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Original Article

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

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ABSTRACT

Background. The inconsistent ability of novel biomarkers to predict acute kidney injury (AKI) across heterogeneous patients and illnesses limits integration into routine practice. We previously retrospectively validated the ability of the renal angina index (RAI) to risk-stratify patients and provide context for confirmatory serum biomarker testing for the prediction of severe AKI.

Methods. We conducted this first prospective study of renal angina to determine whether the RAI on the day of admission (Day$_0$) risk-stratified critically ill children for 'persistent, severe AKI' on Day 3 (Day$_3$-AKI: KDIGO Stage 2–3) and whether incorporation of urinary biomarkers in the RAI model optimized AKI prediction.

Results. A total of 184 consecutive patients (52.7% male) were included. Day$_0$ renal angina was present (RAI ≥8) in 60 (32.6%) patients and was associated with longer duration of mechanical ventilation (P = 0.04), higher number of organ failure days (P = 0.003) and increased mortality (P < 0.001) than in patients with absence of renal angina. Day$_3$-AKI was present in 15/156 (9.6%) patients; 12/15 (80%) fulfilled Day$_0$ renal angina. Incorporation of urinary biomarkers into the RAI model increased the specificity and positive likelihood, and demonstrated net reclassification improvement (P < 0.001) for the prediction of Day$_3$-AKI. Inclusion of urinary neutrophil gelatinase-associated lipocalin increased the area under the curve receiver-operating characteristic of RAI for Day$_3$-AKI from 0.80 [95% confidence interval (CI): 0.58, 1.00] to 0.97 (95% CI: 0.93, 1.00).

Conclusions. We have now prospectively validated the RAI as a functional risk stratification methodology in a heterogeneous group of critically ill patients, providing context to direct measurement of novel urinary biomarkers and improving the prediction of severe persistent AKI.

Keywords: acute kidney injury, biomarkers, critical care, risk stratification, renal angina index

INTRODUCTION

Acute kidney injury (AKI) occurs frequently in critically ill patients and is independently associated with high rates of morbidity and mortality. In a recent meta-analysis of over 3.5 million patients, the incidence of AKI was 21.6% (one in five) in adults and 33.7% in children (one in three), with mortality rates of 23.9 and 13.8%, respectively [1]. Since the adoption of severity-based classifications of injury [2–6], there has been an increasing awareness of the danger of AKI and the associated negative clinical impact of a kidney 'attack' [7, 8]. Unfortunately, no singular effective therapy for AKI has been developed and management consists largely of supportive care [9]. The Kidney Diseases: Improving Global Outcomes (KDIGO) working

group recently described a stage-based best practice AKI management algorithm, notably highlighting recommendations after recognition of a patient with subclinical AKI or AKI risk [4].

Prediction of AKI or risk-stratifying patients in danger of kidney damage offers the opportunity to initiate measures for AKI prevention. Creatinine-based detection strategies, limited in efficacy by time-lag in response to injury and variability with body mass and gender, are particularly problematic in the pediatric population [10]. Novel biomarkers have demonstrated superior performance for the prediction of AKI compared with early, non-stratified changes in serum creatinine (SCr). Validated following cardiopulmonary bypass (CPB) in children, urinary neutrophil gelatinase-associated lipocalin (uNGAL), kidney injury molecule-1 (uKIM-1), interleukin 18 (uIL-18), liver type-fatty acid binding protein (uL-FABP) and cell cycle markers insulin-like growth factor binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases-2 (TIMP-2) detect AKI prior to the functional change heralded by an SCr rise [11–14]. The pediatric CPB population, however, represents an isolated and unique cohort in which to validate AKI biomarkers, as most patients are without other acute illness, have a known time and duration of insult, and are rigorously monitored during and following injury. Unfortunately, a decade after the seminal report describing the exceptional discrimination of urine and serum NGAL for AKI following CPB [15], and despite growing support from the critical care nephrology community [16], AKI biomarkers have yet to be incorporated into routine practice. The reticence to acceptance of the biomarkers may reflect the inconsistent performance of these biomarkers for the prediction of AKI outside of the cardiac

surgery population [17]. In children and adults, a majority of area under the curve receiver-operating characteristic (AUC-ROC) values of novel biomarkers for the prediction of AKI in the non-cardiac surgery population are between 0.6 and 0.8 [17]. Newer biomarkers reflecting injury to different parts of the nephron and injury of different types (i.e. cell cycle arrest) may have potential for utility in the broader population of critically ill patients, and demonstrate superior performance (AUC ~0.9) [18]. Still, even the most widely studied novel biomarker, NGAL, demonstrates inconsistent performance depending on the population tested [e.g. adults in the emergency department, children with sepsis, adult intensive care unit (ICU) patients] [19–22].

