Journal of Critical Care 53 (2019) 1–7

Contents lists available at ScienceDirect

Journal of Critical Care

journal homepage: www.journals.elsevier.com/journal-of-critical-care

Integration of urinary neutrophil gelatinase-associated lipocalin with serum creatinine delineates acute kidney injury phenotypes in critically ill children

Natalja Stanski, MD [a], Shina Menon, MD [b], Stuart L. Goldstein, MD [c], Rajit K. Basu, MD MS [d,*]

[a] Cincinnati Children's Hospital Medical Center, Division of Critical Care Medicine, 3333 Burnet Avenue, MLC 2005, Cincinnati, OH 45229, United States of America
[b] University of Washington School of Medicine, Division of Nephrology, Seattle Children's Hospital, Seattle, WA, United States of America
[c] Cincinnati Children's Hospital Medical Center, Center for Acute Care Nephrology, University of Cincinnati College of Medicine, 3333 Burnet Avenue, MLC 7022, Cincinnati, OH 45229, United States of America
[d] Children's Healthcare of Atlanta, Division of Critical Care Medicine, United States of America

ARTICLE INFO

Keywords:
Acute kidney injury (AKI)
Biomarker
Phenotype
Neutrophil gelatinase-associated lipocalin
Risk stratification

ABSTRACT

Purpose: Acute kidney injury (AKI) is prevalent in critically ill patients and associated with poor outcomes. Current AKI diagnostics— changes to serum creatinine (SCr) and urine output— are imprecise. Integration of injury biomarkers with SCr may improve diagnostic precision.
Methods: We performed a secondary analysis of a study of critically ill children. Measurements of urine neutrophil gelatinase-associated lipocalin (uNGAL) and SCr samples from ICU admission facilitated the creation of four groups for comparison, based on elevation of SCr from baseline and reference NGAL cut-off value: uNGAL-/SCr-, uNGAL+/SCr-, uNGAL-/SCr + and uNGAL+/SCr+. The primary outcome assessed was AKI severity on Day 3.
Results: 178 children were studied. Compared to uNGAL-/SCr-, uNGAL+/SCr- patients had increased risk for all-stage Day 3 AKI (≥ KDIGO stage 1) (OR 3.83, [1.3–11.3], $p = .025$). Compared to uNGAL-/SCr+, uNGAL+/SCr + patients had increased risk for *severe* Day 3 AKI (≥ KDIGO stage 2) (OR 12, [1.4–102], $p = .018$). The only patients to suffer all-stage Day 3 AKI *and* mortality were uNGAL+ (3.2% uNGAL+/SCr-; 6.5% uNGAL+/SCr+).
Conclusions: Unique biomarker combinations on admission are predictive of distinct Day 3 AKI severity phenotypes. These classifications may enable a more personalized approach to the early management of AKI. Expanded study in larger populations is warranted.

© 2019 Elsevier Inc. All rights reserved.

1. Introduction

Acute kidney injury (AKI) is a significant problem in the intensive care unit (ICU). Recent data suggest that over half of critically ill adults, 1 in 4 critically ill children, and 1 in 3 critically ill neonates develop AKI during admission, and that these patients are at increased risk for morbidity and mortality, particularly with increasing duration and severity of AKI [1-3]. The cost of caring for patients with AKI is also substantial, with estimates suggesting a burden of anywhere from 5 to 20 billion dollars each year in the United States alone [4,5]. Although understanding of the consequences of AKI has improved over recent years, cost of care remains high and outcomes remain poor, suggesting that the current strategies for AKI management– whether it be prevention, detection, or treatment– remain suboptimal.

The current diagnostics for AKI are imprecise for a variety of reasons. Increases in serum creatinine (SCr) or decreases in urine output, the current gold standards for diagnosis, are late findings on the continuum of kidney injury. Additionally, in several populations— most notably children— the interpretation of changes to SCr is fraught with complexity, as baseline SCr levels are often unknown and production can vary based on body composition [6-8]. Similarly, critically ill patients of all ages can also experience changes to muscle mass and total body water during acute illness, potentially confounding the diagnosis of AKI by SCr alone [6-8]. Finally, diagnosing AKI by the presence or absence of SCr elevation or decreased urine output assigns a binary categorization to what is likely a more complex and heterogeneous disease process, providing no granularity on the individual patient level. These factors combine to create an inherently imprecise set of diagnostic tools, perhaps contributing to inaccurate or delayed detection of affected patients, and providing barriers to the delivery of appropriate, personalized care.

Abbreviations: uNGAL, urinary neutrophil gelatinase-associated lipocalin; SCr, serum creatinine; AKI_A, any AKI, KDIGO stage 1 AKI or higher; AKI_S, severe AKI, KDIGO stage 2 or 3; FO, fluid overload.

* Corresponding author.
E-mail addresses: natalja.stanski@cchmc.org (N. Stanski), shina.menon@seattlechildrens.org (S. Menon), stuart.goldstein@cchmc.org (S.L. Goldstein), rajit.basu@choa.org (R.K. Basu).

https://doi.org/10.1016/j.jcrc.2019.05.017
0883-9441/© 2019 Elsevier Inc. All rights reserved.

The incorporation of novel biomarkers of AKI may improve the precision of the AKI diagnosis. Tubular damage markers such as neutrophil gelatinase-associated lipocalin (NGAL) have been proposed to allow for early detection and prognostication of existing AKI [9,10]. They may also identify patients with ongoing subclinical AKI— a potentially early form of kidney injury that if promptly recognized could allow for proactive intervention to mitigate further damage— who would otherwise go undetected [11]. The Tenth Consensus Conference of the Acute Dialysis Quality Initiative (ADQI 10) recommended clinical integration of biomarkers to refine and reclassify AKI diagnostic nomenclature [12]. To date, however, insufficient data has been reported studying this concept. Consequently, little remains known about the significance of biomarker positivity in critically ill patients with and without SCr changes [12].

