) diabetes, and 29 (17.9%) CKD.

Characteristics of AKI and accuracy of uNGAL in differential diagnosis and evolution of AKI

Ninety patients (55.6%) had Stage 1 AKI, 39 (24.0%) Stage 2 AKI, 33 (20.4%) Stage 3 AKI; infections were the most frequent precipitant of AKI (54/162–33.3%). Regarding AKI phenotype, 35 patients (21.6%) had prerenal AKI, 64 (39.5%) HRS-AKI, 27 (16.7%) ATN-AKI, and 36 (22.2%) mAKI. The median amount of albumin administered was 160 (70–340) g for prerenal

TABLE 1 Baseline characteristics of included patients

Variable	*N* = 162
Age (years), mean ± SD	62 ± 10
Gender (male), *n* (%)	124 (76.5)
Etiology of cirrhosis, *n* (%)	
Alcohol	78 (48.1)
HCV	45 (27.8)
HBV	21 (13.5)
Autoimmune	8 (4.9)
NASH	27 (16.7)
Other	17 (10.5)
HCC, *n* (%)	49 (30.2)
Diabetes, *n* (%)	62 (38.3)
CKD, *n* (%)	29 (17.9)
Medications, *n* (%)	
Beta-blockers	66 (40.7)
Diuretics	127 (78.4)
Ascites, *n* (%)	140 (86.4)
HE, *n* (%)	37 (22.8)
MELD, mean ± SD	25 ± 8
Child-Pugh score, mean ± SD	9 ± 2
MAP (mm Hg), mean ± SD	85 ± 13
HR (bpm), mean ± SD	80 ± 15
Baseline sCr (μmol/L), median (IQR)	94 (78–108)
White blood cells (×1000/uL), median (IQR)	6.47 (4.53–10.11)
Hemoglobin (g/L), median (IQR)	94 (85–106)
INR, median (IQR)	1.58 (1.35–1.91)
Platelets (×1000/uL)	77 (54–111)
sCr at AKI (μmol/L), median (IQR)	164 (119–239)
BUN (μmol/L), median (IQR)	16.27 (10.85–24.61)
Sodium (mmol/L), median (IQR)	135 (130–137)
Potassium (mmol/L), median (IQR)	4.05 (3.60–4.50)
Bilirubin (μmol/L), median (IQR)	86.7 (32.8–244.8)
Albumin (g/L), median (IQR)	30 (27–35)
CRP (mg/L), median (IQR)	22 (14–49)
uNGAL (μg/ml), median (IQR)	129 (55–463)

Abbreviations: BUN, blood urea nitrogen; CRP, C-reactive protein; HR, heart rate; INR, international normalized ratio; MAP, mean arterial pressure.

AKI, 270 (160–330) g for HRS-AKI, 110 (20–310) g for ATN-AKI, and 130 (90–210) g for mAKI.

The levels of uNGAL were significantly higher in patients with ATN-AKI (1162 ng/ml [423–2105 ng/ml]) compared to those with prerenal AKI, HRS-AKI, and mAKI (87 ng/ml [36–188 ng/ml], 111 ng/ml [54–178 ng/ml], and 109 ng/ml [51–361 ng/ml], respectively; $p < 0.001$) (Figure 1). Moreover, patients with ATN-AKI showed significantly higher levels of uNGAL when compared to those with HRS-AKI at each of the AKI

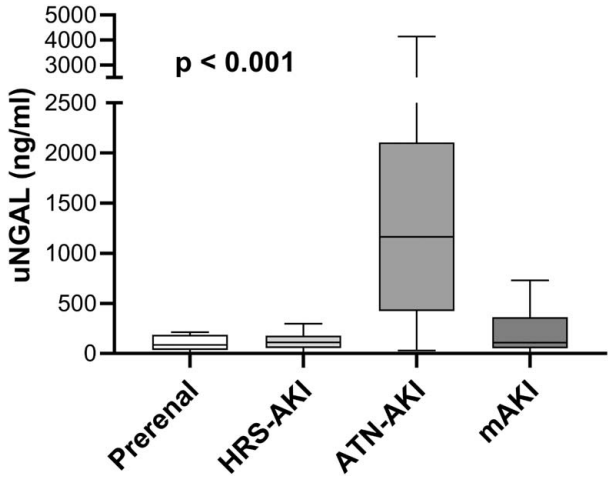

FIGURE 1 uNGAL levels among different AKI phenotypes.

