Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery

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Acute kidney injury (AKI) is a frequent complication of cardiopulmonary bypass (CPB). The lack of early biomarkers for AKI has impaired our ability to intervene in a timely manner. Urinary neutrophil gelatinase-associated lipocalin (NGAL) is recently demonstrated as an early biomarker of AKI after CPB, increasing 25-fold within 2 h and declining 6 h after surgery. In the present study, we tested whether interleukin-18 (IL-18) is a predictive biomarker for AKI in the same group of patients following CPB. Exclusion criteria included pre-existing renal insufficiency and nephrotoxin use. Serial urine samples were analyzed by enzyme-linked immunosorbent assay for IL-18 in 20 patients who developed AKI (defined as a 50% or greater increase in serum creatinine after CPB) and 35 controls (age, race, and gender-matched patients who did not develop AKI after CPB). Using serum creatinine, AKI was detected only 48–72 h after CPB. In contrast, urine IL-18 increased at 4–6 h after CPB, peaked at over 25-fold at 12 h, and remained markedly elevated up to 48 h after CPB. The performance of IL-18 as demonstrated by area under the receiver operating characteristics curve for diagnosis of AKI at 4, 12, and 24 h after CPB was 61, 75, and 73% respectively. Also, on multivariate analysis, both IL-18 and NGAL were independently associated with number of days in AKI among cases. Our results indicate that IL-18 is an early, predictive biomarker of AKI after CPB, and that NGAL and IL-18 are increased in tandem after CPB. The combination of these two biomarkers may allow for the reliable early diagnosis and prognosis of AKI at all times after CPB, much before the rise in serum creatinine.

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In current clinical practice, acute kidney injury (AKI) is typically diagnosed by detecting increases in serum creatinine. Unfortunately, serum creatinine is an unreliable indicator during acute changes in kidney function owing to several reasons.[1] First, serum creatinine concentrations might not change until about 50% of kidney function has already been lost. Second, serum creatinine does not accurately reflect kidney function until a steady state has been reached, which could take several days. Finally, the serum levels of creatinine are affected by several non-renal factors such as age, gender, race, intra-vascular volume, muscle metabolism, drugs, and nutrition. All these reasons contribute to significant delays in the diagnosis of AKI. Also, the serum creatinine value at the time of diagnosis of AKI does not give any reliable information about prognosis of kidney injury as it does not reflect the severity of AKI. Animal studies have shown that although AKI due to ischemia can be prevented or treated by several interventions, these must be started very early after the kidney injury.[1,2] The failure of these interventions in human clinical trials has been attributed to the significant delays in the diagnosis of AKI.[3,4]

Depending on the definition, AKI occurs in up to 10–40% of adults after cardiopulmonary bypass (CPB), with 1–5% needing dialysis, in whom the mortality rate approaches 80%.[5–7] Recent studies have also demonstrated that even a very small increase in creatinine increases the risk of adverse outcomes after cardiac surgery.[8,9] Pathophysiological mechanisms contributing to AKI include diminished renal blood flow, loss of pulsatile flow, hypothermia, atheroembolism, and a generalized inflammatory response. Various clinical algorithms have been proposed for prediction of AKI, based on preoperative risk factors, but objective tests for the early diagnosis of lesser degrees of renal injury are not widely available.[5,10] AKI requiring dialysis also complicates up to 10% of cardiac surgical procedures in infants and children with congenital heart disease.[11] This population is especially vulnerable to development of AKI as many children need multiple surgical procedures for repair of complex congenital anomalies. However, children are unique because comorbid conditions such as advanced age, atherosclerotic disease, and diabetes are usually absent, making them an ideal group for

investigation of biomarkers as predictors of early ischemic renal injury.