Building upon the ADQI 10, the Sixteenth Consensus Conference of the Acute Dialysis Quality Initiative (ADQI 16) advocated for the utilization of biomarkers to aid in the identification of patients at high risk for persistent AKI, a clinical entity that has been shown to be associated with poor outcomes [13]. Defined by the workgroup as kidney injury sustained beyond 48 h (i.e. on day 3), the ADQI 16 provided a consensus time point of clinical relevance that has been utilized as an endpoint of interest since [13]. While this recommendation is supported by the available literature to date and expert opinion, more data is needed to clearly understand the timeline of AKI and its impact on patient outcomes.

Our goal was to operationalize the above outlined recommendations of the ADQI 10 and 16 consensus statements— to utilize a urine kidney injury biomarker in combination with SCr to predict the severity of persistent AKI (at day 3)— in a heterogeneous cohort of critically ill patients. We combined urinary NGAL (uNGAL) and SCr values on the day of admission to create four unique subsets of patients that we proposed could predict Day 3 AKI severity, addressing the existing knowledge gap surrounding the significance of damage biomarker positivity in critical illness. Early identification of these variable risk phenotypes could be the first step towards a more refined and targeted approach to the management of the heterogeneous syndrome of AKI.

2. Materials and methods

2.1. Study design and patient selection

We performed a secondary analysis of Acute Kidney Injury in CHildren Expected by Renal angina and Urinary Biomarkers (AKI-CHERUB, NCT01735162). AKI-CHERUB was a single center, prospective observational study conducted with Institutional Review Board approval with a waiver of the need for informed consent at Cincinnati Children's Hospital Medical Center (CCHMC) from September 2012 to March 2014. CCHMC is a tertiary care pediatric facility admitting patients from all medical and surgical subspecialties. Children and young adults aged 3 months to 25 years with a predicted length of stay of at least 48 h were included. Patients with a history of end stage renal disease and those who were admitted immediately post-renal transplant were excluded.

2.2. Metrics

The original dataset included demographic information, admission diagnoses, comorbid conditions, height, weight, Pediatric Risk of Mortality III Score (PRISM-III) and available laboratory data, including serum creatinine (SCr) and urine neutrophil gelatinase-associated lipocalin (uNGAL), at the time of admission. Baseline SCr was defined as the lowest SCr up to 3 months prior to ICU admission. If no baseline SCr was available, an estimated baseline SCr was derived using the patient's calculated body surface area (m^2) and an eGFR of 120 ml/min per 1.73 m^2, as validated in the literature [1,14]. Data were collected daily from admission through Day 7. Day 28 outcomes assessed included use of renal replacement therapy (RRT), mortality, and ICU and hospital length of stay.

2.3. Definitions

Patients were classified by uNGAL and SCr values measured in the first 24 h of ICU course. uNGAL positivity (uNGAL+) was defined a priori as uNGAL ≥150 ng/ml, a sensitive cut-off that has been previously described in the literature [15,16]. SCr positivity (SCr+) was defined as KDIGO stage 1 AKI or higher (SCr ≥1.5 times higher than measured or calculated baseline). Combining parameters yielded 4 biomarker-based classifications used to cohort patients on the day of ICU admission: uNGAL-/SCr-, uNGAL+/SCr-, uNGAL-/SCr + and uNGAL+/SCr + (Fig. 1).

2.4. Outcome measures

The primary outcome of interest was the severity of AKI on Day 3, as defined by KDIGO staging by the worse of either SCr or urine output criteria. D_3 injury was classified as no AKI, any AKI (AKI_A) (KDIGO stage 1 AKI or higher) or severe AKI (AKI_S) (KDIGO stage 2 or 3). Secondary outcomes included fluid accumulation on D_3, defined as percent fluid overload >20% (FO), ICU and hospital lengths of stay (LOS), RRT use, and mortality.

2.5. Statistical analysis

Analyses were performed comparing the predictive performance of biomarker-based classifications for the outcomes of interest. Single biomarker-based classifications were compared first (uNGAL status versus SCr status). Subsequent analyses compared the four biomarker-based classifications. Additionally, multiple bivariable linear regressions were conducted to predict ICU and hospital LOS based on uNGAL value and severity of illness (PRISM-III scores). These variables were chosen as logical markers of comparison as patients who were uNGAL+ tended to have higher severity of illness scores. A univariate linear regression to evaluate the impact of SCr on both ICU and hospital LOS was also performed for comparison, as SCr is the current gold standard for AKI diagnosis.

Categorical variables were summarized using frequencies and proportions and compared using odds ratios, chi-squares (Fisher's exact when appropriate), relative risk, and 95% confidence intervals. Sensitivity, specificity, positive predictive values and negative predictive values were also calculated to evaluate the impact of uNGAL+ in comparisons. Continuous variables were summarized using medians and compared using the Mann-Whitney test. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using Sigmaplot 13.0 (Systat Software Inc., San Jose, CA, USA).

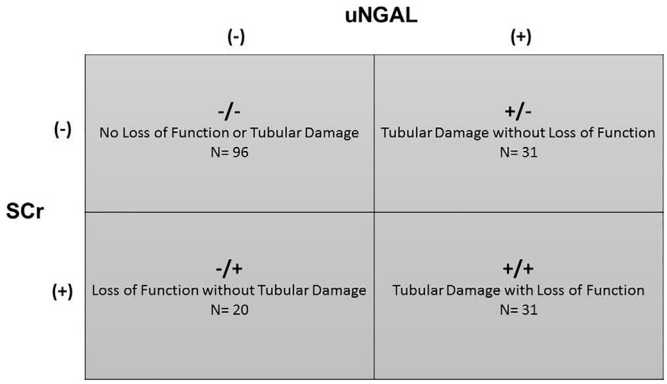

Fig. 1. Four biomarker-based classifications based on uNGAL and SCr status. uNGAL+ was defined as uNGAL ≥150 ng/ml. SCr + was defined as KDIGO stage 1 AKI or higher (SCr ≥1.5 times higher than measured or calculated baseline).