stages (Stage 1 AKI, 1115 [327–2540] vs. 85 [51–167] ng/ml; $p = 0.019$; Stage 2 AKI, 929 [585–1327] vs. 129 [72–156] ng/ml; $p = 0.004$; Stage 3 AKI, 1269 [282–2782] vs. 169 [75–267] ng/ml; $p = 0.002$) (Figure S2).

Out of 35 patients with prerenal AKI, 26 (74.3%) had an initial Stage 1, eight (22.9%) an initial Stage 2, and one (2.8%) an initial Stage 3. Out of 64 patients with HRS-AKI, 34 (53.1%) had an initial Stage 1, 19 (29.7%) an initial Stage 2, and 11 (17.2%) an initial Stage 3. Out of 27 patients with ATN-AKI, six (22.2%) had an initial Stage 1, five (18.5%) an initial Stage 2, and 16 (59.3%) an initial Stage 3. Finally, out of 36 patients with mAKI, 24 (66.7%) had an initial Stage 1, seven (19.4%) an initial Stage 2, and five (13.9%) an initial Stage 3. Patients with ATN-AKI had more frequently an initial AKI Stage 3 when compared to other phenotypes ($p < 0.001$). AKI was solved in 89/162 (55%) of patients, without any significant difference in uNGAL values when compared to patients with persistent AKI (114 ng/ml [51–285 ng/ml] vs. 149 ng/ml [72–921 ng/ml], respectively; $p = 0.152$). AKI progressed in 61/162 (38%), and there was no significant difference in rate of progression among AKI types ($p = 0.063$); patients with ATN-AKI reached more frequently a peak AKI Stage 3 when compared to those with prerenal AKI, HRS-AKI, or mAKI (77.8% vs. 17.1% vs. 48.4% vs. 30.6%, respectively; $p < 0.001$). Table S1 summarizes the characteristics of AKI according to phenotype.

Levels of uNGAL showed a high discrimination ability in detecting ATN-AKI (AUROC, 0.854; 95% CI, 0.767–0.941; $p < 0.001$; Figure 2). The best-performing threshold for detecting ATN-AKI was 220 ng/ml, with a sensitivity of 89% (95% CI, 71–98) and specificity of 78% (95% CI, 71–85). Levels of uNGAL were significantly higher in patients with more severe AKI compared to those with lower stages (88 ng/ml [48–222 ng/ml] vs. 129 ng/ml [72–472 ng/ml] vs. 267 ng/ml [132–1510 ng/ml] for Stages 1, 2, and 3,

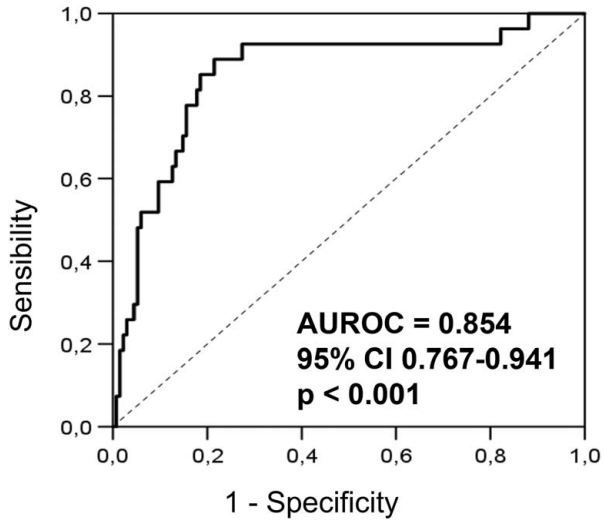

FIGURE 2 Discrimination ability of uNGAL in the diagnosis of ATN-AKI.