Context-driven testing enhances biomarker utility. Cardiac–troponin I (cTi) has a high false positive rate for acute coronary syndrome (ACS) when tested in patients without chest pain and risk factors for heart disease [23]. Akin to heart attack, kidney attack carries a prodrome, providing context for novel biomarker testing [8, 24]. We previously derived and validated the renal angina model to stratify patients by risk of persistent, severe AKI [25]. Modeled on the empiric concept of organ 'torment, suffering, injury' (from the etymology of ankhone or angina), renal angina describes a state of kidney injury identifiable using the renal angina index (RAI). The RAI is an easily calculable measure of renal angina combining objective signs of kidney dysfunction and patient context (i.e. risk factors for AKI) (Figure 1). In a convenience sample population studied retrospectively, the RAI demonstrated an above-average AUC-ROC for the prediction of severe and persistent AKI (0.80), improving significantly with the incorporation of a positive serum AKI biomarker (0.84–0.88) [26]. We now present data from the first

AKI Risk Tranche

Risk Factor	Risk Tranche	Risk Score
ICU Admission	Medium	1
History of Transplantation (Solid Organ or Bone Marrow)	High	3
Vasoactive Support & Mechanical Ventilation	Very High	5

$$X \qquad = \text{Renal Angina Index (Range 1-40)}$$

AKI Injury Tranche

Change in Creatinine	Fluid Overload %	Injury Score
<0	< 0 − 5%	1
1.0 − 1.49x	5 − 9.99%	2
1.5 − 1.99x	10 − 14.99%	4
> 2x	≥ 15%	8

FIGURE 1: The RAI: based on existing pediatric AKI literature, tiered AKI risk strata were assigned point values for 'risk' and 'signs' of injury. The worse parameter between change in estimated creatinine clearance from baseline and %FO was used to yield an injury score. The resultant RAI score can range from 1 to 40. A cutoff of >8 is used to determine renal angina fulfillment (from Basu et al. [25] with permission). ppCRRT, prospective pediatric continuous renal replacement therapy registry.

prospective study of renal angina testing the hypothesis that incorporation of urinary biomarkers in patients who 'rule in' for renal angina (RAI ≥8) results in robust risk stratification for the development of severe, persistent AKI.

MATERIALS AND METHODS

Patients

This investigation was approved by the Institutional Review Board at Cincinnati Children's Hospital Medical Center (CCHMC) with waiver of the need for informed consent. The Acute Kidney Injury in CHildren Expected by Renal angina and Urinary Biomarkers (AKI-CHERUB, NCT01735162) was a prospective observational study conducted in the CCHMC pediatric ICU (PICU) from September 2012 to March 2014. Study procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

We prospectively enrolled all children admitted to the PICU from the ages of 3 months to 25 years, who had a predicted discharge >48 h from PICU admission, and who had a urinary catheter placed by time of first scheduled urine collection and in place for at least first 48 h of PICU admission. Patients with a predicted discharge after 48 h were identified in the admission progress note (prediction discharge date is estimated by the attending ICU provider as part our PICU daily clinical routine). Patients with history of end-stage renal disease and those immediately following renal transplant were excluded from the study. The CCHMC PICU is a tertiary care facility, admitting critically ill patients from all medical subspecialties, following elective and emergent surgery and following trauma, and from one of the busiest pediatric emergency department in North America.

Data Timing. Data were collected from first calendar day of PICU admission (Day_0) for seven consecutive days until 7 days after admission (Day_7). A minimum of 8 h from PICU admission constituted Day_0. Urinary biomarkers were assessed the day after PICU admission, between 12 and 24 h after time of admission (Day_1). Day_3 consisted of the time period between 72 and 96 h after PICU admission.

Collected variables. Demographic information, admission diagnoses, comorbidities, height, weight, available laboratory values, vital signs and Pediatric Risk of Mortality III score (PRISM-III) [27] were collected at the time of admission. Baseline SCr was established as the lowest creatinine up to 3 months before PICU admission on Day_0. Daily collected variables were assessed at 8:00 am for each patient on $Days_{1-7}$ and included vital signs, laboratory values, nephrotoxin use, vasopressor use, mechanical ventilation support level and total ICU fluid intake and output.

Calculated variables and RAI. The RAI score was determined between 8 and 12 h from the time of PICU admission on Day_0 as previously described [25]. Determination of Day_0-RAI required the calculation of percent fluid overload (%FO) [28] from ICU admission and change in creatinine

from baseline. If no baseline SCr was available in the computer system, a reference estimated creatinine clearance of 120 mL/min/m^2 was used and a baseline creatinine was imputed using the patient's height in centimeters [29]. An RAI ≥8 was considered fulfillment of renal angina [25]. Fulfillment or the absence of renal angina was denoted 'RA+' or 'RA−'.

Net ICU fluid balance was determined using simple subtraction of total fluid balance during ICU course. Urine output was calculated as mL/kg/h in 8 h blocks using PICU admission weight.

