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Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness

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Abstract Objective: Sepsis is the most common trigger for acute kidney injury (AKI) in critically ill patients. We sought to determine whether there are unique patterns to plasma and urine neutrophil gelatinase-associated lipocalin (NGAL) in septic compared with non-septic AKI. **Design:** Prospective observational study. **Setting:** Two adult ICUs in Melbourne, Australia. **Patients:** Critically ill patients with septic and non-septic AKI. **Interventions:** None. **Measurements and main results:** Blood and urine specimens collected at enrollment, 12, 24 and 48 h to measure plasma and urine NGAL. Eighty-three patients were enrolled (septic $n = 43$). Septic AKI patients had more co-morbid disease ($p = 0.005$), emergency surgical admissions ($p < 0.001$), higher illness severity ($p = 0.008$), more organ dysfunction ($p = 0.008$) and higher white blood cell counts ($p = 0.01$). There were no differences at enrollment between

groups in AKI severity. Septic AKI was associated with significantly higher plasma (293 vs. 166 ng/ml) and urine (204 vs. 39 ng/mg creatinine) NGAL at enrollment compared with non-septic AKI ($p < 0.001$). Urine NGAL remained higher in septic compared with non-septic AKI at 12 h ($p < 0.001$) and 24 h ($p < 0.001$). Plasma NGAL showed fair discrimination for AKI progression (area under receiver-operator characteristic curve 0.71) and renal replacement therapy (AuROC 0.78). Although urine NGAL performed less well (AuROC 0.70, 0.70), peak urine NGAL predicted AKI progression better in non-septic AKI (AuROC 0.82). **Conclusion:** Septic AKI patients have higher detectable plasma and urine NGAL compared with non-septic AKI patients. These differences in NGAL values in septic AKI may have diagnostic and clinical relevance as well as pathogenetic implications.

Keywords Acute kidney injury · Critical illness · Sepsis · Urinary markers · Fractional excretion of sodium · Microscopy · Neutrophil gelatinase-associated lipocalin

Introduction

Sepsis is an important precipitant of acute kidney injury (AKI) [1]. Observational studies have found sepsis contributes to 30–50% of all AKI encountered in critically ill patients [2–6]. Septic AKI portends a poorer prognosis with lower survival when compared with AKI of non-septic origin [2, 4, 7, 8]. Yet, septic AKI may be associated with higher rates of renal recovery [3]. Considering these differences, the early identification of primarily septic from non-septic AKI may have clinical relevance and prognostic importance.

Experimental studies have suggested that septic AKI may be characterized by a distinct pathophysiology that differs from ischemic/toxic-induced kidney injury [9–15]. These events may be reflected in unique patterns of plasma (p) and urine (u) biomarkers in septic AKI [16, 17]. As a consequence, the application of traditional urinary biochemical and microscopy-based tests in the early diagnosis and differentiation of AKI may be misleading in septic AKI [16–18].

Neutrophil gelatinase-associated lipocalin (NGAL) has emerged as a potentially useful diagnostic biomarker in AKI [19–21]. We hypothesized pNGAL and uNGAL will differ in patients with septic compared with non-septic AKI. Accordingly, we performed a prospective observational study in a cohort of critically ill patients with AKI. Our objectives were to: (1) explore for temporal differences between septic and non-septic AKI in pNGAL and uNGAL; (2) explore for differences between septic and non-septic AKI in peak pNGAL and uNGAL; (3) evaluate whether pNGAL and/or uNGAL could discriminate between septic and non-septic AKI; (4) explore whether any differences in pNGAL and uNGAL are associated with prediction of worsening AKI and/or RRT initiation.

Methods

Study design

This was a prospective observational study of critically ill patients with acute kidney injury (AKI). The Human Research Ethics Committee at the Austin Hospital, Melbourne, approved the study prior to commencement.

Study population

We enrolled patients with AKI and an expected stay in intensive care of ≥ 24 h. Patients were recruited from two participating centers in Melbourne, Australia, from 1 December 2005 to 30 November 2006. Inclusion criteria

were: age ≥ 18 years, AKI (defined by fulfilling RIFLE class, Risk) [22], and either sepsis (cases) [23] or no sepsis (controls). Exclusion criteria were: prior kidney transplant, end-stage kidney disease (K/DOQI Stage V), renal replacement therapy (RRT) prior to ICU admission, or confirmed and/or suspected acute glomerulonephritis, interstitial nephritis, renal vasculitis or obstructive etiology for AKI.

