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Biomarkers for early acute kidney injury diagnosis and severity prediction: a pilot multi-centre Canadian study of children admitted to the intensive care unit.

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Abstract

Objective—Acute kidney injury (AKI) occurs early in pediatric intensive care unit (PICU) admission and increases risks for poor outcomes. We evaluated the feasibility of a multi-centre AKI biomarker urine collection protocol and measured diagnostic characteristics of urine

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Conflicts of interest

The remaining authors have disclosed that they do not have any potential conflicts of interest.

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neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18) and liver fatty acid binding protein (LFABP) to predict AKI and prolonged AKI.

Design: Prospective observational pilot cohort study.

Setting: Four Canadian tertiary healthcare PICUs.

Patients: Eighty-one children 1 month-18 years old. Exclusions: cardiac surgery; baseline severe kidney disease; inadequate urine or serum for PICU days 1 to 3.

Interventions: PICUs performed standardized urine collection protocol to obtain early PICU admission urine samples, with deferred consent.

Measurements and main results: Study barriers and facilitators were recorded. AKI was defined based on Kidney Disease: Improving Global Outcomes (KDIGO) serum creatinine (SCr) criteria (AKI_{SCr}) and by SCr *and* urine output criteria (AKI_{SCr+UO}) Prolonged AKI was defined as AKI duration ≥48 hours. PICU days 1 to 3 NGAL, IL-18 and LFABP were evaluated for AKI prediction (area under the curve [AUC]). Biomarkers on the first day of AKI attainment (day 1 AKI) were evaluated for predicting prolonged AKI. Eighty-two to 95% of subjects had urine collected from PICU days 1 to 3. Sixteen subjects (20%) developed AKI_{SCr}; 38 (47%) developed AKI_{SCr+UO}. On PICU day 1, IL-18 predicted AKI_{SCr} with AUC=0.82, but NGAL and LFABP predicted AKI_{SCr} with AUC's ≤0.69; on PICU day 2, AUC's higher (not shown). IL-18 and LFABP on day 1 AKI predicted prolonged AKI_{SCr} (AUC's 0.74 and 0.83, respectively). When AKI_{SCr+UO} was used to define AKI, biomarker AUC's were globally lower.

Conclusions: Protocol urine collection to procure early admission samples is feasible. Individual biomarker AKI prediction performance is highly variable and modest. Larger studies should evaluate utility and cost effectiveness of using early AKI biomarkers.

Keywords

feasibility; critical care unit; renal; child; diagnostic testing

INTRODUCTION

Acute kidney injury (AKI) occurs in 10–20% of patients in the pediatric intensive care unit (PICU)(1,2). AKI often develops early in PICU admission and is consistently reported to be a risk factor for prolonged ventilation, longer hospital stay and increased PICU mortality(1–4).

AKI definition utilizes serum creatinine (SCr) rise from baseline or urine output (UO) decrease, per the international Kidney Disease: Improving Global Outcomes (KDIGO) definition(5). However, SCr rise with AKI is delayed, resulting in late diagnosis, treatment and prevention of AKI complications(6). Several treatments may attenuate renal tissue injury (7–10) but have been understudied or unsuccessful in humans, at least partially due to the shortcoming of SCr to promptly detect AKI(6,11).

Several early AKI biomarkers have been studied in cardiac surgery patients(12–14) and less so in non-cardiac surgery patients(15–17). Most are urine proteins upregulated in renal tubule cells with cell (or tissue) injury, as opposed to SCr which rises due to renal function

change. Thus, biomarkers have been referred to as “structural” AKI biomarkers(14,18). New AKI biomarkers may rise before functional impairment becomes evident or serve as measures of AKI severity. Neutrophil gelatinase-associated lipocalin (NGAL) is extruded into urine with AKI, involved in injury and repair of renal tubule cells(19). Other recently studied AKI biomarkers include interleukin-18 (IL-18) which plays a role in activating macrophages, T cell-immunity and mediating ischemic tubule injury(20) and liver-type fatty acid binding protein (LFABP), a proximal tubule cytoplasmic fatty acid carrier that binds intermediates of peroxidation(21). These biomarkers have shown promise for early AKI diagnosis in children undergoing cardiac surgery or single-centre studies of non-cardiac populations(12,13,15–17,22–23). Few AKI biomarker studies have been published in the non-cardiac surgery PICU population and no multi-centre PICU biomarker studies have been published. This research gap may be contributed to by the fact that recruiting such patients into early AKI diagnostic biomarker studies is difficult. Because AKI occurs early in PICU admission(1,2), recruiting patients early enough in PICU admission to collect urine specimens for biomarker measurement is challenging.

There is also a knowledge gap regarding the impact of UO criteria of AKI definition, on AKI biomarker performance. Almost all AKI biomarker studies have only applied the KDIGO SCr criteria to define AKI, ignoring UO criteria. AKI ascertainment may differ substantially when SCr criteria alone are used *vs.* applying both SCr and UO criteria to define AKI(3,24). The extent to which applying UO criteria in AKI definition affects AKI biomarker diagnostic performance is unknown.

We hypothesized that urine NGAL, IL-18 and LFABP are early diagnostic markers of AKI and predict prolonged AKI duration (as a marker of AKI severity), in non-cardiac surgery children admitted to the PICU and that inclusion of UO criteria to define AKI modifies biomarker diagnostic performance. We also hypothesized that instituting a PICU clinical urine collection protocol would reduce the challenge of studying of early AKI biomarkers. We performed a pilot multi-centre study to evaluate feasibility of a urine collection protocol on all children admitted to PICU for procurement of early AKI biomarkers. We used this opportunity to evaluate and compare urine NGAL, IL-18, LFABP for early diagnosis of AKI defined using SCr criteria and AKI defined using both SCr and UO criteria.