We have demonstrated that neutrophil gelatinase-associated lipocalin (NGAL) is an early predictive urinary biomarker of AKI after cardiac surgery. NGAL is elevated as early as 2 h after CPB[12] Also, our recently published results implicate the cytokine interleukin-18 (IL-18) as an early biomarker for diagnosis and prognosis of AKI in the critical care setting.[13] With multiple pathophysiological mechanisms contributing to renal injury after cardiac surgery, it is highly likely that not just one biomarker, but rather a collection of strategically selected biomarkers will be required as a part of the panel for the early prediction of AKI.[14] Thus, we hypothesized that both NGAL and IL-18 might represent early predictive biomarkers for AKI following cardiac surgery. The objective of this study was to demonstrate that urine IL-18 is an early biomarker of AKI following cardiac surgery. We also tested the hypothesis that the combination of NGAL and IL-18 can prognosticate the severity of AKI.

RESULTS

Study Population

One hundred children were considered for participation in the study. Twenty-nine were excluded because of nephrotoxin use (ibuprofen, angiotensin-converting enzyme inhibitors, gentamycin, vancomycin) before or soon after surgery. Thus, 71 children were included in the study. AKI occurred in 20 children within a 3-day period and the remaining 51 children who did not have 50% increase in serum creatinine served as potential controls. Thirty-five controls were selected from the group of children without AKI and were matched for race, gender, and age to the AKI cases. Among the 20 patients with AKI, serum creatinine rose 24–48 h after CPB in eight, but in the other 12 the increase happened 48–72 h after the procedure. As seen in Table 1, no differences were noted between the two groups with respect to gender, ethnic origin, or urine output. Children who developed AKI encountered longer CPB times compared with those who did not develop AKI.

Table 1 | Baseline characteristics of the study population

	AKI	Controls	*P*-value
N	20	35	
Age (years)	2±5.1	4.2±5.4	0.14
Boys (%)	38	62	0.7
White ethnic origin (%)	38	62	0.7
Previous heart surgery (%)	3	13	0.12
Cardiopulmonary bypass time (min)	179±60	102±56	<0.0001
Change in creatinine (%)	99±41	9±12	<0.0001
Days in ARF (*n*)	2.8±1.7	0	<0.0001

AKI, acute kidney injury; ARF, acute renal failure.
Continuous data presented as mean±s.d.

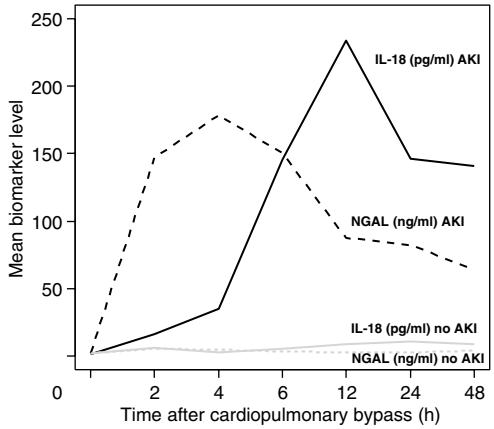

Figure 1 | Pattern of urinary IL-18 and NGAL levels after CPB. AKI (defined as a >50% increase in serum creatinine) developed after 48–72 h in the AKI cases.

Biochemical values of urinary NGAL and IL-18 for early diagnosis of AKI

Serial urine samples were analyzed by enzyme-linked immunosorbent assay for IL-18. The results were combined with the urine NGAL results that are demonstrated in Figure 1 and Table 2. Urinary NGAL increased 25-fold within 2 h and declined after 6 h of CPB. On the other hand, urinary IL-18 increased at 4–6 h after CPB, peaked at a 25-fold increase at 12 h, and remained markedly elevated up to 48 h after CPB. The urine values of both the biomarkers were significantly higher in cases compared to controls at multiple time points after CPB (Table 2).

Performance of Urine IL-18 for diagnosis of AKI

Table 3 demonstrates the sensitivity, specificity, positive predictive value and negative predictive value, and area under the receiver operating characteristic curve for IL-18 at various time points after CPB for diagnosis of AKI. Similar results for NGAL have been published previously.[12] The diagnostic performance is the best at 12–24 h after CPB when the IL-18 biomarker levels peak in the urine.