3. Results

3.1. Patients

Data from 178 patients were analyzed. On the day of admission, 62 patients were uNGAL+ and 116 were uNGAL-, while 51 were SCr + and 127 were SCr-. Four unique biomarker-stratified groups yielded 96 patients who were uNGAL-/SCr-, 31 who were uNGAL+/SCr-, 20 who were uNGAL-/SCr+, and 31 who were uNGAL+/SCr + .

3.2. uNGAL vs. SCr for prediction of day 3 AKI and outcomes

Demographic and outcome data for uNGAL+ compared to SCr + patients and uNGAL- compared to SCr- patients are outlined in Tables 1 and 2, respectively. There were no significant differences in age, gender, presence of sepsis, or PRISM III scores between either set of patients. Patients who were uNGAL+ on ICU admission had similar outcomes to those who were SCr + (Table 1); similarly, uNGAL- patients had comparable outcomes to SCr- patients (Supplementary Table 1).

3.3. uNGAL status predicts AKI severity in SCr + patients

Patients who were uNGAL+ (uNGAL+/SCr+) had higher severity of illness scores and were more likely to have sepsis at the time of PICU admission (Table 2). Patients classified as uNGAL+/SCr + had increased odds of Day-3 AKI_A (OR 10.3, [2.5–43.1], $p = .001$) and Day-3 AKI_S (OR 12, [1.4–102], $p = .018$) when compared to uNGAL-/SCr + patients. Although the associations were not statistically significant, they also demonstrated a trend towards higher odds of FO, in-hospital mortality, and longer ICU and hospital LOS. Furthermore, all patients requiring RRT (6/178) were classified as uNGAL+/SCr + on the day of admission.

Conversely, SCr + patients who were uNGAL- (uNGAL-/SCr+) had uniformly better outcomes than their uNGAL+ counterparts (Table 2). The classification of uNGAL-/SCr + on admission was associated with a negative predictive value of >85% for all Day-3 AKI outcomes, including 95% for AKI_S. Additionally, compared to uNGAL+/SCr+, the uNGAL-/SCr + classification yielded an OR of 10.3 ([2.5–43.1], $p = .001$) for no Day-3 AKI.

3.4. uNGAL+ predicts kidney injury in SCr- patients

For SCr- patients, those who were uNGAL+ (uNGAL+/SCr-) were compared to those who were not (uNGAL-/SCr-) (Table 3). The uNGAL+ patients had higher severity of illness scores compared to uNGAL- on admission. Additionally, these patients had increased odds of Day-3 AKI_A (OR 3.83, [1.3–11.3], $p = .025$), Day-3 FO (OR 4.38, [1.4–14], $p = .023$), and prolonged ICU (10 days (4,15) vs. 4 days (3,9), $p = .002$) and hospital (24 days (11,48) vs. 12 days (7,23), $p = .002$) LOS, compared to uNGAL-/SCr- patients. Though the associations did not reach statistical significance, these patients also had higher odds of Day-3 AKI_S and in-hospital mortality. The side-by-side comparison of Day 3 AKI outcomes for these uNGAL+/SCr- patients and the other biomarker-based groups are shown in Fig. 2. The four groups were also compared with regards to Day-3 AKI_A, RRT use, mortality, and the composite endpoint of Day-3 AKI and mortality (Fig. 3). While the uNGAL+/SCr- group did not require RRT, the incidence of both Day-3 AKI_A and mortality in this group were higher than the uNGAL-/SCr + patients, and closer to that of the uNGAL+/SCr + patients.

3.5. uNGAL is not just a marker of illness severity

Regardless of SCr status, patients who were uNGAL+ on admission had higher severity of illness scores compared to uNGAL- patients (Table 4). These uNGAL+ patients had increased risk for all D_3 AKI outcomes compared to uNGAL-, including increased risk of Day-3 AKI_A (OR 7.86, [3.54–17.5], $p \leq .001$), Day-3 AKI_S (OR 15.1, [3.3–69.6], $p \leq .001$) and Day-3 FO (OR 4.54, [1.7–12], $p = .003$). They also had increased odds of needing RRT, prolonged ICU and hospital LOS as well as a trend towards increased mortality. To adjust for illness severity, multiple linear regressions were calculated to predict ICU and hospital LOS based on uNGAL

Table 1
Comparison of admission uNGAL+ to SCr+.

	All	uNGAL +	SCr +	Comparison uNGAL+ to SCr+
Demographics				
N	178	62	51	–
Age (years)	6.7 (2.7-14)	6.5 (1.9-15)	5.9 (1.5-15)	$p = .92$
Male	92 (51.7)	32 (51.6)	32 (62.7)	$p = .32$
Sepsis	33 (24.2)	26 (41.9)	22 (43.1)	$p = .95$
PRISM III score	7 (3-13)	11 (6-17)	11 (5-20)	$p = .57$
Day 3 AKI outcomes				
Day 3 AKI				
Any AKI	39 (21.9)	28 (45.2)	22 (43.1)	OR 1.09 (0.5-2.3; $p = .98$)
Sensitivity		71.8% (55-85)	59% (42-74)	
Specificity		75.5% (67-82)	79.9% (72-86)	
NPV		90.5% (83-95)	87.4% (80-92)	
PPV		45.2% (33-58)	45.1% (31-60)	
Severe AKI	15 (8.4)	13 (20.6)	13 (25.5)	OR 0.78 (0.3-1.9; $p = .73$)
Sensitivity		86.7% (58-98)	86.7% (58-98)	
Specificity		69.9% (62-77)	76.7% (69-83)	
NPV		98.3% (93-99)	98.4% (94-99)	
PPV		21.0% (12-34)	25.5% (15-40)	
Day 3 FO	21 (11.8)	14 (22.6)	8 (15.7)	OR 1.57 (0.6-4.1; $p = .50$)
Sensitivity		66.7% (43-85)	38.1% (19-61)	
Specificity		69.4% (62-76)	72.6% (65-94)	
NPV		94.0% (88-97)	89.8% (83-94)	
PPV		22.6% (13-35)	15.7% (7.5-29)	
Secondary outcomes				
RRT Use	6 (3.4)	6 (9.7)	6 (11.8)	OR 0.80 (0.24-2.66; $p = .96$)
Mortality	12 (6.7)	7 (11.3)	5 (9.8)	OR 1.17 (0.35-3.94; $p = .96$)
ICU LOS (days)	5 (3-11)	8 (4-16)	6 (3-16)	$p = .21$
Hospital LOS (days)	14 (8-30)	23.5 (10-47)	21 (8-36)	$p = .29$