MATERIALS AND METHODS

Design and setting

This was a prospective, observational cohort study, performed at four Canadian PICUs: the Montreal Children’s Hospital, Montreal (July-October 2013), Children’s Hospital of Winnipeg, Winnipeg (November 2013-January 2014), British Columbia’s Children’s Hospital, Vancouver (January-April 2014), and Stollery Children’s Hospital, Edmonton (June-September 2014). Inclusion criteria were age 1 month-18 years old. Clinical exclusion criteria were: known baseline estimated glomerular filtration rate (eGFR) <60 ml/min/ $1.73m^2$; immediately post-renal transplantation or cardiac surgery; admitted with a primary renal disease (e.g., nephritis); admitted to PICU for <2 calendar days. Enrollement occurred at the latest by PICU day 3. Research ethics board approval was obtained to perform research activities at all institutions.

Recruitment

The subject eligibility evaluation procedure is shown in *Supp.* online Figure 1. To maximize multi-centre staff participation and encourage recruitment, we performed a 2–4 week nurse and physician pre-study education phase (e-mails, in-services, study posters), informing on the study rationale and protocol. Following this, a PICU urine collection protocol was instituted. This protocol included urine collection daily (from existing urinary catheters, urine collection bag or cotton balls in children wearing diapers) for the first 3 PICU days on *all* PICU patients. Urine samples were not used for study purposes until the patient was recruited (deferred consent)(25), but were placed in a 4°C fridge within the PICU until the patient was screened for eligibility. Every 1–3 days, the research team performed eligibility screening (inclusion, exclusion criteria) for all PICU patients. Eligible patients were screened for urine specimen number adequacy and measures of SCr. Of eligible patients (as per criteria described above), only those with a) ≥1 urine specimen if admitted for 2 days or ≥2 urine specimens if admitted for 3 days and b) ≥1 SCr measurement if admitted for 2 days or ≥2 SCr measurements if admitted ≥3 calendar days, were considered for recruitment. Patients fulfilling clinical and specimen eligibility criteria were approached for informed consent and/or patient assent (if appropriate). Urine from patients not eligible or refusing participation was immediately discarded.

Study procedure and clinical data collection

The study occurred over 6 to 10 weeks at each site. Once a patient was recruited (at the latest PICU day 3), daily urine collection continued for up to 5 days from PICU admission. Urine specimens were centrifuged (1000g, 15 minutes, 21°C), aliquoted and stored at −80°C until biomarker measurement. Urine specimens remained in the 4°C fridge ≤72 hours. Prior studies suggest biomarkers are stable under similar conditions(26).

Clinical data was collected for up to 14 days of PICU admission on case report forms with a detailed instructions manual to ensure cross-site data consistency. The central site coordinator performed training for each site coordinator at study initiation, followed by regular phone and email contact throughout study. Clinical variables included: age, gender, weight, height, past medical history, primary PICU diagnosis, fluid balance, sepsis or infection (defined by a positive body fluid culture or infection diagnosis [sepsis, pneumonia, meningitis] in admission or discharge summaries), death, mechanical ventilation, extra-corporeal membrane oxygenation, dialysis, available variables for the Pediatric Risk of Mortality II (PRISM) score (which does not include a renal component)(27), and medications including vasopressors, selected nephrotoxins (including non-steroidal anti-inflammatory agents, vancomycin, aminoglycosides, acyclovir/ganciclovir derivatives and amphotericin) and diuretics. Urine output at every 8 hour interval was recorded (to apply the AKI definition described below) for up to 7 PICU days. Our previously performed single-centre study(28) informed us that UO is very poorly recorded in most patients admitted for over 7 days. Routinely collected SCr values were recorded. When SCr was not measured by the clinical team, if leftover serum from other tests performed that day was available in the hospital laboratory, we obtained this serum to measure SCr when feasible. Data were entered into a secure REDCap database, cleaned every 1–2 months, with 10% data re-entry to capture errors and sites were queried for missing or unclear data.

Baseline SCr was defined as the lowest SCr within 3 months before PICU admission(5). When baseline SCr was not available, baseline estimated glomerular filtration rate (eGFR) of 120 ml/min/1.73m^2 was assumed and baseline SCr was estimated by back-calculating from the Chronic Kidney Disease in Children eGFR formula and height(29). When no height or baseline SCr were available, eGFR was estimated using a height-independent eGFR equation derived in Europe and validated in Canada (30,31), with back-calculation of SCr to estimate baseline SCr.

Throughout the study, sites systematically recorded barriers and facilitators to recruitment, specimen and data collection feasibility.

Laboratory measurements

SCr measurements were performed at each site's central laboratory, to maintain within-subject assay consistency for evaluating SCr change from baseline. All sites report IDMS-traceable SCr. Frozen urine was shipped to the MCH at the end of the study period and stored at −80°C. Biomarkers were measured at the Cincinnati Children's Hospital Medical Center Biomarker Laboratory, Ohio, USA. NGAL was measured using a commercial ELISA kit (Bioporto, Gentogte, Denmark). LFABP was measured using a microbead-based assay (MesoScale Discovery [MSD] Gaithersburg, MD) on a luminex system detected by a Sector Imager 2400 (MSD). IL-18 was measured using an IL-18 ELISA kit (Medical & Biological Laboratories, Nagoya, Japan). Verified inter and intra assay biomarker assay coefficient of variations were ≤10%. Biomarkers were measured in duplicate and corrected for urine creatinine. Personnel measuring biomarkers were blinded to clinical outcomes.