Outcomes

The primary outcome was the presence of severe AKI on Day_3 (Day_3-AKI). This persistent, severe AKI was classified as KDIGO AKI Stage 2 or 3 by change in SCr from baseline, consistent with our previous retrospective publications. Day_3 was chosen since most PICU patients develop AKI within this timeframe, the time highlights the advantage of biomarkers on the PICU admission day to predict the outcome; in addition, Day_3 is a clinically relevant time frame for AKI management. Secondary outcomes included Day_7 and final kidney-specific organ dysfunction, including %FO, the presence of AKI and use of renal replacement therapy (RRT). Tertiary outcomes included ICU-specific endpoints such as duration of mechanical ventilation, length of stay (LOS), organ failure days and incidence of mortality.

Urine collection and biomarker analysis

Urine samples were collected on all enrolled patients. Urine was centrifuged and stored in aliquots at −80°C until measurement. Day_1 biomarker values were used for the purposes of this initial analysis (urine obtained from $Days_{1-4}$ when possible). uNGAL was assayed using a human-specific commercially available enzyme-linked immunosorbent assay (ELISA, AntibodyShop, Grusbakken, Denmark). uIL-18 and uL-FABP were measured using commercially available ELISA kits (Medical and Biological Laboratories Co., Nagoya, Japan and CMIC Co., Tokyo, Japan, respectively) as per manufacturer's instructions. The uKIM-1 ELISA was constructed using commercially available reagents (R&D Systems, Inc., Minneapolis, MN, USA). Optimal cutoff values for biomarkers were determined using sensitivity analysis and Youden's index for the prediction of Day_3-AKI (for each biomarker tested individually) [26, 30].

Statistical analysis

All statistical analyses were performed using SigmaStat version 12.3 software (San Antonio, TX, USA), SAS version 9.2 (SAS Institute, Cary, NC, USA) and R version 2.14.1 (R Development Core Team, Vienna, Austria). R packages pROC and PredictABEL were used [31, 32]. Continuous variables were reported as median with interquartile range and compared using the Mann–Whitney test. Categorical variables were summarized using frequency and proportion and compared by χ^2 or Fisher's exact tests. An RAI cutoff of ≥8 was used to define renal angina fulfillment (RA+) and this cutoff was used for operative characteristics [25]. Simple and multivariable logistic models were used to risk-stratify patients for Day_3-AKI using RAI and its combinations with AKI biomarker(s)

concentration levels for the prediction of Day_3-AKI. AUC-ROC values were calculated for each prediction model (RAI and biomarker concentrations used as continuous variables) and compared using DeLong's method [33]. The Akaike Information Criterion (AIC), net reclassification improvement (NRI) and integrated discrimination improvement (IDI) quantified additional changes in discrimination after incorporation of AKI biomarkers [34, 35]. No risk categories were selected for the calculation of NRI. R package Hmisc was used for the calculation of NRI and IDI. Sample size was initially projected in multiple ways. In designing the study, we determined size based on the requirements of a reliable logistic model. *A priori*, we assumed four variables would be included in a comparative prediction model—the RAI index, age, PRISM-II score and the presence of sepsis. Ten events per variable means we needed 40 events (patients with AKI). Assuming a 10% incidence rate based on existing epidemiology reports, 400 patients needed to be enrolled. On initiation of the study, we had already begun to plan the multi-center AWARE study (NCT01987921) and realized we would not have 2 years to complete this pilot study. Based on our previous studies of RAI, we estimated that between 150 and 200 patients provided a large enough sample size to perform our analysis. In all analyses, a P-value of <0.05 was considered statistically significant.

RESULTS

Patients

A total of 184 consecutive patients were enrolled for study and all were used to derive demographic and outcome data; 156 (84.7%) remained in the ICU on Day_3. Their data were used for Day_3-AKI analyses. Of the 156, Day_1 urinary biomarkers were measured in 114 patients representing 62% of the original sample (114/184).

Renal angina occurs frequently in a heterogeneous patient population and is associated with poor outcome

Approximately one in three patients (32.6%) was RA+ within the first 12 h of PICU admission. These patients were younger, had a higher prevalence of sepsis or history of transplant, and higher PRISM-III scores (Table 1). Additionally, RA+ on Day_0 was associated with a longer duration of mechanical ventilation, greater number of organ failure days and higher mortality.

Fulfillment of renal angina on admission is associated with an increased incidence of subsequent, severe AKI

In the 156 patients remaining in the ICU on Day_3, 52 (33.3%) were RA+ on Day_0. Day_3-AKI incidence was 9.6% (15/156); 12/15 (80%) of Day_0 RA+ were AKI+ on Day_3 (Table 2). Day_0 RA+ status was associated with a greater incidence of Day_3-AKI than Day_0 RA− status (23.1 versus 2.9%, χ^2 = 16.3, P < 0.001). Additionally, Day_0 RA+ patients carried significantly greater positive net fluid balance and higher %FO on Day_3 than RA− patients.

The RAI is independently associated with risk of Day_3 – AKI

Multivariate logistic regression performed on the univariate day of admission variables associated with Day_3-AKI identifies high odds ratios for severe injury with higher severity of illness score (PRISM-III), the presence of sepsis, history of transplant and renal angina on admission (Table 3).