Study definitions

AKI was defined according to the RIFLE criteria [22]. Worsening and/or progressive AKI was defined as transition from RIFLE class-Risk to class-Injury, Failure or RRT initiation during the 5 days following enrollment. Sepsis was defined according to consensus guidelines [23]. Shock was defined as a mean arterial pressure (MAP) < 60 mmHg and/or need for vasoactive support. Pre-existing co-morbid illnesses were defined by the Charlson index [24].

Study protocol

Patients were identified by daily surveillance of the participating ICUs. Eligible patients underwent a medical record review. Data were extracted on standardized data forms. Clinical data included: demographics, co-morbidities, diuretic and/or nephrotoxin exposure, mechanical ventilation, RRT details and clinical outcomes. Physiologic and laboratory data included: weight, hemodynamics, vasoactive drugs, illness severity scores, and details of pre-morbid and enrollment kidney function. Estimated glomerular filtration rate (eGFR) was calculated by the modification of diet in renal disease (MDRD) equation [25] using the lowest documented creatinine in the 6 months prior to hospitalization. All patients had indwelling urinary catheters. Patients had urine drawn at enrollment, 12 and 24 h, and blood drawn at enrollment and at 12, 24 and 48 h.

Urine neutrophil gelatinase-associated lipocalin (uNGAL)

Urine samples for uNGAL testing were centrifuged at 1,500 rpm \times 10 min and supernatant stored at -70°C for batched analysis. uNGAL was measured by a chemiluminescent microparticle assay using the ARCHITECT platform (Abbott Diagnostics Inc., Abbott Park, IL), as previously described [26]. uNGAL was expressed as ng/mg creatinine, to standardize and correct for changes in urine concentration [21].

Plasma neutrophil gelatinase-associated lipocalin (pNGAL)

Blood samples for pNGAL were collected in EDTA anticoagulated tubes, centrifuged at 5,000 rpm \times 5 min and plasma stored at -70°C for batched analysis. We used the Triage NGAL Test (Biosite Inc., San Diego, CA), a point-of-care, fluorescence immunoassay for quantitative measurement of pNGAL, as previously described [27].

Statistical analysis

Statistical analysis was performed with STATA release 8.2 (StataCorp, College Station, TX). Normally distributed variables are reported as means with standard deviations (SD) and compared with Student's *t* test or one-way repeated-measures analysis of variance (ANOVA). Non-normally distributed data are reported as medians with inter-quartile range (IQR) and compared with Mann–Whitney U test, Kruskal–Wallis test or Friedman's test. Categorical data are reported as proportions and compared using Fisher's exact test. pNGAL and uNGAL were assessed for ability to discriminate septic from non-septic AKI, to predict worsening AKI and RRT initiation, as defined by nonparametric calculation of the area under the receiver-operating characteristic (AuROC) curve. An AuROC value of 0.90–1.0 indicated excellent, 0.80–0.89 good, 0.70–0.79 fair, 0.60–0.69 poor and 0.50–0.59 no useful performance for discrimination of the outcome under assessment [28]. For selected comparisons, we estimated an ideal threshold for pNGAL or uNGAL by visual inspection of the AuROC curve for

values that optimized both sensitivity (1.0) and specificity (1.0). This value was then used as a cutoff value to calculate sensitivity and specificity estimates. A *p*-value of <0.05 was considered significant.

Results

Eighty-three critically ill patients with AKI were enrolled. Mean (SD) age was 64.4 (16.6) years, 60.2% were male, and pre-morbid Charlson co-morbidity score was 3.3 (2.8). Mean (SD) APACHE II scores were 21.4 (7.6), 71.1% were mechanically ventilated, 71.1% required vasoactives, and 73.2% were in shock.

Clinical characteristics

Forty-three (51.8%) patients had septic AKI. Septic AKI patients, compared to those with non-septic AKI, had more co-morbid illness ($p = 0.005$), including cancer ($p = 0.002$), had a greater likelihood of emergency surgery ($p < 0.001$), and had APACHE II score ($p = 0.008$) (Table 1). The most common sources of sepsis were thoracic and intra-abdominal (Table 2).