Outcomes

The primary study feasibility outcome was achieving urine collection on Days 1, 2 and 3 of PICU admission. The primary outcome for biomarker analyses was AKI based on the KDIGO definition(5). Because few AKI biomarker studies have used the UO criteria to define AKI, and few pediatric studies have evaluated the UO criteria for associations with clinical outcomes(24), we defined AKI in two ways. The first was to only apply KDIGO SCr criteria (hereafter, AKI_{SCr}). Peak SCr during PICU admission was divided by baseline SCr to calculate percentage SCr rise. Subjects with SCr rise ≥50% or ≥26.5 umol/L from baseline were classified as AKI_{SCr} stage 1; subjects with SCr doubling were classified as stage 2; those with SCr tripling, requiring dialysis or eGFR <35 ml/min/1.73m^2 were classified as stage 3. We also defined AKI using both SCr and UO criteria for AKI definition, whereby a patient may be classified as having AKI if they either fulfill SCr criteria (described above) *or* UO criteria. The UO criteria were stage 1 AKI, if UO was <0.5 ml/kg/hour for 8 hours, stage 2 if UO was <0.5 ml/kg/hour for 16 hours, or stage 3 if the patient was anuric for 12 hours or had UO < 0.3 ml/kg/hour for 24 hours. When evaluating consecutive 8-hour intervals, we performed this in a contiguous manner for different PICU days (e.g., evaluating the last 8 hours of the prior day and the first 8 hours of the current day to determine that 16 hour UO severity stratum). Of note, the KDIGO guideline defines UO AKI in adults using 6-hour, rather than 8-hour intervals. However, almost all published pediatric AKI studies have defined AKI using the 8-hour intervals used in this study, from the original published pediatric AKI modified definition (3, 24). For consistency with past

research, we utilized these pediatric-specific UO criteria, referring to the combined SCr *or* UO-based definition as AKI_{SCr+UO}, (or "complete" KDIGO AKI definition).

A secondary biomarker outcome was prolonged AKI duration, defined as AKI criteria fulfillment for ≥48 hours from the day of first evidence of AKI (e.g., the first day that SCr rises by at least 50%)(3).

Statistical Analysis

Continuous variables were compared between groups using t-tests, one-way analysis of variance, Mann-Whitney-U or Kruskal-Wallis tests. Categorical variables were compared using Fisher's exact or Chi-square tests. The multi-centre recruitment goal was 15–20 patients/site within 4–6 weeks. Analyses were performed using STATA® version 10, College Station, Texas, USA. Analyses were performed separately using AKI_{SCr} and AKI_{SCr+UO} definitions.

Biomarkers for early AKI diagnosis

Biomarker concentrations on PICU days 1 to 3 were plotted and compared between AKI and non-AKI groups, in subjects with biomarkers available from all three days.

Calculation of area under the receiver operating characteristic curve (AUC, 95% confidence interval [CI]) was used to evaluate biomarkers from PICU days 1 to 3 (in patients with biomarkers available on those individual days), to predict AKI development. All non-AKI subjects with biomarkers available were included in this AUC calculation; only AKI subjects who had *not* yet developed AKI by the day of assessment were included in these analyses.

Biomarker associations with prolonged AKI

In AKI subjects, biomarkers measured on the first day of AKI attainment were evaluated for predicting prolonged AKI duration using AUC analysis.

RESULTS

Study population

Our final cohort included 81 children (Figure 1, study flow). Sixteen subjects (20%) developed AKI_{SCr} (stage 1, n=11; stage 2 or worse, n=5); 38 (47%) developed AKI_{SCr+UO} (stage 1, n=25; stage 2 or worse, n=13). Baseline SCr was available in 37 (46%) patients. About 95% of all AKI developed by PICU day 4. There was 73% agreement between AKI_{SCr} and AKI_{SCr+UO} designations (Kappa statistic = 0.44; *Supp.* Table 1 shows AKI staging severity by each definition). Primary reasons for PICU admission were respiratory (n=21, 26%), neurosurgical (n=15, 19%), orthopedic or trauma (n=14, 17%), hematologic-oncologic (n=6, 7%), or other (n=25, 31%), with no significant difference between AKI and non-AKI groups (not shown). In general, differences in patient characteristics between AKI *vs.* non-AKI groups were more striking, with increased illness severity markers (i.e., higher PRISM, more vasopressor use, longer stay, Table 1) when AKI was defined by SCr criteria, compared to defining AKI by SCr or UO criteria (Table 1).

PICU urine collection protocol/study feasibility

Sites easily attained recruitment goals (Figure 1). Study barriers and facilitators were identified from discussions with PICU staff, coordinators and investigators (Table 2). In general, barriers were related to inter-site differences in data availability, care processes, impeding on protocol adherence. These were mostly resolvable by inter-site regular communication, clarifying instructions. Study facilitators were mainly related to open, consistent communication between study and clinical staff and considering site-specific processes during protocol development. Figure 1 displays that the urine collection protocol resulted in a urine specimen available 82%, 95% and 83% of subjects on PICU days 1 to 3, respectively. Sixty-seven percent of subjects had urine specimens available all 3 days. There was no significant difference in protocol urine collection between AKI_{SCr} and non-AKI_{SCr} subjects (2.3±0.7 *vs.* 2.6±0.5 urines within the first 3 PICU days, respectively) nor between AKI_{SCr+UO} and AKI_{SCr+UO} subjects (2.5 *vs.* 2.7 urine samples, respectively). There were no significant differences in patient characteristics between subjects who did *vs.* did not have a PICU day 1 urine specimen available (not shown). On different PICU days, up to 14% of SCr measurements were performed using leftover samples from other blood tests performed by the clinical team.