Data for categorical variables are n(%) and for continuous variables are median (IQR); odds ratios (OR) are presented as OR (95% CI; *p*-value).
AKI- acute kidney injury; NPV- negative predictive value; PPV- positive predictive value; FO- fluid overload; RRT- renal replacement therapy; LOS- length of stay.

Table 2
The significance of uNGAL positivity in SCr positive patients.

	All	uNGAL−/ SCr+	uNGAL+/SCr+	Comparison uNGAL+ to uNGAL-
Demographics				
N	178	20	31	–
Age (years)	6.7 (2.7-14)	6.87 (1.4-18)	5.46 (1.8-13)	$p = .78$
Male	92 (51.7)	14 (70)	18 (58.1)	$p = .57$
Sepsis	33 (24.2)	3 (15)	19 (61.3)	$p = .003$
PRISM III score	7 (3-13)	7.5 (3-15)	15 (8-20)	$p = .026$
DAY 3 AKI outcomes				
Day 3 AKI				
Any AKI	39 (21.9)	3 (15)	20 (64.5)	OR 10.3 (2.5-43.1; $p = .001$)
Sensitivity			87% (65-97)	RR 4.3 (1.5-12.6; $p = .001$)
Specificity			60.7% (41-78)	
NPV			85% (61-96)	
PPV			64.5% (46-80)	
Severe AKI	15 (8.4)	1 (5)	12 (38.7)	OR 12 (1.4-102; $p = .018$)
Sensitivity			92.3% (62-99)	RR 7.74 (1.1-55; $p = .018$)
Specificity			50% (34-99)	
NPV			95% (73-99)	
PPV			38.7% (22-58)	
Day 3 FO	21 (11.8)	1 (5)	7 (22.6%)	OR 5.54 (0.63-49; $p = .13$)
Sensitivity			87.5% (47-99)	RR 4.52 (0.6-34; $p = .13$)
Specificity			44.2% (29-60)	
NPV			95% (73-99)	
PPV			22.6% (10-42)	
Secondary outcomes				
RRT use	6 (3.4)	0 (0)	6 (19.4)	OR (+inf) ($p = .07$)
				RR (+inf) ($p = .07$)
Mortality	12 (6.7)	1 (5)	4 (12.9)	OR 2.82 (0.3-27.2; $p = .64$)
				RR 2.58 (0.3-21.4; $p = .64$)
ICU LOS (days)	5 (3-11)	4 (3-10)	7 (3-18)	$p = .12$
Hospital LOS (days)	14 (8-30)	15.5 (8-24)	23 (8-47)	$p = .2$

Data for categorical variables are n(%) and for continuous variables are median (IQR); odds ratios (OR) and relative risks (RR) are presented as OR/RR (95% CI; *p*-value).
AKI- acute kidney injury; NPV- negative predictive value; PPV- positive predictive value; FO- fluid overload; RRT- renal replacement therapy; LOS- length of stay.

Table 3
The significance of uNGAL positivity in SCr negative patients.

	All	uNGAL-/SCr-	uNGAL+/SCr-	Comparison uNGAL+ to uNGAL-
Demographics				
N	178	96	31	–
Age (years)	6.7 (2.7-14)	7.09 (3-14)	11.1 (2-15)	$p = .88$
Male	92 (51.7)	46 (47.9)	14 (45.2)	$p = .95$
Sepsis	33 (24.2)	14 (14.6)	7 (22.6)	$p = .45$
PRISM III score	7 (3-13)	5 (1.3-11)	8 (4-13)	$p = .045$
DAY 3 AKI outcomes				
Day 3 AKI				
Any AKI	39 (21.9)	8 (8.33)	8 (25.8)	OR 3.83 (1.3-11.3; $p = .025$)
Sensitivity			50% (26-75)	RR 3.1 (1.3-7.6; $p = .025$)
Specificity			79.3% (70-86)	
NPV			91.7 (84-96)	
PPV			25.8% (13-45)	
Severe AKI	15 (8.4)	1 (1.04)	1 (3.2)	OR 3.17 (0.2-52; $p = .98$)
Sensitivity			50% (27-97)	RR 3.1 (0.2-48; $p = .98$)
Specificity			76% (67-83)	
NPV			99% (94-99.9)	
PPV			3.23% (0.2-19)	
Day 3 FO	21 (11.8)	6 (6.25)	7 (22.6)	OR 4.38 (1.4-14; $p = .023$)
Sensitivity			53.8% (26-80)	RR 3.61 (1.3-9.9; $p = .023$)
Specificity			78.9% (70-86)	
NPV			93.4% (86-97)	
PPV			22.6% (10-42)	
Secondary outcomes				
RRT Use	6 (3.4)	0 (0)	0 (0)	–
Mortality	12 (6.7)	4 (4.17)	3 (9.7)	OR 2.46 (0.5-11.7; $p = .47$)
				RR 2.32 (0.6-9.8; $p = .47$)
ICU LOS (days)	5 (3-11)	4 (3-9)	10 (4-15)	$p = .002$
Hospital LOS (days)	14 (8-30)	12 (7-23)	24 (11-48)	$p = .002$

Data for categorical variables are n(%) and for continuous variables are median (IQR); odds ratios (OR) and relative risks (RR) are presented as OR/RR (95% CI; *p*-value).
AKI- acute kidney injury; NPV- negative predictive value; PPV- positive predictive value; FO- fluid overload; RRT- renal replacement therapy; LOS- length of stay.