Baseline physiology and laboratory values

Septic AKI patients were more tachycardic, more likely to receive vasoactives and had greater organ dysfunction (Table 3). Pre-morbid creatinine and eGFR were not

Table 1 Summary of baseline characteristics

Characteristic	Septic AKI (<i>n</i> = 43)	Non-septic AKI (<i>n</i> = 40)	<i>p</i> -value
Age (years) [mean (SD)]	67.9 (16.3)	60.6 (16.3)	0.04
Male sex (%)	46	54	0.26
Weight (kg) [mean (SD)]	71.2 (15.4)	81.1 (17.8)	0.01
Charlson co-morbidity score [mean (SD)]	4.1 (3.0)	2.4 (2.2)	0.005
Cardiac disease (%)	41.9	55	0.28
COPD (%)	27.9	12.5	0.11
Diabetes mellitus (%)	20.9	25.0	0.80
Liver disease (%)	11.6	17.5	0.54
Any cancer (%)	44.2	12.5	0.002
Surgical admission (%)	48.8	65.0	0.18
Cardiac surgical (%)	9.1	69.2	<0.001
Emergency surgery (%)	90.5	15.4	<0.001
APACHE II score [mean (SD)]	23.5 (5.4)	19.2 (8.9)	0.008
SAPS II score [mean (SD)]	47.6 (10.8)	35.3 (18.3)	<0.001
SOFA score [mean (SD)]	8.2 (3.1)	6.3 (3.4)	0.008
Mechanical ventilation (%)	60.5	82.5	0.03
Vasoactive drugs (%)	79.1	62.5	0.15
Shock (%)	79.1	66.7	0.22

COPD chronic obstructive pulmonary disease, APACHE acute physiology and chronic health evaluation, SAPS simplified acute physiology score, SOFA sequential organ failure assessment

Table 2 Summary details of septic source and diagnosis

Characteristic	<i>n</i> = 43 (%)
Source of sepsis	
Intra-abdominal	37.2
Pulmonary/thoracic	34.9
Urogenital	11.6
Skin/soft tissue/bone	7.0
Endovascular	7.0
Central nervous system	2.3
Nosocomial	34.9
Bloodstream infection	30.2
Immuno-suppressed	25.6

different between septic and non-septic AKI (Table 4). At enrollment, creatinine, urine output and RIFLE class were not different between the groups. Septic patients were more likely to have received aminoglycosides, whereas non-septic patients were more likely to have had cardiac surgery or rhabdomyolysis.

Plasma and urine NGAL

Septic AKI was associated with higher pNGAL and uNGAL at enrollment compared with non-septic AKI (Figs. 1, 2).

pNGAL was higher in septic compared with non-septic patients at 12 h; however, there was no difference at 24 and 48 h. There was no trend in pNGAL over time. Septic compared with non-septic AKI was associated with significantly higher peak pNGAL values [381 (253–585) ng/ml vs. 176 (92–269) ng/ml, $p = 0.002$].

uNGAL was higher for septic compared with non-septic AKI at both 12 and 24 h (Fig. 2). There was no trend in uNGAL over time. Peak uNGAL was significantly higher for septic compared with non-septic AKI

[396 (97–2,552) ng/mg creatinine vs. 50 (26–287) ng/mg creatinine, $p = 0.001$].

Peak pNGAL showed slightly better but non-significant discrimination of septic and non-septic AKI when compared with peak uNGAL ($p = 0.67$) (Fig. 3). A pNGAL ≥ 280 ng/ml was associated a sensitivity 75.0% and specificity 76.2% for the diagnosis of septic versus non-septic AKI (AuROC 0.77; 95% CI, 0.63–0.90). A uNGAL ≥ 150 ng/mg creatinine was associated with a sensitivity 69.1% and specificity 60.0% for a diagnosis of septic AKI (AuROC 0.70; 0.59–0.82).

Peak pNGAL showed a correlation with white blood cell count for septic ($R = 0.43$, $p = 0.02$) but not non-septic patients ($R = 0.31$, $p = 0.17$). There was no correlation between peak uNGAL and white cell count for either septic ($R = -0.09$, $p = 0.59$) or non-septic patients ($R = -0.16$, $p = 0.33$). Septic shock patients had higher peak pNGAL [05 (289–585) vs. 255 (164–520) ng/ml, $p = 0.36$] and uNGAL [486 (138–3,074) vs. 113 (15–418) ng/mg creatinine, $p = 0.03$], compared with sepsis only; however, differences in pNGAL were non-significant. Similar non-significant trends were observed in the non-septic group. In the septic group, there was no significant difference in pNGAL [481 (376–585) vs. 299 (217–497) ng/ml, $p = 0.21$] or uNGAL when stratified by cancer diagnosis ($p = 0.29$) Likewise, there was no difference by cancer diagnosis in the non-septic group ($p = 0.81$, $p = 0.98$).