Table 1. Demographics and outcomes of patients stratified by Day_0 renal angina fulfillment

	Category	Overall	RA−	RA+	P-value (RA+ versus RA−)
Demographics	n (%)	184	124 (67.4)	60 (32.6)	—
	Male (%)	98 (52.7)	63 (50.8)	34 (56.7)	ns
	Age on admission (years)	7.7 (2.7, 14.9)	11.1 (3.1, 15.9)	4.6 (1.9, 12.9)	0.03
	Weight (kg)	27.2 (13.4, 53.9)	31.4 (14.1, 54.9)	17.4 (11.9, 49.3)	0.06
	Height (cm)	127 (88, 158)	130 (93, 159)	106 (82, 152)	0.05
	BSA (m^2)	0.99 (0.57, 1.55)	1.05 (0.61, 1.55)	0.71 (0.52, 1.53)	0.06
	PRISM-III	7 (3, 13.8)	5 (2, 9)	14 (7.3, 21)	<0.001
	Admitted from				
	Surgical post-op	77 (41.2)	65 (52.4)	12 (20.0)	
	ED	53 (28.8)	30 (24.2)	23 (38.3)	
	General Peds	18 (9.6)	12 (9.7)	6 (10.0)	
	Transport	14 (7.5)	6 (4.8)	8 (13.3)	
	Liver/GI	16 (8.6)	7 (5.6)	9 (15.0)	
	Heme-Onc	4 (2.1)	4 (3.2)	0 (0)	
	BMT	2 (1.1)	0 (0)	2 (3.3)	
	Sepsis Dx (%)	45 (24.5)	20 (16.1)	25 (41.7)	χ^2 = 14.6, P < 0.001
	Transplant history (%)	21 (11.4)	6 (4.8)	15 (25)	χ^2 = 16.1, P < 0.001
Outcomes	Mechanical Vent days	2 (0, 6)	1 (0, 5)	3 (1, 6.8)	0.04
	CRRT use (%)	6 (3.3)	1 (0.8)	5 (8.3)	χ^2 = 6.9, P = 0.009
	Organ failure days	2 (0, 7)	1 (0, 6)	4 (1, 8)	0.003
	Hospital LOS (days)	14 (8, 30)	14 (8, 29)	16 (8, 41)	0.08
	ICU LOS (days)	5 (3, 11)	4 (3, 11)	6 (4, 13)	0.19
	ICU mortality (%)	12 (6.5)	1 (0.8)	11 (18.3)	χ^2 = 17.1, P < 0.001

Patients in AKI-CHERUB dichotomized by the fulfillment or the absence of renal angina (RA+ or RA−) on the day of ICU admission. All values are expressed as medians with interquartile ranges except where designated. n, sample size; BSA, body surface area; ED, emergency department; General Peds, general pediatric ward; GI, gastrointestinal; Heme-Onc, hematology-oncology; BMT, bone marrow transplant unit; Dx, diagnosis; Vent, ventilation; CRRT, continuous renal replacement therapy; ns, not significant.

Table 2. Day$_3$ kidney health and ICU outcomes stratified by renal angina

	Category	All patients	Day$_0$ RA−	Day$_0$ RA+	P-value (RA− versus RA+)
Demographics	n (%)	156	104 (66.6)	52 (33.3)	—
	Male (%)	82 (52.6)	52 (50)	30 (57.7)	ns
	Age (years)	8.4 (2.7, 15.5)	11.7 (2.8, 16.0)	5 (2.0, 13.9)	0.13
	Day$_0$ PRISM-III	8 (3, 14)	5 (2, 10)	14 (8.3, 20)	<0.001
Day$_3$ kidney health	AKI by SCr-KDIGO 2–3	15 (9.6)	3 (2.9)	12 (23.1)	$\chi^2 = 16.3$, P < 0.001
	Net fluid balance (mL)	2311 (613, 4333)	1831 (485, 3904)	2768 (1040, 5026)	0.03
	%FO	9.3 (3.2, 15.3)	6.8 (2.6, 15.3)	11.8 (6.8, 15.7)	0.009
ICU outcomes	Mechanical Vent days	2 (0, 7)	1.5 (0, 6)	3.5 (1, 7.8)	0.05
	CRRT use (% group)	6 (3.8)	1 (1)	5 (9.6)	$\chi^2 = 4.8$, P = 0.03
	Organ failure days	3 (1, 8)	2 (0, 7)	6 (2, 8)	0.007
	Hospital LOS (days)	17 (8, 34)	16 (8, 29)	21 (9, 47)	0.20
	ICU LOS (days)	6 (3, 12)	5 (3, 11)	7 (4, 16)	0.19
	Mortality (% group)	9 (5.7)	1 (1)	8 (15.4)	$\chi^2 = 17.1$, P < 0.001

Patients in AKI-CHERUB remaining in the ICU on Day$_3$ of admission to the PICU dichotomized by the fulfillment or the absence of renal angina (RA+ or RA−) on the day of ICU admission (Day$_0$). All values are expressed as medians with interquartile ranges except where designated. n, sample size; AKI by SCr-KDIGO 2–3, AKI diagnosed by change in SCr from baseline as per the KDIGO staging criteria Stages 2–3; Vent, ventilation; CRRT, continuous renal replacement therapy; ns, not significant.