Biomarkers excretion from PICU days 1 to 3 by AKI status

Figure 2A shows that patients who developed AKI_{SCr} during PICU admission had higher PICU days 1 to 3 biomarker concentrations than non-AKI_{SCr} patients (statistically significant [$p<0.05$] for PICU days 2 and 3 NGAL, day 1 IL-18, day 2 LFABP, Figure 2A). Figure 2B shows that there was no significant difference in biomarker concentrations between subjects with *vs.* without AKI_{SCr+UO} on PICU days 1 to 3.

Biomarkers from PICU days 1 to 3 for predicting AKI development

Table 3 shows the AUC's for biomarkers to predict AKI, when measured on PICU days 1, 2 and 3, if AKI had not yet occurred. Results differed by biomarker and PICU day. Of note, on PICU days 2 and 3, only 2–3 AKI_{SCr} subjects were available for analysis. On PICU day 1, IL-18 predicted AKI with AUC 0.82 (detailed, Table 3); NGAL and LFABP predicted AKI with AUC's< 0.7 (Table 3). AKI_{SCr+UO} prediction was poor for all 3 PICU day 1 biomarkers (AUC 0.43–0.65, Table 3). For NGAL and LFABP, AKI_{SCr} prediction was substantially higher when measured on PICU days 2 or 3 (e.g., AUC 0.9 for day 2 NGAL; AUC 0.77 for day 2 LFABP). A similar phenomenon was seen (i.e., better performance of biomarkers) for predicting AKI_{SCr+UO} when measured on PICU days 2 or 3, *vs.* day 1 (Table 3).

Biomarker associations with prolonged AKI

Thirteen subjects had biomarkers available on the first day of AKI_{SCr} attainment (Day 1 AKI_{SCr}); 7 (54%) had AKI_{SCr} duration ≥48 hours. Biomarker concentrations from Day 1 AKI_{SCr} were higher in patients with AKI_{SCr} duration ≥48 hours (statistically significant for LFABP, Figure 3A). IL-18 and LFABP predicted AKI_{SCr} ≥48 hours (AUC's= 0.74 and 0.83, respectively), whereas AUC for NGAL was 0.69 (detailed, Figure 3A). Fourteen of 33 subjects with AKI_{SCr+UO} (and biomarkers available on Day 1 AKI_{SCr+UO}) had AKI_{SCr+UO} duration ≥48 hours. Figure 3B, shows biomarkers were overall higher in subjects with

prolonged AKI_{SCr+UO}, but AUC's for predicting prolonged AKI_{SCr+UO} were lower than AUC's for predicting prolonged AKI_{SCr} (Figure 3B).

DISCUSSION

This is one of the first multi-centre studies evaluating AKI biomarkers in non-cardiac PICU children. A urine collection protocol to obtain early PICU admission urine with deferred consent, was feasible. Ability of biomarkers to predict AKI was highly variable and modest.

The fact that AKI occurs early in PICU complicates biomarker research. To measure biomarkers during the short therapeutic AKI window (before SCr rise), collecting urine in the first 1–3 PICU days is critical but not easy. This problem may explain the paucity of PICU AKI biomarker studies. We proposed a protocol-based urine collection, with later eligibility screening, to maximize recruitment in AKI biomarker studies. Barriers often related to differences in site practice (Table 1). Facilitators generally related to open communication and clear, labour-minimizing actions (e.g., pre-labeled tubes). Deferred consent has mainly been limited to studies involving time sensitive, life-threatening treatments(25); we propose that to move PICU-AKI research forward, deferred consent may help obtain needed specimens in patients most likely to develop AKI. We demonstrate such a study is feasible and provide insights on successful performance, at least in the Canadian setting. Despite our protocol, however, several patients were excluded from analysis due to early AKI occurrence and/or lacking specimens. Of note, we obtained ethics approval for deferred consent, *only* by not handling urine in *any* way before obtaining consent. Although biomarkers have been shown to be stable under conditions of our study(26), urine would ideally be processed immediately, before deferred consent occurs. This may be feasible in other settings or countries.

We investigated the impact of UO AKI criteria on biomarker performance. We confirmed previous pediatric studies showing discrepancy between SCr- and UO-defined AKI(3,24), as shown by a substantial number of patients with AKI by one method, but not the other (*Supp.* Table 1). Although adult data suggest that UO-defined AKI is associated with clinical outcomes(5,32) little is known on this matter in children. UO criteria are challenging to collect, particularly in patients without urinary catheters. Biomarker performance was worse when including UO criteria. It is possible that a small, temporary UO reduction (i.e., stage 1) may not reflect tubular injury, but rather be a sensitive indicator of temporary volume depletion. It may be that in children, more severe UO reductions are more relevant, in terms of outcome associations or renal tissue injury. These UO criteria were based on AKI definition empirically derived for adults. A pediatric-specific, outcome-based UO definition is needed.

There is little data on biomarkers in non-cardiac surgery patients. Early PICU biomarker diagnostic performance was highly variable. On PICU day 1, where the sample size was highest, IL-18 was the best predictor of AKI_{SCr} development (AUC = 0.82), comparable to or better than what was previously reported in cardiac and non-cardiac PICU patients(12,13,16); PICU day 1 NGAL AKI_{SCr} prediction was moderate and LFABP performed poorly, unlike some previous reports(12,15,17). There is high diagnostic

heterogeneity in the PICU, increasing the likelihood of variability in biomarker performance. Different biomarkers may perform variably by primary disease etiology. For instance, NGAL and IL-18 were shown to be higher in sepsis, possibly since IL-18 is a cytokine and NGAL is neutrophil-derived(16,17). In those studies, NGAL predicted AKI in sepsis, but IL-18 was only diagnostic of AKI without sepsis. Biomarkers appear to be higher in younger children(12), possibly due to tubular immaturity. Biomarkers have also been shown to predict more severe (e.g., stage 2) AKI better than stage 1 AKI(12). It is likely that there is more AKI misclassification in stage 1 AKI (i.e., patients without renal *injury*, but only reduced GFR due to temporary volume depletion). Our sample size precluded analysis of diagnostic, age or AKI severity subgroups. Future studies should be powered to evaluate other factors potentially affecting biomarkers and control for these in multivariate analyses. Finally, studies in cardiac and non-cardiac surgery patients have shown that early PICU admission clinical variables may be useful for predicting AKI (clinical models predicting AKI with AUC's from ~0.7 to ~0.8)(12,15). Future studies should evaluate the extent to which adding biomarkers to clinical prediction models significantly improves prediction *and* ultimately is cost-effective, for future AKI therapeutic trial selection and clinical care.