The ratios of plasma/urine NGAL stratified by group are shown in Table 5. The ratio for septic AKI showed a decreasing trend over time, whereas in non-septic AKI, the ratio showed an increasing trend. There was no statistical association between plasma/urine NGAL ratio and the diagnosis of septic versus non-septic AKI.

uNGAL in patients with urinary tract (UTI) sources of sepsis were non-significantly higher when compared with other sources. At enrollment, uNGAL values were

Table 3 Summary of baseline physiology and laboratory values

Characteristic	Septic AKI (<i>n</i> = 43)	Non-septic AKI (<i>n</i> = 40)	<i>p</i> -value
MAP (mmHg) [mean (SD)]	64.5 (14.1)	64.4 (12.8)	0.97
Heart rate (beats/min) [mean (SD)]	121 (24)	107 (25)	0.01
CVP (cmH ₂ O) [mean (SD)]	10.6 (3.4)	11.5 (4.0)	0.31
≥ 2 SIRS criteria (%)	100	85.0	0.01
PaO ₂ /FiO ₂ ratio [mean (SD)]	206 (110)	294 (110)	<0.001
White cell count (10 ⁹ /ml) [median (IQR)]	16.6 (11–25.1)	12.7 (10.0–15.5)	0.01
Platelets (10 ⁹ /ml) [mean (SD)]	192 (117)	162 (89)	0.20
Albumin (g/l) [mean (SD)]	21.2 (6.8)	27.2 (6.4)	<0.001
Bilirubin (mmol/l) [median (IQR)]	21 (15–38)	17 (12–27)	0.03
pH [mean (SD)]	7.32 (0.11)	7.33 (0.13)	0.75
Sodium (mmol/l) [mean (SD)]	137 (4.1)	139 (5.4)	0.04
Potassium (mmol/l) [mean (SD)]	4.3 (1.0)	4.5 (0.9)	0.42
Bicarbonate (mmol/l) [mean (SD)]	23.7 (5.3)	24.3 (5.2)	0.63
Creatine kinase (U/l) [median (IQR)]	168 (38–55)	582 (336–2367)	0.006
Lactate (mmol/l) [median (IQR)]	2.5 (1.8–4.3)	2.5 (1.9–3.6)	0.96

MAP mean arterial pressure, CVP central venous pressure, SIRS systemic inflammatory response syndrome

Table 4 Summary of kidney function at enrollment and clinical outcomes

Parameter	Septic AKI (n = 43)	Non-septic AKI (n = 40)	p-value
Baseline kidney function			
Serum creatinine (μmol/l) [mean (SD)]	75 (65–85)	76 (68–90)	0.73
Serum urea (mmol/l) [mean (SD)]	5.6 (2.8)	5.8 (2.3)	0.22
eGFR (ml/min/1.73 m ²) [mean (SD)]	87.9 (27.2)	92.5 (34.4)	0.50
eGFR < 60 mL/min/1.73 m ² (%)	7.0	15	0.30
Enrollment kidney function			
Serum creatinine(μmol/l) [median (IQR)]	142 (94–191)	102 (84–136)	0.06
Serum urea (mmol/l) [median (IQR)]	12.2 (7.1)	10.1 (8.5)	0.25
RIFLE category at enrollment			
Risk (%)	60.5	70	0.72
Injury (%)	23.3	17.5	
Failure (%)	16.3	12.5	
Urine output (ml/h) [mean (SD)] ^a	63 (46–132)	69 (44–144)	0.74
Oliguria prior to enrollment (%) ^b	12.2	19.4	0.53
Nephrotoxins prior to enrollment (%)	55.8	72.5	0.17
Radiocontrast media (%)	27.9	27.5	1.0
Aminoglycosides (%)	37.2	10.0	0.005
Cardiopulmonary bypass (%)	4.7	45.0	<0.001
Rhabdomyolysis ^c (%)	2.3	17.5	0.03
Amphotericin (%)	2.3	2.5	1.0
Diuretics prior to enrollment (%)	67.4	52.5	0.19
RRT after enrollment (%)	18.6	12.5	0.55
Hospital death (%)	44.2	15.0	0.004