respectively; $p < 0.001$; Figure S3). Higher values of uNGAL were observed in patients with AKI progression when compared to those without, but no statistical significance was found (121 ng/ml [67–562 ng/ml] vs. 96 ng/ml [40–188 ng/ml]; $p = 0.09$).

uNGAL and response to treatment with terlipressin and albumin in patients with HRS-AKI

Sixty-four patients had a diagnosis of HRS-AKI and were treated with terlipressin and albumin for a median of 9 (IQR, 3–14) days, and 38 (59%) responded to treatment. A complete response to treatment was observed in 29 (76%) patients, while nine (24%) patients showed a partial response. Demographical, clinical, and laboratory characteristics of responders and nonresponders are listed in Table S2. Responders, considered as a whole, had significantly lower uNGAL values than nonresponders (94 ng/ml [53–165 ng/ml] vs. 149 ng/ml [79–679 ng/ml]; $p = 0.043$; Figure 3A). Furthermore, a significantly higher rate of response to treatment was found in patients with HRS-AKI whose uNGAL values were < 220 ng/ml compared to those with uNGAL ≥ 220 ng/ml (70% vs. 33%, respectively; $p = 0.015$; Figure 3B). After adjusting the analysis for sCr and ACLF grade, uNGAL ≥ 220 ng/ml was independently associated with nonresponse (adjusted OR [aOR], 6.17; 95% CI, 1.41–27.03; $p = 0.016$; Table S3). It is noteworthy that 11 out of 36 (30.5%) patients with mAKI were treated with terlipressin and albumin, and three of them (27.3%) showed a full response to treatment. Likewise, it should be highlighted that in four out of five (80%) patients with HRS-AKI on CKD, a response to treatment was observed (a complete response in two and a partial response in two). Nine out of 64 (14.1%) patients with HRS-AKI had a recurrence. There was no difference in uNGAL values between patients with recurrence and those without (104 [86–154] vs. 111 [53–195] ng/ml, respectively; $p = 0.915$).

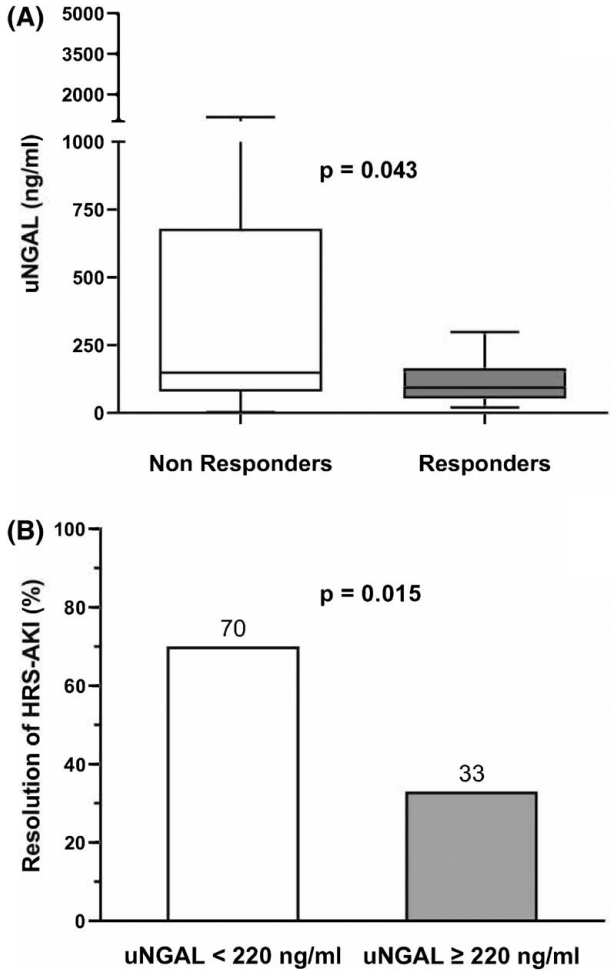

FIGURE 3 (A) uNGAL values and response to treatment with terlipressin and albumin in patients with HRS-AKI. (B) Rate of response to treatment with terlipressin and albumin in patients with HRS-AKI according to baseline values of uNGAL.