Our sample size was too small to make strong conclusions on biomarker performance from PICU days 2 or 3. However, AKI prediction was much stronger for NGAL and LFABP on these days. It is possible that after the initial high acuity resuscitation phase, other factors affecting biomarker concentrations (e.g., inflammation, fluid administration) are less prominent after day 1. Early PICU biomarker excretion patterns may be distinct for different biomarkers. Future larger studies should elucidate the extent to which *timing* of biomarker measurement may impact diagnostic performance (as has been shown in cardiac surgery patients).

LFABP and less so, IL-18, predicted AKI duration >48 hours, supporting this previously shown utility of biomarkers(17). When SCr rises, there is often uncertainty on whether there is mild, temporary SCr rise *vs.* an early sign of significant AKI (i.e., true cell injury). Biomarkers may identify tissue injury and help make timely decisions on nephrotoxins and fluid management. We used AKI >48 hours to represent prolonged AKI, based previous studies. Future studies may identify alternative "AKI duration" thresholds.

Study limitations included low sample size (described above), and multiple testing. Baseline SCr was missing for ~50% patients and had to be estimated, which may have resulted in AKI misclassification. Lack of baseline SCr in PICU patients is common, highlighting an inherent issue with SCr-based AKI definitions. We did not collect detailed UO criteria after PICU day 7, based on our previously research. Though it is conceivable that some patients may have developed UO-defined AKI later, this proportion would unlikely be high enough to substantially affect results and also, biomarkers may not be useful to predict such delayed UO-AKI.

CONCLUSION

Validating early AKI biomarkers may improve AKI management and trial performance. Better characterization of biomarker performance (timing, subgroups) and more discovery

research to identify valid AKI biomarkers in the PICU are needed. An early PICU urine collection protocol, likely critical for PICU AKI biomarker studies, is feasible and beneficial for obtaining early PICU urine specimens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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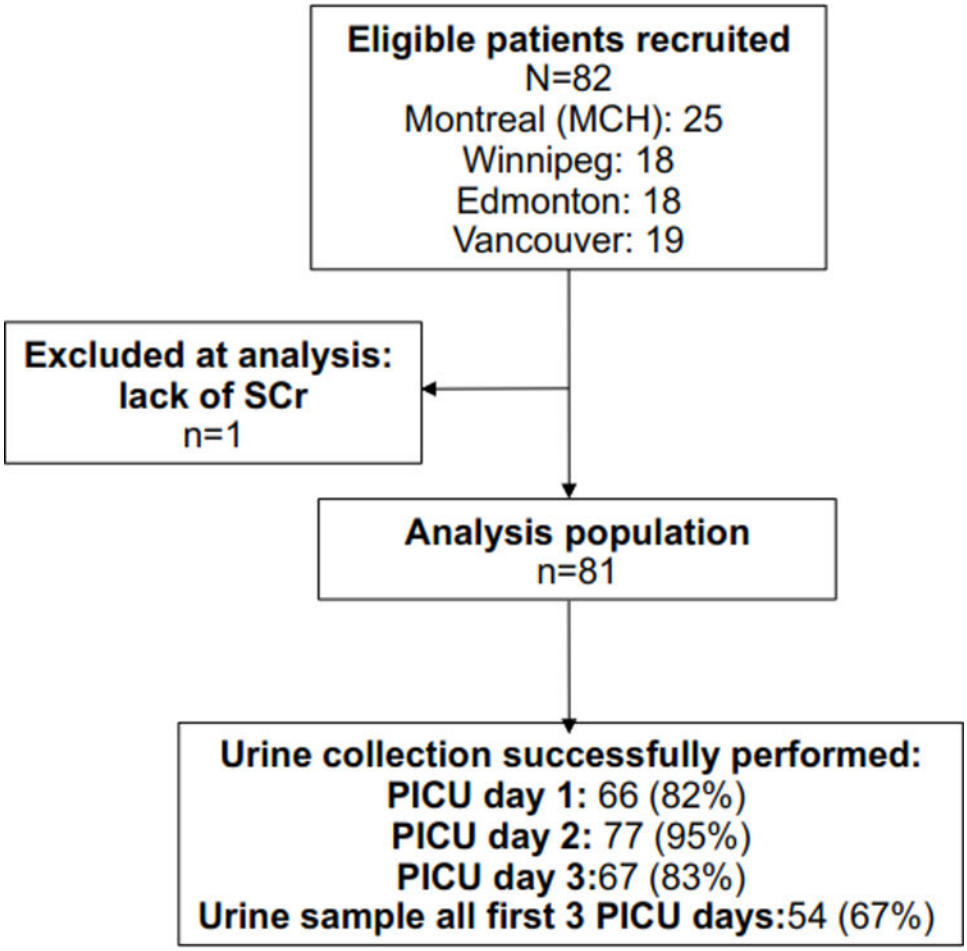

Figure 1. Study flow to analysis population and urine collection protocol performance.
Describes the performance of urine collection protocol, as measured by proportions of patients with a urine specimen collected on each of the first 3 PICU admission days. *Abbreviations*: *SCr*: serum creatinine; *PICU*: pediatric intensive care unit.