eGFR estimated glomerular filtration rate, *RRT* renal replacement therapy

^a Urine output average over 6 h prior to study enrollment

^b Oliguria defined as <500 ml urine output in 24 h prior to enrollment

^c Rhabdomyolysis defined by serum CK >1,500

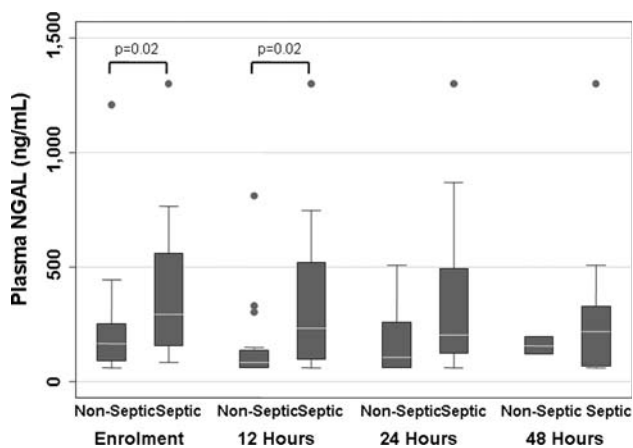


Fig. 1 Plasma NGAL concentration stratified sepsis versus non-sepsis and duration after enrollment

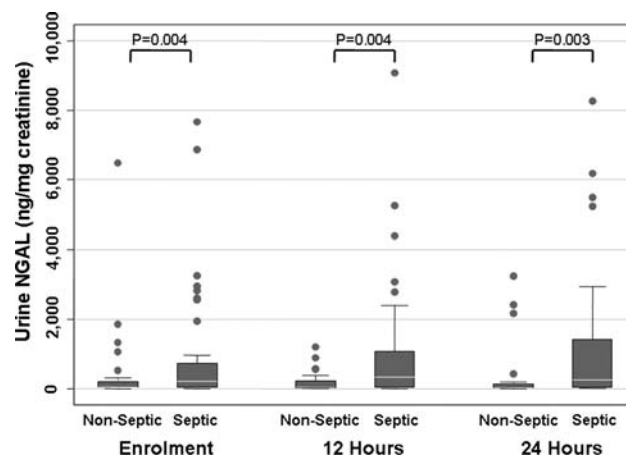


Fig. 2 Urine NGAL concentration stratified by sepsis versus non-sepsis and duration after enrollment

821 ng/mg creatinine in UTI patients versus 201 ng/mg creatinine in other septic sources ($p = 0.16$). No statistical differences were evident by UTI status in pNGAL.

Injury progression, renal replacement therapy and mortality

Twenty patients (24.1%) developed worsening AKI at 48 h. Of these, 13 (65%) received RRT. The peak NGAL

preceded AKI progression in only ten patients (50%). Worsening RIFLE class in the first 48 h was predictive of subsequent RRT initiation (AuROC 0.95; 0.91–0.99). In this cohort, there were no statistical differences in pNGAL or uNGAL between septic and non-septic groups for worsening RIFLE class or RRT initiation. Peak pNGAL and uNGAL were higher for those developing worsening RIFLE class and for those receiving RRT (Table 6). Analysis of the AuROC curve for peak pNGAL showed fair discrimination for predicting AKI progression