In-hospital and 90-day mortality

Fifty-seven (35.2%) patients died during hospitalization, 11 (6.8%) underwent LT, and 94 (58.0%) were discharged. In univariable analysis, in-hospital mortality was associated with diagnosis of HRS-AKI, progression of AKI, development of ACLF Grade 2 or 3, and higher levels of white blood cells, C-reactive protein, and uNGAL (Table 2). In multivariable analysis (Table 3), independent predictors of in-hospital mortality were a diagnosis of HRS-AKI (aOR, 4.81; 95% CI, 1.84–12.57; $p = 0.001$), older age (aOR, 1.043; 95% CI 1.01–1.09; $p = 0.045$), ACLF grade (aOR, 8.39; 95% CI, 2.55–27.59; $p < 0.001$), and uNGAL values (aOR,

TABLE 2 Characteristics of patients by vital status after hospitalization

Variable	Alive at discharge ($n = 94$)	Died during hospitalization ($n = 57$)	p
Age (years), mean ± SD	62 ± 9	63 ± 9	0.495
Gender (male), *n* (%)	76 (81)	40 (70)	0.132
Etiology of cirrhosis, *n* (%)			
Alcohol	45 (47.9)	30 (52.6)	0.571
HCV	24 (25.5)	17 (29.8)	0.565
HBV	11 (11.7)	7 (12.3)	0.915
Autoimmune	3 (3.2)	4 (7)	0.423
NASH	21 (22.3)	5 (8.8)	0.032
Other	9 (9.6)	6 (10.5)	0.850
HCC, *n* (%)	28 (29.8)	17 (29.8)	0.996
Diabetes, *n* (%)	41 (43.6)	18 (31.6)	0.142
CKD, *n* (%)	21 (22.3)	7 (12.3)	0.123
Ascites, *n* (%)	83 (88.3)	48 (84.2)	0.473
HE, *n* (%)	21 (22.3)	15 (26.3)	0.578
MELD, mean ± SD	22 ± 6	28 ± 7	< 0.001
Child-Pugh, mean ± SD	9 ± 2	10 ± 2	< 0.001
MAP (mm Hg), mean ± SD	85 ± 13	84 ± 13	0.165
HR (bpm), mean ± SD	80 ± 15	79 ± 14	0.306
Infections *n* (%)	35 (37.2)	26 (45.6)	0.309
ACLF, *n* (%)	50 (53.2)	41 (71.9)	0.023
ACLF grade			< 0.001
No ACLF	44 (46.8)	16 (28.1)	
Grade 1	44 (46.8)	19 (33.3)	
Grade 2	4 (4.3)	18 (31.6)	
Grade 3	2 (2.1)	4 (7.0)	
Precipitating event	41 (43.6)	27 (47.4)	0.653
AKI stage			0.652
Stage 1	54 (54.5)	32 (56.2)	
Stage 2	24 (25.5)	11 (19.3)	
Stage 3	16 (17.0)	14 (24.6)	
Nosocomial AKI, *n* (%)	54 (57.4)	36 (63.2)	0.488
AKI phenotype, *n* (%)			0.012
Prerenal	27 (28.7)	6 (10.5)	
HRS-AKI	29 (30.9)	31 (54.4)	
ATN-AKI	14 (14.9)	9 (15.8)	
mAKI	24 (25.5)	11 (19.3)	
AKI progression, *n* (%)	19 (20.2)	39 (68.4)	< 0.001
AKI resolution, *n* (%)	60 (63.8)	22 (38.6)	0.003
White blood cells (×1000/μl), median (IQR)	6.00 (4.00–9.14)	8.83 (5.56–12.93)	0.001
Hemoglobin (g/l), median (IQR)	94 (84–103)	95 (87–118)	0.232
INR, median (IQR)	1.5 (1.3–1.7)	1.8 (1.5–2.1)	< 0.001
Platelets (×1000/μL)	81 (61–120)	71 (48–96)	0.024
sCr at AKI (μmol/l), median (IQR)	166 (130–251)	179 (131–265)	0.530
BUN (μmol/l), median (IQR)	15.3 (10.0–24.2)	19.1 (13.9–26.0)	0.016
Sodium (mmol/l), median (IQR)	136 (132–138)	134 (128–137)	0.100
Potassium (mmol/l), median (IQR)	4.1 (3.7–4.5)	4 (3.3–4.3)	0.081