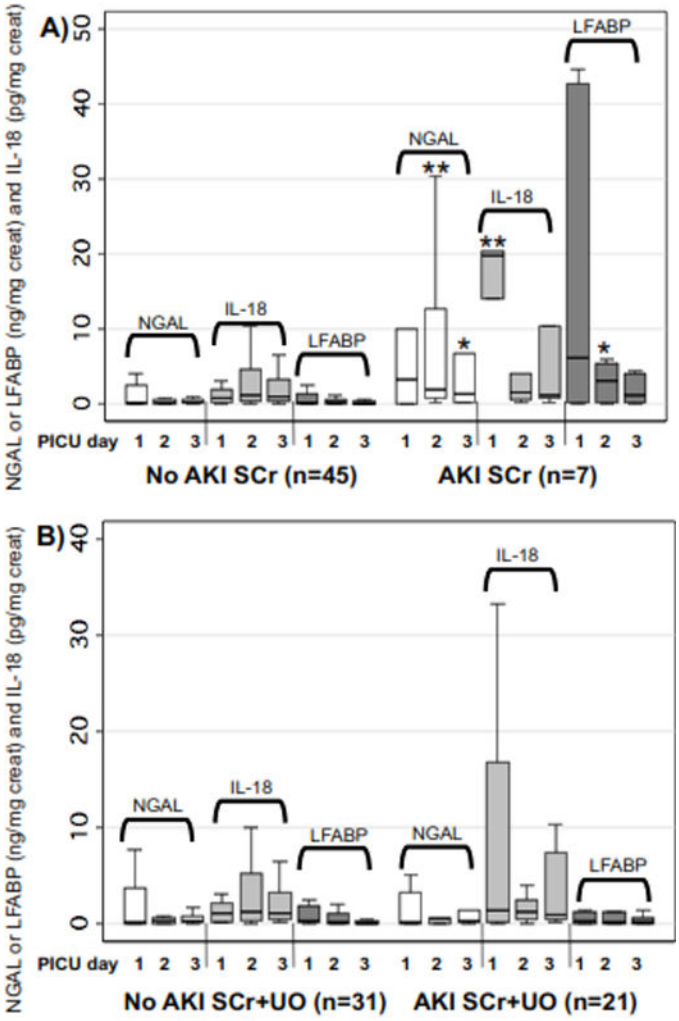

Figure 2. Biomarker excretion in the first 3 days of pediatric intensive care unit (PICU) admission by acute kidney injury status.

Boxplots of urinary NGAL, IL-18 and LFABP on days 1, 2 and 3 of PICU admission, *only* including subjects with biomarkers available on all three PICU days. Left side of both graphs are biomarkers in subjects without AKI; on the right side of graphs are subjects who developed AKI. A) AKI defined using the serum creatinine criteria. B) AKI defined using both serum creatinine or urine output criteria. * indicates statistically significant difference in biomarker concentration between AKI *vs.* non-AKI on that PICU day (* = $p<0.05$; ** = $p<0.005$). *Abbreviations*: *NGAL*: neutrophil gelatinase associated lipocalin; *LFABP*: liver fatty acid binding protein' *creat*: urine creatinine; *IL-18*: interleukin-18; *PICU*: pediatric intensive care unit; *AKI SCr*: acute kidney injury defined using serum creatinine criteria; *AKI SCr+UO*: acute kidney injury defined using serum creatinine criteria or urine output criteria.