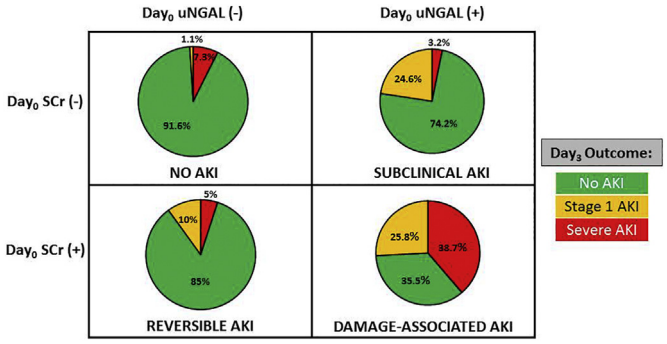

Fig. 2. Day-3 AKI phenotypes predicted by Day_0 biomarker-based classification. uNGAL+ identifies unique subsets of patients with higher risk of Day-3 AKI, regardless of SCr status.

value and PRISM-III score. Only uNGAL was a significant predictor of ICU LOS ($\beta = 4.480$, $p \leq .001$), while PRISM-III was not ($\beta = 0.0697$, $p = .289$). The overall mode fil was $R^2 = 0.110$. Similar results were seen for hospital LOS, as uNGAL was a significant predictor of longer LOS ($\beta = 16.412$, $p = .014$), and again, PRISM-III was not ($\beta = -0.0480$, $p = .903$). The overall model fit for hospital LOS was $R^2 = 0.0363$. To assess if SCr— the current gold standard for AKI diagnosis— impacted LOS in a significant manner, univariate linear regression was performed. Unlike uNGAL, admission SCr was not a significant predictor of either ICU LOS ($\beta = 1.430$, $p = .225$) or hospital LOS ($\beta = 8.543$, $p = .207$), with overall model fits of $R^2 = 0.00835$ and $R^2 = 0.00909$, respectively.

4. Discussion

Our data demonstrate that prediction of AKI severity on Day 3 can be made more precise via the integration of an AKI biomarker with SCr. Utilization of a combination biomarker-based approach can delineate unique patient classifications which ultimately predict distinct Day-3 AKI severity phenotypes, each with their own associated morbidity profile. Although SCr-based AKI on admission identifies patients at risk for Day-3 AKI and its associated sequelae, that risk *changes* based on the tubular biomarker classification. Notably, a negative tubular damage biomarker (i.e. uNGAL-/SCr+) identifies a group of patients likely to have transient, or reversible, AKI. The limited data to-date combining AKI biomarkers with functional markers of kidney injury (i.e. SCr) for better diagnostic and prognostic precision has primarily reported on adult and pediatric post-bypass populations [12,17-20]. Our study demonstrates that even in a heterogeneous population of critically ill patients, this construct can be operationalized to risk stratify patients for the likelihood of AKI and poor outcomes. Finally, our work highlights a potential

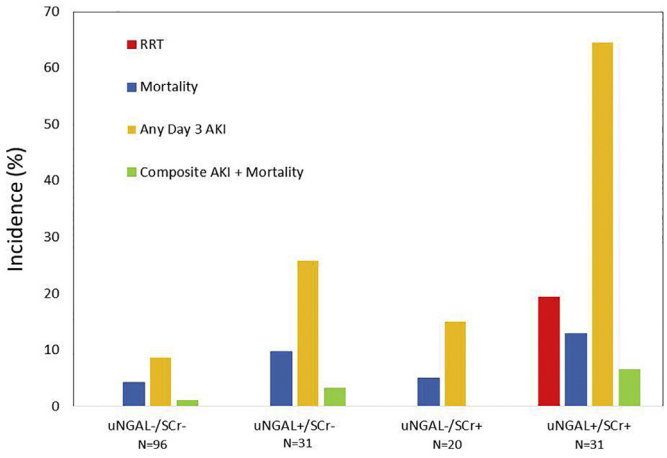

Fig. 3. Incidence of RRT, mortality, AKI_A, and a composite of AKI_A + mortality by admission biomarker-based classification.

Table 4
Comparison of admission uNGAL+ to uNGAL-.

	All	uNGAL +	uNGAL−	Comparison uNGAL+ to uNGAL−
Demographics				
N	178	62	116	–
Age (years)	6.7 (2.7-14)	6.5 (1.9-15)	7.1 (2.9-14)	$p = .71$
Male	92 (51.7)	32 (51.6)	60 (51.7)	$p = .89$
Sepsis	33 (24.2)	26 (41.9)	17 (14.7)	$p \leq .001$
PRISM III score	7 (3-13)	11 (6-17)	5 (2-11)	$p \leq .001$
Day 3 AKI outcomes				
Day 3 AKI				
Any AKI	39 (21.9)	28 (45.2)	11 (9.5)	OR 7.86 (3.54-17.5; $p \leq .001$)
Severe AKI	15 (8.4)	13 (20.6)	2 (1.7)	OR 15.1 (3.3-69.6; $p \leq .001$)
Day 3 FO	21 (11.8)	14 (22.6)	7 (6)	OR 4.54 (1.7-12; $p = .003$)
Secondary outcomes				
RRT use	6 (3.4%)	6 (9.7)	0	OR + inf ($p = .003$)
Mortality	12 (6.7%)	7 (11.3)	5 (4.3)	OR 2.86 (0.86-9.3; $p = .15$)
ICU LOS (days)	5 (3-11)	8 (4-16)	4 (3-9)	$p = <0.001$
Hospital LOS (days)	14 (8-30)	23.5 (10-47)	12 (7-23)	$p \leq 0.001$

Data for categorical variables are n(%) and for continuous variables are median (IQR); odds ratios (OR) are presented as OR/RR (95% CI; *p*-value).
AKI- acute kidney injury; FO- fluid overload; RRT- renal replacement therapy; LOS- length of stay.

significance of a positive biomarker in the absence of SCr elevation (i.e. uNGAL+/SCr-), a proposed state of subclinical AKI that appears to be associated with worse outcomes compared to injury biomarker negative patients.