<div style="writing-mode: vertical-rl">ORIGINAL ARTICLE</div>

Day$_3$-AKI is associated with poor outcome in a heterogeneous population of critically ill children

Day$_3$-AKI was associated with ICU mortality (Table 4). Day$_3$-AKI increased duration of ICU LOS, prolonged mechanical ventilation and was associated with a greater number of organ failure days than the absence of AKI. Similar associations with worse longer term outcomes are seen when patients remaining in the ICU on Day$_3$ are dichotomized by Day$_0$ '±' RA− or '±' Day$_3$-AKI (Tables 2 and 4).

Incorporation of urinary biomarkers into the RAI model optimizes Day$_3$-AKI prediction

Biomarker incorporation into the RAI significantly increased the specificity for severe AKI on Day$_3$. Measured on the day after admission (Day$_1$), NGAL, KIM-1 and IL-18 increased the specificity and likelihood for severe AKI and also demonstrated NRI (Table 5). Notably, after inclusion of NGAL in the model, the AUC-ROC of Day$_0$-RAI to predict Day$_3$-AKI increased from 0.80 [95% confidence interval (CI): 0.58, 1.00] to nearly unity (0.97, 95% CI: 0.93, 1.00), decreased the AIC and demonstrated significant NRI (1.64, P < 0.001) and IDI (0.419, P = 0.02). Odds ratios for the development of Day$_3$-AKI were calculated for biomarkers in combination with the RAI for Day$_3$-AKI (Table 5) but for NGAL positivity in the context of renal angina fulfillment was not calculable because of a non-existent negative event rate. Similarly, no statistical analysis was possible on biomarker prediction of Day$_3$-AKI in patients without Day$_0$ renal angina given the low event rate.

DISCUSSION

In this study, we prospectively validate the concept that provision of clinical context to direct biomarker testing enhances the ability to predict clinically significant AKI in critically ill children. In our earlier, retrospective derivation and validation studies [25, 26], the advantage of the RAI methodology for AKI risk stratification was clear: the RAI is easily calculable

Table 3. Multivariate regression for Day$_3$ AKI

Criterion	Odds ratio	
	All Day$_0$ patients	Patients with Day$_3$ data
PRISM-III	1.12 (1.05, 1.19)	1.09 (1.04, 1.16)
Sepsis	7.1 (2.26, 22.3)	7.6 (2.44, 23.7)
Transplant history	8.62 (2.69, 27.6)	9.63 (3.04, 30.5)
Renal angina	10.1 (2.71, 37.7)	10.1 (2.7, 37.3)

Multivariate regression analysis for Day$_3$-AKI as predicted by the criteria with univariate association on admission (P < 0.10). Criterion included PRISM-III score, diagnosis of sepsis, history of transplant and the fulfillment of renal angina on Day$_0$. Results expressed with 95% CI.

(versus severity of illness scores), early (on ICU admission day) and offers comparable or superior AKI risk stratification versus traditional markers of illness or patient demographics. Another important difference from our previous retrospective analyses of RAI [26] is that CHERUB incorporated urinary biomarkers. Prospective testing in patients for the specific purpose of analyzing AKI prediction demonstrates incorporation of uNGAL into the RAI model predicts severe and persistent AKI with an AUC-ROC of 0.97. 'CHERUB' models a relevant, clinical time course: a critically ill child is admitted to the ICU, resuscitated and stabilized over a period of 8–12 h. The RAI can then be used to rule in risk of AKI, triggering measurement of a highly specific AKI biomarker for the prediction of subsequent severe AKI. The timing of this predictive model, within 8–12 h of admission for nearly all patients, could be used in a targeted trial examining AKI management involving hemodynamic interventions, nephrotoxic medications and targets for fluid balance (Figure 2).

The RAI risk stratifies patients for subsequent, severe AKI—an injury at a point in time of ICU admission with significant clinical consequence. As our data demonstrate, Day$_3$-AKI is associated with worse clinical outcomes including prolonged LOS, longer time on mechanical ventilation and increased rate of death. This novel finding supports our *a priori* hypothesis in both the derivation/validation studies of RAI and CHERUB, that AKI on Day$_3$ is a clinically relevant marker of illness

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Table 4. Outcomes stratified by Day$_3$-AKI