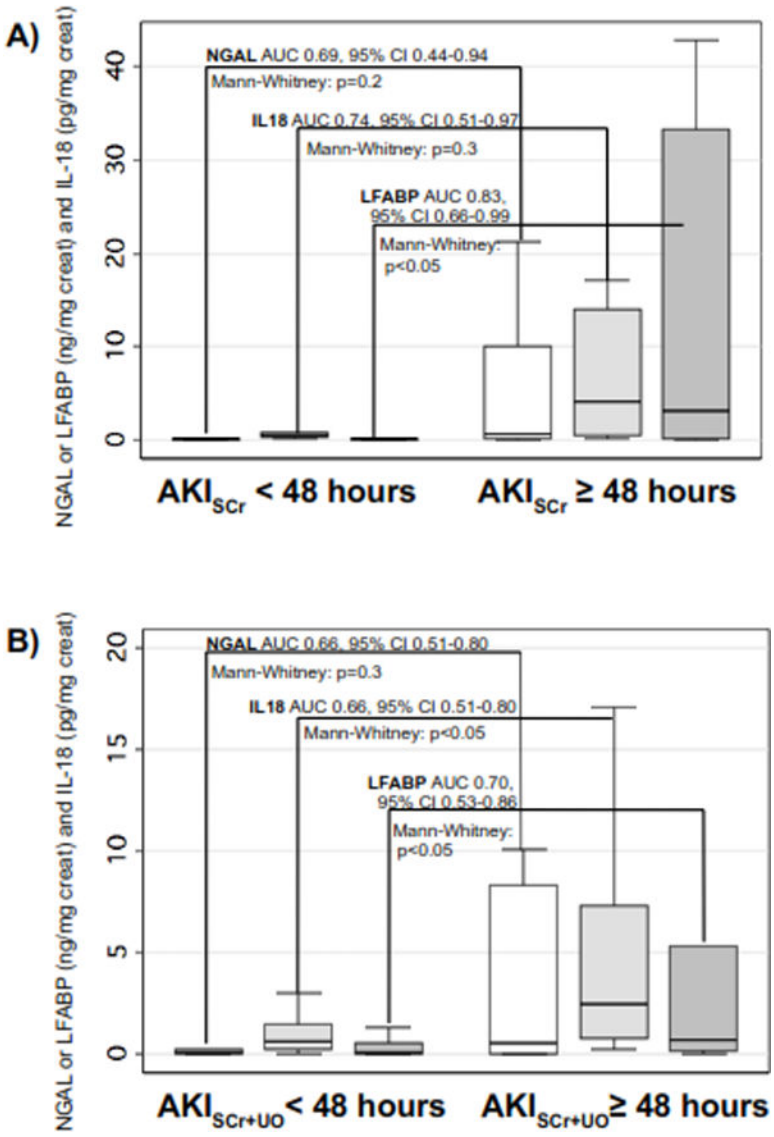

Figure 3. Association of biomarkers from the first day of AKI attainment with AKI duration at least 48 hours.

Boxplots of biomarker concentrations (y-axis) measured on the first PICU day the patient fulfilled criteria for AKI, in subjects with AKI duration (fulfilling criteria for AKI) less than *vs.* greater than or equal to 48 hours (indicated on x-axis). Within graph statistics shown are the p-value for biomarker concentration differences between the two groups (Mann-Whitney U test) and the area under the receiver operating characteristic curve for each biomarker to predict AKI duration at least 48 hours (with lower and upper 95% confidence interval). A) AKI defined using serum creatinine criteria. B) AKI defined using both serum creatinine or urine output criteria. *Abbreviations*: *NGAL*: neutrophil gelatinase associated lipocalin; *IL-18*: interleukin-18; *LFABP*: liver fatty acid binding protein; *AUC*: area under the curve; AKI_{SCr}: acute kidney injury defined using serum creatinine criteria; AKI_{SCr+UO}: acute kidney injury defined using serum creatinine criteria or urine output criteria.

Table 1.

Patient characteristics by acute kidney injury (AKI) status, as defined by serum creatinine (SCr) criteria and by SCr or urine output (UO) criteria.

Variable		No AKI SCr (n=65)	AKI SCr (n=16)	No AKI SCr+UO (n=43)	AKI SCr+UO (n=38)
Age (years)		7.7 (6.2)	10.7 (5.9)	6.5 (6.3)	10.3 (5.5)*
Male gender		35 (53.9%)	8 (50.0%)	22 (51.2%)	21 (55.3%)
PRISM score		6.4 (5.7)	12.1 (6.1)*	6.6 (5.9)	8.6 (6.4)
Baseline eGFR(ml/min/1.73m^2)		104.4 (65.4)	138.8 (26.3)*	92.3 (48.4)	131.4 (65.3)*
Bladder catheter	**PICU day 1**	43 (67.2%)	13 (81.2%)	27 (64.3%)	29 (76.3%)
	PICU day 2	48 (73.9%)	12 (75.0%)	30 (69.8%)	30 (79.0%)
	PICU day 3	27 (69.2%)	8 (72.7%)	19 (79.1%)	16 (61.5%)
Infection	**Primary diagnosis**	2 (3.1%)	2 (12.5%)	2 (4.7%)	2 (5.3%)
	During PICU	11 (16.9%)	8 (50.0%)*	10 (23.3%)	9 (23.7%)
Number of nephrotoxins in PICU		0.7 (0.8)	0.9 (1.2)	0.8 (0.8)	0.7 (0.9)
Diuretics	**Diuretics first 2 PICU days**	15 (23.1%)	7 (43.8%)	13 (30.2%)	9 (23.7%)
	# PICU Days with diuretics	0.9 (2.4)	2.8 (3.9)*	1.1 (2.5)	1.5 (3.1)
Mechanical ventilation	**Ventilated**	30 (46.2%)	11 (68.8%)	19 (44.2%)	22 (57.9%)
	# days of mechanical ventilation	2.3 (3.6)	4.1 (4.5)	2.7 (4.1)	2.6 (3.5)
Vasopressors used		6 (9.2%)	5 (31.3%)*	6 (14.0%)	5 (13.2%)
Death		1 (1.5%)	0 (0%)	1 (2.3%)	0 (0%)
Dialysis		0 (0%)	1 (6.3%)*	0 (0%)	1 (2.6%)
PICU length of stay (days)		7.9 (16.7)	10.1 (10.5)	10.0 (20.2)	6.5 (7.5)
Hospital length of stay (days)		17.2 (26.3)	37.3 (67.2)	19.5 (30.4)	22.9 (45.6)

Abbreviations: *PRISM*: pediatric risk of mortality score; *eGFR*: estimated glomerular filtration rate; *PICU*: pediatric intensive care unit;

Continuous variables are expressed as Mean (Standard deviation); categorical values are expressed as number and column percent.

Table 2.

Representative examples of barriers and facilitators to performing the multi-centre prospective biomarker study and PICU institutional urine collection protocol.