Prediction of D_3 AKI severity may be advantageous for acute management. Kidney dysfunction persisting beyond 48 h is known to be associated with increased morbidity and mortality in both adults and children [1,13,21-23], and as such, those at risk warrant early recognition. The desire for the early detection of these patients is heightened by the fact that there are data supporting the efficacy of early, proactive therapies to improve outcomes in those at risk [24-26]. With this in mind, modeling adjuncts such as the renal angina index (RAI) have been derived and validated to identify high-risk patients in whom confirmatory biomarker testing is optimally used [15,27-29]. Our data suggest that biomarker-based classifications can also be used on the day of admission to identify patients likely to have a severe AKI phenotype. Therefore, combining these two strategies may be the next logical step, allowing for further refinement of the granularity of AKI prediction. For example, this approach to AKI severity prognostication could allow for the detection of patients at highest risk for needing RRT, as the only patients in our study who required this level of care were classified as uNGAL+/SCr + on the day of admission. Identifying these patients early could prove useful given the ongoing debate surrounding the optimal timing of RRT initiation [30,31], with some evidence suggesting earlier RRT improves outcomes [32].

The prediction of reversible renal dysfunction could also impact clinical management. In our study, patients classified as uNGAL-/SCr + on admission had significantly lower odds for all D_3 AKI outcomes when compared to biomarker positive patients, again highlighting the importance of operationalizing the recommendations of ADQI 10 in concert with the AKI timeline delineated in ADQI 16. The elevation of SCr in isolation, therefore, is an indiscriminate finding that gains more specificity — including the ability to predict the clinically relevant D_3 AKI severity— with the incorporation of a tubular damage marker. For example, previously expected elevations of SCr seen post-operatively can now be objectively categorized as reversible changes (i.e. injury biomarker negative), or changes associated with tubular injury that are likely to

be persistent (i.e. injury biomarker positive). Accurate recognition of lower-risk patients may enable providers to identify a unique therapeutic target– or in this case, a non-target– as their likelihood of progressing to persistent AKI and requiring intervention is low. This information could allow for the delivery of higher value care, as unnecessary and potentially costly interventions could be avoided in this low risk population.

Tubular injury without concurrent evidence of decreased glomerular filtration may represent a state of subclinical AKI. This state of biomarker positivity in the absence of associated changes in kidney function (i.e. SCr rise) has been previously described but remains limited in study [11,17,18,20]. Our data indicate that these patients had higher rates of Day-3 AKI and associated poor outcomes when compared to biomarker negative patients. Importantly, patients with subclinical AKI were also prevalent, representing almost one quarter of the patients without SCr elevation at time of PICU admission, and would be missed by the current gold standard AKI definitions. Although the significance of this state is unknown, because uNGAL has been shown in both animal models and patients post-bypass (i.e. patients in whom exact time of injury is known) to rise early in response to kidney injury [9,10,33,34], subclinical AKI has been proposed to be an early subtype of AKI (13). Regardless of the exact pathophysiology of these patients, given the trend towards poor outcomes in patients with *and* without Day-3 AKI, our data suggest that this cohort is clinically significant and important to detect.

We propose that the use of these biomarker-based classifications for AKI severity prediction could allow for proactive, and possibly more effective, interventions for critically ill patients. This is supported by existing evidence suggesting that early recognition of post-surgical patients at high risk for AKI followed by the implementation of standardized KDIGO management bundles can decrease AKI disease burden [24,25]. The electronic health record (EHR) could be used to communicate these risk categories to providers, as several studies have already demonstrated its ability to detect patients at high risk for AKI and provide appropriate clinical decision support to improve outcomes [35-42]. Similarly, biomarker-based classifications could be easily integrated into the EHR, alerting providers to subsets of patients who may benefit from early, targeted therapies (i.e. uNGAL+ patients), and identifying those who may not require intervention at all (i.e. uNGAL-). Finally, delineation of AKI subtypes in this way may allow for predictive enrichment that could inform patient selection for future interventional trials for AKI, possibly increasing the likelihood of positive results.

Our study operationalizes the directives of the ADQI consensus panel to combine a novel AKI biomarker (in this case, uNGAL) with SCr changes to predict AKI severity on Day 3 in a heterogeneous group of critically ill patients. Previous work in this area has focused on the homogeneous post-bypass population. However, our work does have limitations. This was a small, single center cohort which may not be generalizable to other populations of critically ill children. Furthermore, uNGAL was measured in all patients but was not utilized by providers in clinical decision making; as such, while we are suggesting that using this information early in admission may improve patient outcomes and lead to more cost-effective care, our study does not provide direct evidence for that claim. These preliminary data support further study in larger populations.

Acknowledgements

We would like to acknowledge Dr. Michael Bennett, PhD, for his work processing samples for the original study performed by Dr. Menon.

Declarations of interest

RKB is a consultant for Bioporto Diagnostics. SLG serves as a consultant and receives grant funding from BioPorto Diagnostics, Inc., which has licensed NGAL.

Funding

The original data collection was performed by Dr. Menon during her Acute Care Nephrology and Dialysis Fellowship at CCHMC, which was funded in part by Gambro Renal Products, Inc. Urinary biomarker processing for the original data set was supported by a Nephrology Center of Excellence Grant (P50 DK096418-01, Devarajan).

Conflict of interest statement

Both RKB and SLG are consultants for BioPorto Diagnostics, Inc., which has licensed NGAL.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcrc.2019.05.017.