TABLE 2. (*continued*)

Variable	Alive at discharge ($n = 94$)	Died during hospitalization ($n = 57$)	p
Bilirubin (μmol/l), median (IQR)	48 (28–137)	136 (71–454)	< 0.001
Albumin (g/l), median (IQR)	31 (27–35)	30 (25–35)	0.497
CRP (mg/l), median (IQR)	18 (11–43)	28 (19–59)	0.001
uNGAL (μg/ml)	53 (128–256)	267 (96–1259)	0.011

Abbreviations: BUN, blood urea nitrogen; CRP, C-reactive protein; HR, heart rate; INR, international normalized ratio; MAP, mean arterial pressure.

1.74; 95% CI, 1.26–2.38; $p < 0.001$). At 90 days, 74 (45.7%) patients died, 15 (9.3%) underwent LT, 60 (37.0%) survived, and 13 (8.0%) were lost to follow-up. uNGAL values were independent predictors of 90-day mortality (sHR, 1.35; 95% CI, 1.12–1.62; $p = 0.001$; Table S4).

DISCUSSION

The study suggests that uNGAL is an excellent urinary biomarker in the differential diagnosis between ATN-AKI and HRS-AKI and can play a significant role in refining the diagnosis of AKI in patients with cirrhosis. The best threshold for differentiating ATN-AKI from other forms of AKI was found to be 220 ng/ml, with a sensitivity of 89% and a specificity of 78%. These data are in keeping with the results of other studies which evaluated the diagnostic and prognostic role of uNGAL in patients with cirrhosis and AKI.[10,11,12,13,23]

Yet the most relevant result of the study is that uNGAL values predict response to treatment with terlipressin and albumin in patients with HRS-AKI, higher uNGAL values being associated with significantly lower rates of response. Thus, the combination of these two findings has important clinical implications. An improved differential diagnosis between HRS-AKI and ATN-AKI and a more careful prediction of the response to terlipressin plus albumin in those with HRS-AKI will reduce the risk of exposing patients to unsuitable or ineffective treatment. Because the use of terlipressin and albumin can be associated with the development of serious adverse effects,[24] the avoidance of an unsuitable or ineffective treatment is an important advancement in clinical practice respecting one of its guiding principles, which is *primun non nocere* ("first, do not harm"). This message, however relevant, must be taken with a little caution when applied to patients with a final diagnosis of HRS-AKI because 33% of patients with a uNGAL value > 220 ng/ml still responded to treatment. First, the use of uNGAL has limitations in the distinction between AKI etiologies. Previous research suggests that combining it with cystatin C may be superior to use of it alone in identifying HRS-AKI.[25] Second, although uNGAL was found to be the only independent predictor of response to terlipressin and albumin, it is well established that other powerful predictors of response are the starting level of sCr and the presence and stage of ACLF.[26,27]

TABLE 3 Multivariable analysis of in-hospital mortality

Variable	OR	95% CI	p
Age	1.043	1.01–1.09	0.045
ACLF Grade 2–3[a]	8.39	2.55–27.59	< 0.001
WBC[b]	2.16	0.99–4.4	0.053
HRS-AKI	4.81	1.84–12.57	0.001
uNGAL[b]	1.74	1.26–2.38	< 0.001

Abbreviation: WBC, white blood cells.
[a]No ACLF and ACLF Grade 1 were used as reference.
[b]Log-transformed.