Barriers		
Barrier	**Explanation and Implication**	**Rectification/Improvements**
Data issues		
PICU admission time discrepancy between sources	Discrepant in electronic records (EMR) and paper chart. Affects eligibility and Pediatric Risk of Mortality (PRISM) score window	PICU clerk keeps time log. Use if available. If not, chart, then EMR
Glascow coma score (GCS): sedated/non-sedated assessment	Sedation status not always collected, thus unable to be certain recorded GCS was definitely non-sedated	Queries sent; sedatives list added to case report form (CRF) instructions
Daily drugs, events data Incomplete	Clarity issues on which study day corresponded to a PICU day	CRF table titles and instructions modified, simpler. Queries sent
CRF instructions lacking	Assuming research assistant (RA) knowledge for some variables causes data variability	Every data point described in detail in final CRF instructions
Data entry errors	Certain variables more error prone	Monthly data clean; 10% reentry
Height not always available	Crucial to estimate baseline glomerular filtration rate (GFR); often not recorded in chart	Systematically measure height at recruitment or shortly after
Clinical staff issues		
Day 1 –3 urine not always collected per protocol	PICU nurses did not always collect daily urine during the clinical protocol (in most cases, stated to have forgotten)	Order form to collect daily urine, added to admission package
Urine not collected on PICU discharge day	Ward nurses not all informed of study. PICU nurse sometimes did not collect urine on day of PICU to ward transfer	RA sees patient every study day; Involve parents; reminders; Fridays, speak to nurse and clerk
Sites differ in nurse “shift intervals”	Affects study day start time. Affects time needed for data collection	A uniform CRF to account for inter-site variations in shift times
PICU staff schedule changes	Nurse and resident rotations, “new faces” not always familiar with the study	Study reminders; urine collection order sheet; new staff tracking
Lab issues		
Site lab results reporting (units) differ	E.g., bilirubin, FiO2, Mostly affected PRISM calculation	Conversion tables added to CRF instructions
Leftover serum use, when no routine SCr measured	Leftover serum from central lab used to measure SCr when not available. Some sites not always able to easily obtain	Some sites need specific process to obtain serum from central lab. Site-specific process set up
Other		

Missing weekend patients potential	Mostly Friday admission with Monday discharge to home and not ward. Reviewed: small number	Protocol dictates urine collection 7 days/week. Monday AM priority to recruit such patients first
No routine PICU blood draws	Results in no SCr. Reviewed: small number, low-risk, short stay patients	Minimum SCr criteria included in inclusion criteria; draw study blood if not routinely drawn
Parent accessibility, significant "back and forth to PICU"	Parents leave PICU often, challenge for consent; patient tests (e.g. imaging); time and cost concern	Provide RA/site investigator pager to nurses; involve site investigator on non-RA hours.
	Facilitators	
Facilitator	**Explanation**	**Product**
Involving Head Nurse in planning	Re: study initiation and maintenance	Nurses more aware, reminded of urine protocol. Head nurses is research team member or involved in designing site-specific study feasibility methods
RA/site investigator PICU presence	Having one study RA puts a face to the study; helped remind staff of study	Prefer consistent RA over "hospital research service", unless research service has constant presence.
Deferred consent, during/after PICU-wide urine protocol collection	At recruitment, urine often available from the first 2–3 PICU days; i.e. protocol successful to obtain early PICU urines; accepted by nurse staff	The PICU-wide urine collection protocol will be used for future studies
Simple PICU-wide urine collection protocol for nurses	Focus on urine collection without nurse screening work; all patients; PICU staff not confused by the protocol	The PICU-wide urine collection protocol will be used for future studies.

Table 3.

Comparison of AKI *vs.* non-AKI biomarker concentrations from days 1 to 3 of PICU admission, with associated area under the curve (AUC) for AKI prediction.

Biomarker PICU Day[£]		**AKI_{SCr}**			**AKI_{SCr+UO}**		
		No AKI (n=63)	AKI (n=8) [£]	AUC (95% CI)	No AKI (n=41)	AKI (n=11) [£]	AUC (95% CI)
NGAL (ng/mg cr)	Day 1	0.10 (0.81)	2.11 (6.01)[§]	0.69 (0.51–0.88)	0.14 (1.33)	0.28 (3.21)	0.53 (0.34–0.73)
	Day 2	0.16 (0.48)	15.53 (29.69)[§]	0.90 (0.75–1.00)	0.23 (0.59)	0.38 (30.30)	0.60 (0.29–0.90)
	Day 3	0.21 (0.51)	0.70 (68.67)	0.74 (0.42–1.00)	0.21 (0.51)	2.42 (36.61)	0.66 (0.29–1.00)
IL-18 (pg/mg cr)	Day 1	0.81 (1.90)	3.06 (18.60) [*]	0.82 (0.68–0.96)	0.74 (1.96)	1.64 (10.19)	0.65 (0.45–0.84)
	Day 2	0.79 (3.37)	3.28 (1.58)	0.74 (0.63–0.85)	0.97 (4.17)	2.61 (10.41)	0.63 (0.38–0.88)
	Day 3	0.87 (1.76)	10.33 (9.25)[§]	0.83 (0.64–1.00)	0.96 (1.76)	5.71 (24.31)	0.69 (0.35–1.00)
LFABP (ng/mg cr)	Day 1	0.26 (1.20)	0.23 (20.45)	0.59 (0.33–0.86)	0.28 (1.64)	0.20 (0.68)	0.43 (0.22–0.63)
	Day 2	0.10 (0.46)	0.71 (1.04)	0.77 (0.54–1.00)	0.12 (0.92)	0.80 (1.12)	0.67 (0.43–0.90)
	Day 3	0.08 (0.21)	0.12 (1.26)	0.69 (0.42–0.96)	0.08 (0.20)	0.41 (0.84)	0.76 (0.56–0.97)

Biomarker concentrations are expressed as Median (interquartile range).

[£]On PICU day 1, 8 subjects with AKI_{SCr} were available for analysis (had not yet developed AKI and had a urine specimen available); On PICU days 2 and 3, only 3 and 2 AKI subjects were available for analysis. On PICU day 1, 11 subjects with AKI_{SCr+UO} were available for analysis; 6 and 4 AKI_{SCr+UO} subjects respectively were available for PICU days 2 and 3.

[§]indicates p-value for difference between AKI *vs.* non-AKI groups is between 0.05 and 0.07.

[*]indicates p-value for difference between AKI *vs.* non-AKI groups is <0.05.