	Category	Overall	No AKI	AKI	P-value (no AKI versus AKI)
Demographics	n (%)	156	141 (90.4)	15 (9.6)	—
	Male (%)	98 (52.7)	71 (50.4)	11 (73.3)	ns
	Age (years)	8.4 (2.7, 15.5)	8.2 (2.7, 15.1)	12.8 (2.9, 18.0)	ns
	Day$_0$ PRISM-III	8 (3, 14)	7 (3, 13)	15 (11, 23)	<0.001
	Day$_0$ renal angina	52 (33.3)	40 (28.4)	12 (80)	$\chi^2 = 16.2$, $P < 0.001$
	Sepsis Dx (%)	41 (26.3)	31 (21.9)	10 (66.7)	$\chi^2 = 13.9$, $P < 0.001$
	Transplant history (%)	20 (12.8)	13 (9.2)	7 (46.7)	$\chi^2 = 17.1$, $P < 0.001$
Outcomes	Mechanical Vent days	2 (0, 7)	2 (0, 6)	6 (3, 15)	0.03
	CRRT use (%)	6 (85.7)	0	6 (100)	$\chi^2 = 48.8$, $P < 0.001$
	Organ failure days	3 (1, 8)	3 (1, 7)	7 (3, 16)	0.03
	Hospital LOS (days)	17 (8, 34)	16 (8, 30)	38 (16, 69)	0.02
	ICU LOS (days)	6 (3, 12)	6 (3, 12)	8 (6, 18)	0.02
	Mortality (%)	9 (7.1)	6 (4)	3 (20)	$\chi^2 = 7.4$, $P = 0.007$

Patients in AKI-CHERUB remaining in the ICU on Day$_3$ of admission to the PICU dichotomized by the presence or the absence of severe AKI on Day$_3$ of ICU admission. All values are expressed as medians with interquartile ranges except where designated. n, sample size; AKI by SCr-KDIGO 2–3, AKI diagnosed by change in SCr from baseline per the KDIGO Staging Criteria Stages 2–3; Dx, diagnosis; Vent, ventilation; CRRT, continuous renal replacement therapy; LOS, length of stay; ns, not significant.

and disease trajectory. Severity of illness scores such as PRISM-III and the Pediatric Logistic Organ Dysfunction score in pediatrics and adult severity of illness are not calibrated for severity of illness outside of time of admission [24]. Creation of complex models for AKI risk stratification or prediction to be used in real-time is cumbersome and not practical for bedside care. Traditional 'AKI prediction' scores such as the Lianos, Bullocks, Mehta and SHARF 2 scores work well for population study but are not feasible for individual patient analysis. The RAI is designed to be a practical, bedside tool to assess whether a patient is, or is not at-risk for developing AKI.

Optimized biomarker assessment can significantly impact time to therapeutic intervention and associated outcome. Mortality from ACS began to decline with the advent of both care units dedicated to cardiac patients and a diagnostic biomarker test with high discrimination for injury in patients demonstrating cardiac angina. The modern ACS gold standard biomarker, cTi, loses specificity when measured in patients without risk factors or signs of illness. Fortunately for those patients, heart attack recognition has a clinical advantage over patients suffering from kidney attack [8]. Simply stated, AKI does not 'hurt'. We suggest, however, that renal angina is easily identifiable and offers diagnostic direction for biomarker use that parallels chest pain for troponin. The RAI is a risk discrimination model that optimizes the pre-test probability of disease. Biomarkers tested using this context then have an optimized post-test probability of detecting AKI. Just as indiscriminate use of troponin leads to overall poor prediction of the ACS, capricious testing of AKI biomarkers will lead to poor diagnostic performance and lessened cost-effectiveness, and will delay integration into routine bedside clinical practice. In patients without Day$_0$ renal angina, there is very low risk of Day$_3$-AKI (i.e. a very low probability of Day$_3$-AKI) even with a positive biomarker test on Day$_1$ (Supplementary Table S1). Judicious AKI biomarker assessment, using the context of patient risk, increases predictive performance and clinical efficacy, and offers hope of more expeditious acceptance into management algorithms [36, 37].

We report the highest discrimination of uNGAL for the prediction of severe AKI in the general critical care population in either adults or children. Outside of the original paper describing NGAL for AKI prediction after CPB (AUC-ROC 0.99), very few reports demonstrate such a high level of discrimination [15]. In a recent critical evaluation of NGAL performance in critical care and emergency department settings, the combined AUC-ROC for uNGAL in 5347 patients was 0.80 and for serum NGAL in 3154 was 0.79 [22]. Discrimination calculation using the AUC-ROC to test NGAL in patients without Day$_0$ renal angina in our study was impossible due to the infrequency of Day$_3$-AKI in this population [only 2.9% (3/104) of patients negative for Day$_0$ renal angina had Day$_3$-AKI compared with 23.1% (12/52) patients for positive for renal angina on Day$_0$]. The performance characteristics of the RAI incorporating urinary biomarkers demonstrate increases in the specificity and positive likelihood ratios for the primary outcome of Day$_3$-AKI (Table 5). These results support the argument that testing of a biomarker in a targeted, risk-stratified patient (i.e. renal angina positive) offers a real-time predictive advantage for a treating clinician.