References

[1] Kaddourah A, Basu RK, Bagshaw SM, Goldstein SL, AWARE Investigators. Epidemiology of acute kidney injury in critically ill children and young adults. N Engl J Med 2017;376:11–20. https://doi.org/10.1056/NEJMoa1611391.
[2] Hoste EAJ, Bagshaw SM, Bellomo R, Cely CM, Colman R, Cruz DN, et al. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. Intensive Care Med 2015;41:1411–23. https://doi.org/10.1007/s00134-015-3934-7.
[3] Jetton JG, Boohaker LJ, Sethi SK, Wazir S, Rohatgi S, Soranno DE, et al. Incidence and outcomes of neonatal acute kidney injury (AWAKEN): a multicentre, multinational, observational cohort study. Lancet Child Adolesc Health 2017;1:184–94. https://doi.org/10.1016/S2352-4642(17)30069-X.
[4] Silver SA, Chertow GM. The economic consequences of acute kidney injury. NEF 2017;137:297–301. https://doi.org/10.1159/000475607.
[5] Silver SA, Long J, Zheng Y, Chertow GM. Cost of acute kidney injury in hospitalized patients. J Hosp Med 2017;12:70–6. https://doi.org/10.12788/jhm.2683.
[6] Siew ED, Ware LB, Ikizler TA. Biological markers of acute kidney injury. J Am Soc Nephrol 2011;22:810–20. https://doi.org/10.1681/ASN.2010080796.
[7] Doi K, Yuen PST, Eisner C, Hu X, Leelahavanichkul A, Schnermann J, et al. Reduced production of creatinine limits its use as marker of kidney injury in sepsis. J Am Soc Nephrol 2009;20:1217–21. https://doi.org/10.1681/ASN.2008060617.
[8] Waikar SS, Bonventre JV. Creatinine kinetics and the definition of acute kidney injury. J Am Soc Nephrol 2009;20:672–9. https://doi.org/10.1681/ASN.2008070669.
[9] Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol 2003;14:2534–43.
[10] Singer E, Markó L, Paragas N, Barasch J, Dragun D, Müller DN, et al. Neutrophil gelatinase-associated lipocalin: pathophysiology and clinical applications. Acta Physiol (Oxf) 2013;207:663–72. https://doi.org/10.1111/apha.12054.
[11] Ronco C, Kellum JA, Haase M. Subclinical AKI is still AKI. Crit Care 2012;16:313. https://doi.org/10.1186/cc11240.
[12] McCullough PA, Bouchard J, Waikar SS, Siew ED, Endre ZH, Goldstein SL, et al. Implementation of novel biomarkers in the diagnosis, prognosis, and management of acute kidney injury: executive summary from the tenth consensus conference of the Acute Dialysis Quality Initiative (ADQI). Contrib Nephrol 2013;182:5–12. https://doi.org/10.1159/000349962.
[13] Chawla LS, Bellomo R, Bihorac A, Goldstein SL, Siew ED, Bagshaw SM, et al. Acute kidney disease and renal recovery: consensus report of the Acute Disease Quality Initiative (ADQI) 16 Workgroup. Nat Rev Nephrol 2017;13:241.
[14] Zappitelli M, Parikh CR, Akcan-Arikan A, Washburn KK, Moffett BS, Goldstein SL. Ascertainment and epidemiology of acute kidney injury varies with definition interpretation. Clin J Am Soc Nephrol 2008;3:948–54. https://doi.org/10.2215/CJN.05431207.
[15] Basu RK, Wang Y, Wong HR, Chawla LS, Wheeler DS, Goldstein SL. Incorporation of biomarkers with the renal angina index for prediction of severe AKI in critically ill children. Clin J Am Soc Nephrol 2014;9:654–62. https://doi.org/10.2215/CJN.09720913.
[16] Wheeler DS, Devarajan P, Ma Q, Harmon K, Monaco M, Cvijanovich N, et al. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. Crit Care Med 2008;36:1297–303. https://doi.org/10.1097/CCM.0b013e318169245a.
[17] Haase M, Devarajan P, Haase-Fielitz A, Bellomo R, Cruz DN, Wagener G, et al. The outcome of neutrophil gelatinase-associated lipocalin-positive subclinical acute kidney injury: a multicenter pooled analysis of prospective studies. J Am Coll Cardiol 2011;57:1752–61. https://doi.org/10.1016/j.jacc.2010.11.051.
[18] Albert C, Albert A, Kube J, Bellomo R, Wettersten N, Kuppe H, et al. Urinary biomarkers may provide prognostic information for subclinical acute kidney injury after cardiac surgery. J Thorac Cardiovasc Surg 2018;155:2441–2452.e13. https://doi.org/10.1016/j.jtcvs.2017.12.056.
[19] Basu RK, Wong HR, Krawczeski CD, Wheeler DS, Manning PB, Chawla LS, et al. Combining functional and tubular damage biomarkers improves diagnostic precision for