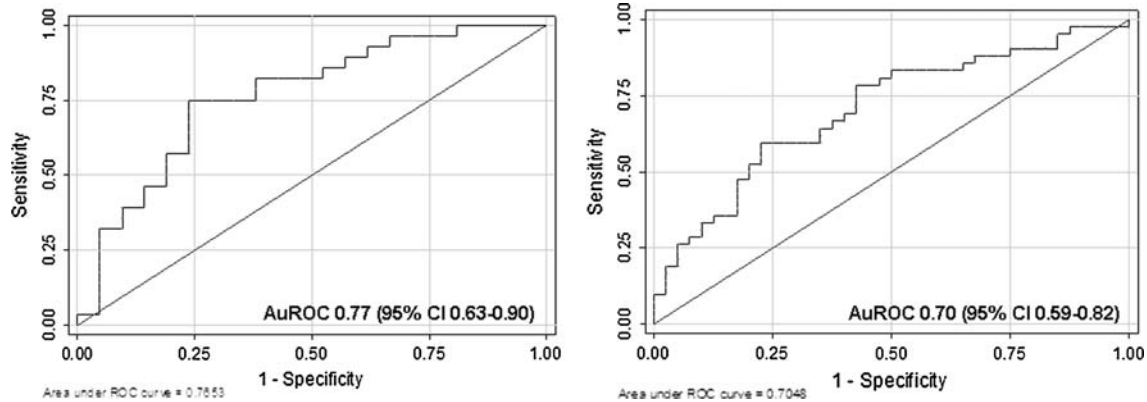


Fig. 3 Area under receiver-operator characteristic curve for diagnosis of septic versus non-septic AKI for: **a** peak plasma NGAL and **b** peak urine NGAL

Table 5 Summary of plasma/urine NGAL ratios stratified by septic versus non-septic AKI

Sampling time	Plasma/urine NGAL ratio (median [IQR])	
	Septic AKI	Non-septic AKI
Baseline	3.1 (1.1–5.0)	1.9 (0.3–3.9)
12 h	1.1 (0.2–2.4)	6.4 (1.0–9.8)
24 h	0.6 (0.3–12.8)	7.9 (1.7–13.3)

NGAL neutrophil gelatinase-associated lipocalin

(0.71, 0.55–0.88) and need for RRT (0.78, 0.61–0.95) (Fig. 4). Peak uNGAL showed slightly worse discrimination compared with pNGAL for AKI progression (0.70, 0.58–0.81) and need for RRT (0.70, 0.58–0.82). However, peak uNGAL had better performance for predicting AKI progression in non-septic AKI compared with septic AKI. A uNGAL ≥ 230 ng/mg creatinine was associated with a sensitivity of 77.8% and specificity of 80.7% for predicting AKI progression in non-septic AKI and performed better when compared with septic AKI (0.82 vs. 0.59, $p = 0.04$). No other differences between septic and non-septic AKI were noted.

In-hospital mortality was 30.2% ($n = 25$). Worsening RIFLE class had fair discrimination for in-hospital mortality (0.70; 0.59–0.81). Peak pNGAL and uNGAL alone had poor discrimination for in-hospital death (pNGAL, 0.69; 0.48–0.74; uNGAL, 0.62; 0.49–0.76).

Discussion

We conducted a prospective observational study comparing pNGAL and uNGAL in critically ill patients with septic and non-septic AKI. We found that septic AKI was associated with higher initial values of pNGAL and uNGAL, and this difference generally persisted for the study duration when compared with non-septic AKI. We did not find

significant trends in pNGAL or uNGAL for either the septic or non-septic AKI groups. We found septic AKI was associated with higher peak pNGAL and uNGAL compared with non-septic AKI. We also found peak pNGAL and uNGAL showed fair discrimination for a diagnosis of septic versus non-septic AKI. Interestingly, we found a significant difference in the p/u NGAL ratio over time between septic (decreasing) and non-septic AKI (increasing). Overall, there was no difference in worsening AKI or RRT initiation between the groups. However, higher peak pNGAL and uNGAL were associated with worsening AKI and/or RRT initiation, and AuROC analysis showed fair discrimination. We also found peak uNGAL had better performance for predicting AKI progression in non-septic compared with septic AKI. We found critically ill patients with septic, when compared with non-septic AKI, had considerable differences across numerous clinical, physiologic and laboratory parameters. Despite similar AKI severity, septic patients were generally older, had more comorbid chronic illness, higher illness severity and organ dysfunction, and were more likely to have undergone emergency surgery. These differences have likewise been described in prior studies [2, 3, 29].