Patients with early fulfillment of renal angina demonstrate a significantly higher degree of fluid accumulation in their subsequent ICU course. Because initial %FO is included in the calculation of the RAI (the worse of %FO or change from baseline SCr is used to denote injury score), separating early fluid accumulation from persistent severe fluid accumulation ('fluid overload') may be an important sub-analysis, particularly in patients with AKI. In the scope of this report, however, we suggest that regardless of initial fluid accumulation, higher net fluid balance and increased %FO in RA+ patients on Day$_3$ may be a clinically important prognostic marker of outcome. In the context of oligo-anuric AKI requiring RRT, observational studies performed originally in pediatrics demonstrate that high %FO at the time of RRT initiation is strongly associated with poor outcome (each 1% in FO increases mortality by 3%) [38]. Outside of patients receiving renal support, association of positive net fluid balance with increased morbidity in mechanically ventilated patients has been highlighted in adults [39] and in several recent pediatric reports [40–42]. Additionally, significant fluid overload

Table 5. Discrimination for Day$_3$-AKI by the RAI in combination with urinary biomarkers

Terms	Sensitivity	Specificity	PPV	NPV	+LR	OR[a] (95% CI)	AUC-ROC (95% CI)	AIC	NRI (P-value)	IDI (P-value)
Day$_0$ RAI	80 (52–97)	72 (64–78)	20 (11–32)	98 (93–100)	2.8 (2.0–4.0)	n/a	0.80 (0.58, 1.00)	48.8	n/a	n/a
Day$_0$ RAI+ NGAL	86 (42–99)	85 (77–90)	23 (9–44)	99 (95–100)	5.6 (3.4–9.2)	NC[b]	0.97 (0.93, 1.00)	28.5	1.64 (<0.001)	0.42 (0.02)
Day$_0$ RAI+ KIM-1	43 (10–82)	95 (90–98)	33 (8–70)	97 (92–99)	9.3 (2.9–30)	5.1 (0.5, 50.4)	0.77 (0.53, 1.00)	48.8	0.69 (0.007)	0.039 (0.12)
Day$_0$ RAI+ L-FABP[c]	86 (57–98)	56 (58–64)	15 (8–25)	98 (92–100)	1.9 (1.5–2.6)	2.5 (0.2, 43.2)	0.82 (0.69, 0.95)	82.0	0.49 (0.04)	0.04 (0.31)
Day$_0$ RAI+ IL-18[c]	57 (29–82)	97 (92–99)	62 (32–86)	96 (91–99)	17.4 (6.6–46)	5.5 (0.6, 47.1)	0.79 (0.65, 0.92)	81.1	0.87 (0.001)	0.05 (0.05)

Predictive performance and discrimination was determined for the primary outcome: severe AKI diagnosed by KDIGO Stage 2–3 change of creatinine from baseline. Sensitivity, specificity, PPV, NPV, positive likelihood ratio (+LR), AUC-ROC, change in AIC, NRI and IDI were performed on the RAI on Day$_0$ of admission to the PICU. Effect of including urinary biomarkers NGAL, KIM-1, L-FABP and IL-18 measured on Day$_1$ of PICU admission was then determined. Cutoff values used for the biomarkers were determined based on optimal Youden's index results on individual urinary biomarkers for the population tested (in ng/mL): NGAL >150, KIM-1 >4000, L-FABP >150 and IL-18 >1000. Patients included in this analysis: $n = 137$. Results expressed with 95% CI. n/a, not available.

[a]Odds ratio—analysis done for impact of biomarkers in renal angina positive patients.

[b]Not calculable.

[c]167.

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may actually mask the true incidence of AKI [43, 44]. Pooled data indicate that AKI diagnosed by both increases in SCr and oliguria has significantly worse outcomes than either alone [45].

There are several potential limitations to our study. Like many others, we describe the limitations of using changes in SCr as an early sign of injury (i.e. a risk stratification biomarker), particularly in children. However, these limitations exist using creatinine without stratified, objective patient context. The RAI is a creatinine-based risk stratification methodology derived and validated in a number of critically ill patient populations, novel because of the stratified, objective use of creatinine changes in patient context. Despite the excellent discrimination for Day$_3$-AKI afforded by biomarker positivity in the context of renal angina positivity, the positive predictive value (PPV) remains low (15–62%). The reliance on PPV as a marker of statistical predictive efficacy should be limited, however, as the incidence of AKI in the cohort was 10%. In a population with higher AKI prevalence, e.g. critically ill septic children only, the PPV would be higher [26]. The practical use of the RAI is to rule out disease—and the very high negative predictive value (NPV) supports our suggestion that the index serves to increase the pre-test probability of disease. As a single-center study, management of patients is subject to the inherent bias of our institution. However, both the AUC of RAI for Day$_3$-AKI and AKI incidence rate in this study were essentially identical to our previous derivation and validation studies of the RAI [25] and other epidemiology studies performed in tertiary PICU settings [46]. Given our sample size, our positive event rate (Day$_3$-AKI) limited some of the statistical analyses (e.g. AUC). As listed in the Materials and methods, in the initial study design nearly 400 patients were targeted for enrollment and we had to curtail the study to initiate our broader, multi-center prospective observational study. Additionally, although we had over 180 patients enrolled in the study from the time of admission, many patients were transferred from the ICU earlier than anticipated and did not remain in the ICU on Day$_3$ as was expected. Patient safety initiatives to reduce the rate of catheter-associated urinary tract infections were instituted in the middle of the study and impacted the rate of both patient enrollment and the ability to capture urine samples for biomarker testing. Although we collected biomarkers on Days$_{2–4}$, this initial analysis investigated only the Day$_1$ biomarkers. This selection was intentional as a feasible clinical workflow, described earlier, is illustrated in Figure 1. The temporal profile of the biomarkers in response to injury may be more clearly demonstrated with future analysis of Days$_{2–4}$ urine specimens.