Prognostication of severity of AKI with urinary NGAL and IL-18

Severity of AKI was assessed by the number of days until recovery of renal function (fall in serum creatinine to <50% of baseline) after the diagnosis among cases (Table 4). There was a significant correlation between biomarker values at different time points with days in AKI. This correlation ranged from 1 to 18% in IL-18 group and 1–22% in the NGAL group at different time points ($P<0.05$). Urine NGAL at 4 h and IL-18 at 4 h had the best correlation with days in AKI ($r^2=0.22$ and 0.18 respectively). On multiple linear regression, both IL-18 and NGAL were significant after adjusting for other baseline and clinical factors. The biomarkers explained 40% of the variability in the severity of AKI from the total of 66% explained by the model. None of the patients with AKI required renal replacement therapy.

DISCUSSION

AKI is a common heterogeneous disease that complicates several medical and surgical conditions. Several risk factors

Table 2 | Urine NGAL (ng/ml) and IL-18 (pg/ml) following cardiopulmonary bypass

	0 h	2 h	4 h	6 h	12 h	24 h	48 h
NGAL control	1.7±0.5	5.0±1.1	4.8±1.1	3.2±0.8	2.4±0.6	2.3±0.7	3.8±1.3
NGAL AKI	1.8±0.5	**147±23.1**	**178±36.2**	**150.2±30.9**	**88±17.7**	**82±17**	**64±10.6**
IL-18 control	1.8±0.9	6.2±3.3	2.3±1.7	5.0±2.1	9±3.6	11±5.4	9±5.0
IL-18 AKI	1.4±1.2	16.1±8.2	**35±15.5**	**146±85.5**	**234±91.1**	**146.3±53.2**	**141±55.4**

AKI, acute kidney injury; IL-18, interleukin-18; NGAL, neutrophil gelatinase-associated lipocalin.
Values are means±s.e.
Values in bold are significantly different ($P<0.05$) compared to controls by analysis of variance.

Table 3 | Performance of urine IL-18 (pg/ml) for diagnosis of AKI at various time points after CPB

	Sensitivity %	Specificity %	Positive predictive value[a] %	Negative predictive value[a] %	Area under ROC curve %
Urine IL-18 at 4 h					
>70	20	100	100	69	61
>50	25	97	83	69	61
>20	25	94	71	69	61
Urine IL-18 at 12 h					
>70	40	94	80	73	75
>50	50	94	83	77	75
>20	55	83	65	76	75
Urine IL-18 at 24 h					
>70	30	94	75	70	73
>50	40	94	80	73	73
>20	60	89	75	79	73

AKI, acute kidney injury; CPB, cardiopulmonary bypass; IL-18, interleukin-18; ROC, receiver operating characteristic.
[a]Positive predictive value and negative predictive values will vary based on the AKI prevalence. The AKI prevalence for this table is 36%.

Table 4 | Significant predictors of severity of AKI after pediatric cardiac surgery on multivariate analysis[a]

Variable	Estimate	Error	*P*-value
Log IL-18 pg/ml at 4 h	0.16	0.06	0.01
Log NGAL ng/ml at 4 h	0.58	0.18	0.005
Female gender	−0.55	0.26	0.05
Prior history of cardiac surgery	−0.86	0.37	0.03

AKI, acute kidney injury; IL-18, interleukin-18; NGAL, neutrophil gelatinase-associated lipocalin.
The biomarkers NGAL and IL-18 have combined r^2 of 0.4 out of the total r^2 of 0.66 with all the significant variables in the model for predicting severity of AKI.
[a]The other variables that were included in the analysis were age, race, and cardiopulmonary bypass time and serum creatinine at diagnosis.

and etiologic mechanisms contribute individually or in combination to the ensuing kidney tubular injury. Even in the relatively homogenous setting of cardiac surgery, AKI is caused by several pathophysiological mechanisms such as ischemia, hypotension, hypoperfusion, cytokine release, and atheroemboli. Thus, it is very unlikely that a single biomarker would be sufficient for accurate and reliable diagnosis and risk stratification of AKI. Several biomarkers representing different injury pathways would be necessary for screening and prognosis of kidney injury.