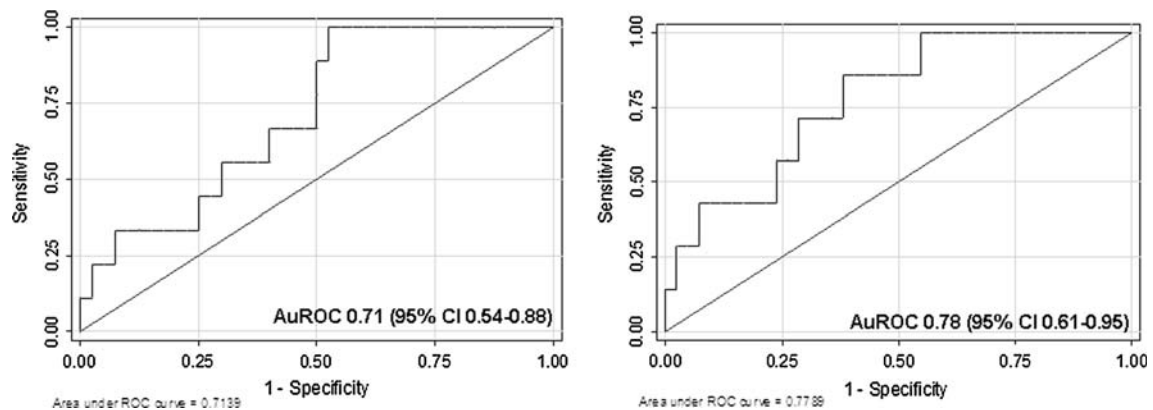
Sepsis is now recognized as the most common precipitant for AKI in critically ill patients [1–4]. Moreover, septic AKI patients have considerably higher mortality when compared with non-septic AKI patients [2, 3]. Such a high incidence and marked disparity in outcome implies a need for increasing our understanding of the pathophysiology, diagnosis and treatment options for septic AKI [9].

Animal data indicate septic AKI may have distinct pathophysiologic features that differ from ischemia/toxin-induced kidney injury [10]. For example, in a mammalian model of *E. coli*-induced hyperdynamic sepsis, renal blood flow was increased nearly 300% from baseline at 48 h [14]. This renal hyperemia was evident despite severe reductions in kidney function. This phenomenon has been similarly described in septic humans [30–32].

Table 6 Association between plasma and urine NGAL and AKI progression, RRT and mortality

Parameter	<i>n</i> (%)	Peak plasma NGAL (ng/ml)	<i>p</i> -value	Peak urine NGAL (ng/mg creatinine)	<i>p</i> -value
Worsened AKI					
Yes	20 (24.1)	401 (269–613)	0.047	411 (217–1,371)	0.008
No	63 (75.9)	267 (122–452)		122 (27–821)	
RRT					
Yes	13 (15.7)	445 (336–869)	0.019	423 (247–1,068)	0.024
No	70 (84.3)	264 (122–437)		143 (28–883)	
Death					
Yes	25 (30.1)	496 (192–750)	0.051	418 (201–1,068)	0.078
No	58 (69.9)	269 (133–401)		106 (30–883)	

All values presented as median (IQR) unless specified
 NGAL neutrophil gelatinase-associated lipocalin

**Fig. 4** Area under receiver-operator characteristic curve for peak plasma NGAL to predict: **a** worsening RIFLE class; **b** RRT

These observations may have implications for the critically ill septic patient. First, unique patterns of urine biochemistry and/or biomarkers may exist in septic AKI that can be detected early, guide therapy, aid in clinical decision-making (early renal support) and/or provide prognostic information (renal recovery) [16]. Second, emerging literature has challenged the conventional paradigm on the value of urine biochemistry and derived indices in septic AKI [16–18]. This widely accepted paradigm for classifying AKI (i.e., pre-renal azotemia or acute tubular necrosis) may be flawed in septic AKI, and novel methods for evaluation are needed [16, 33].

NGAL is a 25-kD polypeptide that is up-regulated, secreted and detected early in AKI [20]. The detection of NGAL has been described in several studies as an early sensitive biomarker of AKI that precedes the lag-time effect needed to detect abnormalities in traditional measures of kidney function [19, 21, 26–28, 34–37]. Only two studies have evaluated NGAL in critical illness, both in pediatric populations [38, 39]. In a prospective observational study, Zappitelli et al. measured daily uNGAL in 140 mechanically ventilated children [39]. Increased uNGAL was detected approximately 2 days prior to onset of AKI, and peak uNGAL correlated well with AKI

severity. Wheeler et al. performed a multi-center observational study evaluating pNGAL within 24 h of ICU admission to predict AKI in 143 critically ill children with systemic inflammatory response syndrome or septic shock [38]. AKI occurred in 15.4% after a median 24 h, and pNGAL was significantly higher in AKI compared with no AKI (355 vs. 186 ng/ml, $p = 0.009$).

Our study is the first to evaluate for differences in pNGAL and uNGAL in critically ill patients with septic compared with non-septic AKI. We found that septic AKI was associated with higher levels of both pNGAL and uNGAL. One hypothesis to potentially explain this observation is that sepsis induces a greater “injury” to the kidney compared with other contributing factors. While higher pNGAL levels have been associated with greater severity of AKI [39], no investigation as of yet has examined for differences in expression of NGAL by contributing factor for AKI.