We conclude that the prediction of severe and persistent AKI produced by biomarkers analyzed in the context of renal angina fulfillment identifies the patients at the highest risk of injury. As we previously demonstrated, the high NPV of the RAI supports its use to rule out AKI. In this study, the ability to rule in AKI using novel biomarkers is optimized by a clinical model. While larger and multi-center studies of RAI and AKI biomarkers should be performed to bolster our findings, we suggest that the ease of use and performance of the RAI supports integration into clinical practice at this time.
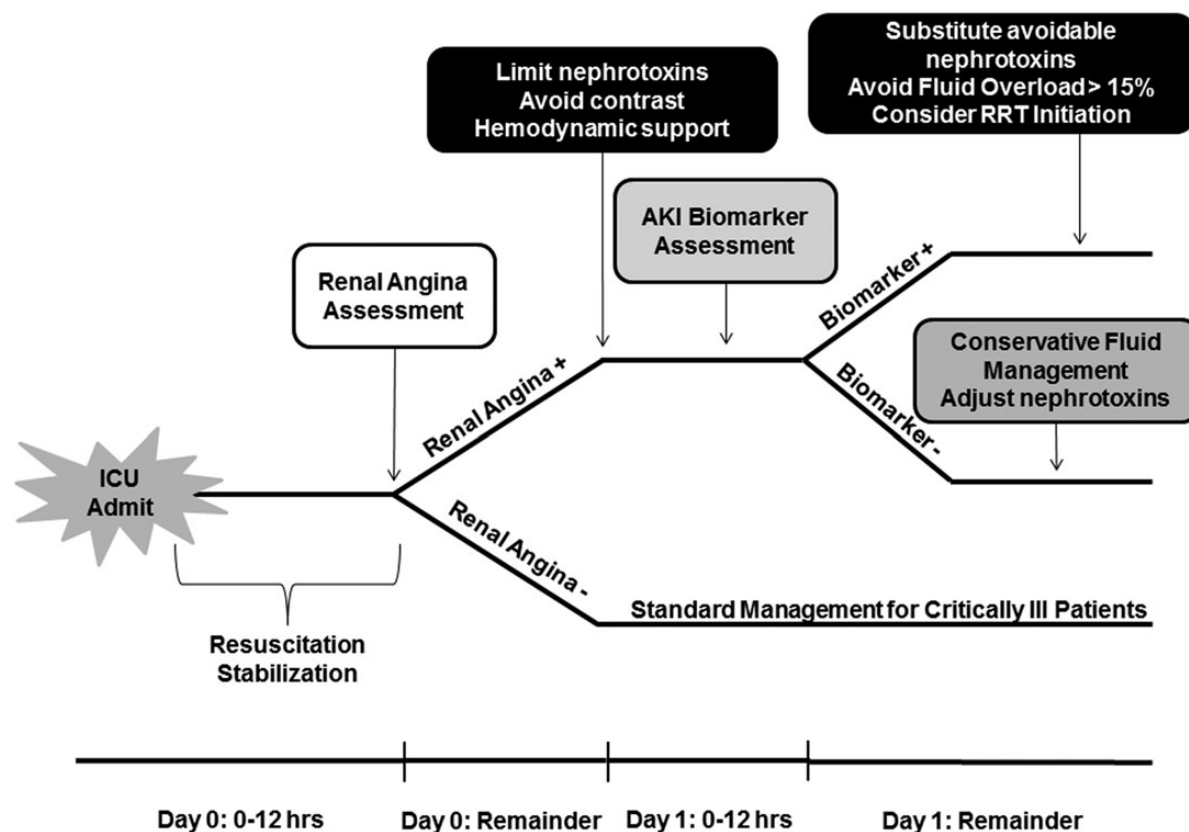
FIGURE 2: Schema depicted represents a potential trial of prospective evaluation on outcome based on use of the RAI for AKI risk stratification after admission to the ICU.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxford journals.org.

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AUTHORS' CONTRIBUTIONS

S.M.: Co-Principal Investigator, primary data collection and analysis, manuscript editing; S.G.: Co-Principal Investigator, data analysis, manuscript editing; T.M.: data collection, entry and urinary specimen collection; T.T.: data collection, entry and urinary specimen collection; L.F.: Statistician; A.K.: data collection, analysis and urinary specimen collection; P.A.: data entry, urinary specimen collection; M.B.: urinary biomarker processing; R.B.: Co-Principal Investigator, data entry, analysis, manuscript preparation and editing.

CONFLICT OF INTEREST STATEMENT

The results presented in this paper have not been published previously in whole or in part, except in abstract format.

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