Despite these limitations, the results of the study reinforce the utility of uNGAL in clinical practice, suggesting that its measurement could be included as a step in an updated algorithm for the management of AKI. This would help clinicians to better differentiate patients with HRS-AKI from those with ATN-AKI or those with HRS-AKI in whom treatment with terlipressin and albumin could be more effective.

These conclusions are further supported by two secondary findings of the study. First, in this setting it should be highlighted that values of uNGAL < 220 ng/ml may help the clinicians in the decision to treat patients with a diagnosis of HRS-AKI on CKD with terlipressin and albumin. In fact, all four patients with HRS-AKI on CKD who responded to the treatment had a uNGAL < 220 ng/ml, while the only nonresponder had a value far above this threshold. These data follow the proposal to consider the possibility of making a diagnosis of HRS-AKI in patients with CKD.[18]

Second, it should be considered that uNGAL was found to be an independent predictor of in-hospital mortality in patients with AKI, which is probably related to its ability to identify patients with a higher AKI stage or those with either ATN-AKI or HRS-AKI without response to medical therapy.[10,28] Again, the predictive value of uNGAL for hospital survival is not absolute for, at least, two reasons. First, it has to be shared with that of other powerful predictors such as age, diagnosis and stage of ACLF, and level of systemic inflammation. Second, it has been observed that uNGAL may predict outcomes unrelated to kidney

dysfunction, for example, ACLF[28] or development of a second infection in patients with cirrhosis hospitalized with infections.[29]

Our study has some limitations. The diagnosis of ATN-AKI was based not on the gold standard (kidney biopsy) but on surrogate validated parameters,[]; however, on clinical grounds, kidney biopsy would be unfeasible and risky in patients with cirrhosis and AKI. Anyway, considering the limitations of some of the criteria used (e.g., sodium excretion) we cannot exclude that some patients classified as mAKI may have had ATN-AKI. This was a single-center study, and our findings need external validation.

In conclusion, between strengths and limitations, the study supports the use of uNGAL in the management of AKI in patients with cirrhosis, suggesting that a threshold value of 220 ng/ml can be used in the formulation of prognosis in patients with AKI, in the differential diagnosis between HRS-AKI and ATN-AKI, and in predicting response to terlipressin and albumin in those with HRS-AKI.

AUTHOR CONTRIBUTIONS

Carmine Gambino, Salvatore Piano: Conceptualization; methodology; investigation; formal analysis; writing–original draft. Matteo Stenico, Marta Tonon, Alessandra Brocca, Valeria Calvino, Simone Incicco, Nicola Zeni, Roberta Gagliardi, Chiara Cosma, Martina Zaninotto: Investigation. Patrizia Burra, Daniela Basso, Umberto Cillo: Writing–review & editing. Paolo Angeli: Conceptualization; methodology; supervision, formal analysis; writing–original draft; writing–review & editing.

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CONFLICT OF INTEREST

Paolo Angeli consults for Sequana. He advises Mallinckrodt. He received grants from Grifols and Behring. He owns intellectual property rights in BioVie. Patrizia Burra is on the speakers' bureau for Kedrion, Biotest, Sandoz, Chiesi Farmceutici, and MSD Italia. Salvatore Piano advises Mallinckrodt.

DATA AVAILABILITY STATEMENT

The data sets of this study are available from the corresponding author upon reasonable request. The data that support the findings of this study are available from the corresponding author, PA, upon reasonable request.

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