Alternatively, NGAL may have higher expression in inflammatory states such as sepsis or in selected malignancies. Indeed, Wheeler et al. found higher values of pNGAL in children with septic shock compared to those with either SIRS or healthy controls [38]. NGAL is constitutively expressed in several tissues including lung,

liver, kidney and the gastrointestinal tract [40]. Moreover, NGAL expression has been found to be significantly up-regulated in several forms of cancer [41–43]. Likewise, NGAL expression has been found to be increased during acute inflammatory and/or infectious processes [41, 42, 44]. These observations suggest NGAL may represent an emerging global biomarker for inflammation, tissue injury, illness severity and organ failure, and correlate with survival in sepsis. This was recently shown by Shapiro et al. in a multi-center observational study of 971 patients with suspected sepsis presenting to the ED. These investigators developed a biomarker panel for the early diagnosis of septic shock and multi-organ failure [45]. Three plasma biomarkers, including interleukin-1 receptor antagonist, protein C and NGAL, were found to optimally predict subsequent septic shock within 72 h (AuROC 0.80).

We were not able to detect a significant trend in pNGAL or uNGAL over the study duration. This may be more challenging in an unselected ICU cohort with established AKI where the “time of injury” is less certain [38, 39]. However, we speculate that relative changes in pNGAL and uNGAL over well-defined points in time may have diagnostic/prognostic value and should be evaluated in further investigations. Interestingly, we observed a reversal in the p/uNGAL ratio over time when comparing septic and non-septic AKI. Likewise, we observed that peak uNGAL had reasonable performance for predicting AKI progression in non-septic, but not septic AKI. These data signify a differential response in pNGAL and/or uNGAL, or perhaps variable thresholds for correlation with clinical outcomes across syndromes of AKI (i.e., septic vs. non-septic). Moreover, the early appearance of pNGAL and/or uNGAL holds promise for enabling the prediction of the natural history, clinical course and outcomes for these patients, such as worsening or persistent AKI, RRT initiation and mortality. Zappatelli et al. found uNGAL had fair discrimination for critically ill children developing AKI persisting >48 h (AuROC 0.79) [39]. Nicholas et al. found uNGAL ≥ 130 $\mu\text{g/g}$ creatinine was associated with higher likelihood of RRT initiation [21]. While both peak pNGAL and uNGAL showed fair discrimination for worsening AKI or RRT initiation, a uNGAL ≥ 230 ng/mg creatinine was more predictive of AKI progression in non-septic AKI (AuROC 0.82).

There are limitations to our study. First, while prospective, our study was small and is potentially prone to

type I error, and it has limited statistical power. In addition, critically ill patients are often exposed to numerous concurrent sources for kidney injury. Accordingly, as noted, there were differences between groups in exposure to aminoglycosides, cardiopulmonary bypass and rhabdomyolysis. Similarly, the septic AKI patients had a higher prevalence of cancer, which may impact the observed differences in NGAL between septic and non-septic groups. In addition, we did not include a septic non-AKI control group for comparison. Therefore, our data are not able to discriminate the ability of pNGAL or uNGAL for the early diagnosis of AKI per se. Future investigations of novel AKI biomarkers, such as NGAL, should continue to explore whether there is differential expression across AKI syndromes, evaluate the potential role of NGAL in therapeutic monitoring in response to interventions, their role during weaning from renal support and their association with renal recovery and/or progression to chronic kidney disease.

In summary, pNGAL and uNGAL are higher in septic compared to non-septic AKI patients. In general, septic AKI patients have a higher burden of illness and are sicker when compared with non-septic AKI patients. Both pNGAL and uNGAL showed a significant association with worsening AKI and RRT initiation. Peak uNGAL was a better predictor of AKI progression in non-septic AKI. These observations suggest there are differential biomarker patterns in septic AKI that may have clinical relevance and prognostic importance. Larger comparative studies appear justified.

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Conflicts of interest statement Drs. Bellomo and Devarajan have acted as paid consultants for Abbott Diagnostics and Inverness Medical. Dr. Haase has received an honorarium for speaking for Abbott Diagnostics and Biosite Incorporated. Dr. Bagshaw has received an honorarium for speaking for Inverness Medical. Both companies are involved in the development of NGAL assays to be applied in clinical practice.

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