acute kidney injury after cardiac surgery. J Am Coll Cardiol 2014;64:2753–62. https://doi.org/10.1016/j.jacc.2014.09.066.
[20] Fang F, Hu X, Dai X, Wang S, Bai Z, Chen J, et al. Subclinical acute kidney injury is associated with adverse outcomes in critically ill neonates and children. Crit Care 2018;22:256. https://doi.org/10.1186/s13054-018-2193-8.
[21] Sood MM, Shafer LA, Ho J, Reslerova M, Martinka G, Keenan S, et al. Early reversible acute kidney injury is associated with improved survival in septic shock. J Crit Care 2014;29:711–7. https://doi.org/10.1016/j.jcrc.2014.04.003.
[22] Brown JR, Kramer RS, Coca SG, Parikh CR. Duration of acute kidney injury impacts long-term survival after cardiac surgery. Ann Thorac Surg 2010;90:1142–8. https://doi.org/10.1016/j.athoracsur.2010.04.039.
[23] Mehta S, Chauhan K, Patel A, Patel S, Pinotti R, Nadkarni GN, et al. The prognostic importance of duration of AKI: a systematic review and meta-analysis. BMC Nephrol 2018;19:91. https://doi.org/10.1186/s12882-018-0876-7.
[24] Meersch M, Schmidt C, Hoffmeier A, Van Aken H, Wempe C, Gerss J, et al. Prevention of cardiac surgery-associated AKI by implementing the KDIGO guidelines in high risk patients identified by biomarkers: the PrevAKI randomized controlled trial. Intensive Care Med 2017;43:1551–61. https://doi.org/10.1007/s00134-016-4670-3.
[25] Göcze I, Jauch D, Götz M, Kennedy P, Jung B, Zeman F, et al. Biomarker-guided intervention to prevent acute kidney injury after major surgery: the prospective randomized BigpAK study. Ann Surg 2018;267:1013–20. https://doi.org/10.1097/SLA.0000000000002485.
[26] Schanz M, Wasser C, Allgaeuer S, Schricker S, Dippon J, Alscher MD, et al. Urinary [TIMP-2]·[IGFBP7]-guided randomized controlled intervention trial to prevent acute kidney injury in the emergency department. Nephrol Dial Transplant 2018. https://doi.org/10.1093/ndt/gfy186.
[27] Basu RK, Chawla LS, Wheeler DS, Goldstein SL. Renal angina: an emerging paradigm to identify children at risk for acute kidney injury. Pediatr Nephrol 2012;27:1067–78. https://doi.org/10.1007/s00467-011-2024-5.
[28] Basu RK, Zappitelli M, Brunner L, Wang Y, Wong HR, Chawla LS, et al. Derivation and validation of the renal angina index to improve the prediction of acute kidney injury in critically ill children. Kidney Int 2014;85:659–67. https://doi.org/10.1038/ki.2013.349.
[29] Menon S, Goldstein SL, Mottes T, Fei L, Kaddourah A, Terrell T, et al. Urinary biomarker incorporation into the renal angina index early in intensive care unit admission optimizes acute kidney injury prediction in critically ill children: a prospective cohort study. Nephrol Dial Transplant 2016;31:586–94. https://doi.org/10.1093/ndt/gfv457.
[30] Wald R, Adhikari NKJ, Smith OM, Weir MA, Pope K, Cohen A, et al. Comparison of standard and accelerated initiation of renal replacement therapy in acute kidney injury. Kidney Int 2015;88:897–904. https://doi.org/10.1038/ki.2015.184.
[31] Yang X-M, Tu G-W, Zheng J-L, Shen B, Ma G-G, Hao G-W, et al. A comparison of early versus late initiation of renal replacement therapy for acute kidney injury in critically ill patients: an updated systematic review and meta-analysis of randomized controlled trials. BMC Nephrol 2017;18:264. https://doi.org/10.1186/s12882-017-0667-6.
[32] Zarbock A, Kellum JA, Schmidt C, Van Aken H, Wempe C, Pavenstädt H, et al. Effect of early vs delayed initiation of renal replacement therapy on mortality in critically ill patients with acute kidney injury: the ELAIN randomized clinical trial. JAMA 2016;315:2190–9. https://doi.org/10.1001/jama.2016.5828.
[33] Kaucsár T, Godó M, Révész C, Kovács M, Mócsai A, Kiss N, et al. Urine/plasma neutrophil gelatinase associated lipocalin ratio is a sensitive and specific marker of subclinical acute kidney injury in mice. PLoS ONE 2016;11:e0148043. https://doi.org/10.1371/journal.pone.0148043.
[34] Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 2005;365:1231–8. https://doi.org/10.1016/S0140-6736(05)74811-X.
[35] Kirkendall ES, Spires WL, Mottes TA, Schaffzin JK, Barclay C, Goldstein SL. Development and performance of electronic acute kidney injury triggers to identify pediatric patients at risk for nephrotoxic medication-associated harm. Appl Clin Inform 2014;5:313–33. https://doi.org/10.4338/ACI-2013-12-RA-0102.
[36] Goldstein SL, Kirkendall E, Nguyen H, Schaffzin JK, Bucuvalas J, Bracke T, et al. Electronic health record identification of nephrotoxin exposure and associated acute kidney injury. Pediatrics 2013;132:e756–67. https://doi.org/10.1542/peds.2013-0794.
[37] Goldstein SL, Mottes T, Simpson K, Barclay C, Muething S, Haslam DB, et al. A sustained quality improvement program reduces nephrotoxic medication-associated acute kidney injury. Kidney Int 2016;90:212–21. https://doi.org/10.1016/j.kint.2016.03.031.
[38] Selby NM, Crowley L, Fluck RJ, McIntyre CW, Monaghan J, Lawson N, et al. Use of electronic results reporting to diagnose and monitor AKI in hospitalized patients. Clin J Am Soc Nephrol 2012;7:533–40. https://doi.org/10.2215/CJN.08970911.
[39] Park S, Baek SH, Ahn S, Lee K-H, Hwang H, Ryu J, et al. Impact of electronic acute kidney injury (AKI) alerts with automated nephrologist consultation on detection and severity of AKI: a quality improvement study. Am J Kidney Dis 2018;71:9–19. https://doi.org/10.1053/j.ajkd.2017.06.008.
[40] Porter CJ, Juurlink I, Bisset LH, Bavakunji R, Mehta RL, Devonald MAJ. A real-time electronic alert to improve detection of acute kidney injury in a large teaching hospital. Nephrol Dial Transplant 2014;29:1888–93. https://doi.org/10.1093/ndt/gfu082.
[41] Chertow GM, Lee J, Kuperman GJ, Burdick E, Horsky J, Seger DL, et al. Guided medication dosing for inpatients with renal insufficiency. JAMA 2001;286:2839–44.
[42] Colpaert K, Hoste EA, Steurbaut K, Benoit D, Van Hoecke S, De Turck F, et al. Impact of real-time electronic alerting of acute kidney injury on therapeutic intervention and progression of RIFLE class. Crit Care Med 2012;40:1164–70. https://doi.org/10.1097/CCM.0b013e3182387a6b.