IL-18 is a novel biomarker that has been studied in detail in preclinical ischemia–reperfusion models followed by successful translation of the findings in human kidney diseases. Using caspase-1-deficient mice and anti-IL-18 antiserum, we demonstrated that IL-18 mediates ischemic acute renal failure in mice.[15,16] These preclinical observations suggested that IL-18 in the urine has the potential to serve as a biomarker for AKI in humans. In a subsequent cross-sectional study in humans, we measured urine IL-18 in patients with AKI and other kidney diseases. Urine IL-18 levels were significantly increased in patients with AKI compared to urinary tract infection, chronic renal insufficiency, and nephrotic syndrome.[17] Urinary IL-18 levels displayed sensitivity and specificity of >90% for diagnosis of established AKI in humans. Urine IL-18 was also increased in kidney transplant patients who had delayed kidney graft function.[17] Furthermore, urine IL-18 was demonstrated to appear up to 48 h earlier in patients with acute lung injury and acute respiratory distress syndrome who develop AKI in the intensive care unit.[13]

In contrast, NGAL was identified as one of the most prominent upregulated genes in the kidney after ischemia. NGAL protein was also markedly induced in kidney tubule cells early after ischemia in mouse models.[18] Importantly, NGAL protein was easily detected in the urine very early after ischemic injury in animals.[18,19] NGAL was first demonstrated as an early biomarker of AKI in the present cohort of pediatric patients receiving cardiac surgery.[12] It was found to be highly predictive with excellent performance characteristics for diagnosis of AKI.

As IL-18 and NGAL are important mediators of ischemic renal injury, it is not very surprising that they assist in prognostication of severity of renal injury. IL-18 was demonstrated to predict mortality in the ICU in the population with acute respiratory distress syndrome.[13] No other biomarker has demonstrated the risk-stratification potential in AKI. Several severity prediction models, using clinical and demographic parameters have been suggested for AKI.[10,20] However, none of them are easy to use clinically at the bedside and have not been validated by other investigators in different settings. Availability of non-invasive biomarkers for selection of patients based on intensity of kidney injury will be immensely helpful for interventional trials and allocation of resources.[14,21]

In clinical medicine, a single biomarker is seldom a stand-alone test. Even outstanding biomarkers such as troponin for cardiac injury and B-type natriuretic peptide are much more accurate and valuable when combined with other biomarkers or clinical data.[22,23] Thus, it is likely that not just one biomarker such as NGAL or IL-18, but rather a combination of biomarkers and clinical parameters will emerge as powerful tools for the early prediction and risk stratification of AKI. Several other potential biomarkers such as kidney injury molecule (KIM-1), IL-6, IL-8, serum, and urine cystatin C should be tested in different clinical settings of AKI in an effort to develop a panel consisting of reliable AKI markers that are easy to measure and offer ability for prognostication of kidney injury.

A limitation of this study is that it represents the results from a relatively small number of patients from a single center. It is acknowledged that our results, although of clear clinical and statistical significance, will need to be validated in a larger population. Also, the current cohort of patients was homogenous with no significant comorbidities with AKI related to CPB. The results of this study will need to be confirmed in other settings of AKI where the etiology of AKI is multifactorial in patients with other comorbid conditions such as diabetes and chronic kidney disease.

The present study of urinary IL-18 along with the previous publication of urinary NGAL in the same cohort identifies two important markers, IL-18 and NGAL, that when combined has an added advantage for prognosis of AKI. NGAL increases as early as 2 h and peaks at 6 h after CPB and then tapers down. IL-18 increases at 4–6 h after CPB and peaks at 12 h and then remains elevated for next 24–48 h. NGAL and IL-18 also predict severity of AKI at the time of diagnosis of AKI. These biomarkers will pave the way for future interventional studies in AKI as they identify renal injury early and assist with stratification of patients based on severity of AKI.

In summary, our results indicate that both NGAL and IL-18 are early predictive urinary biomarkers of AKI after CPB, and that these markers increase in tandem after CPB. The combination of these two biomarkers may allow for the reliable early prediction of AKI at all times after CPB, much before the rise in serum creatinine. These markers also demonstrate the ability for risk stratification and predict days with kidney organ failure in patients with AKI.

MATERIALS AND METHODS

Patients

All children undergoing CPB at Cincinnati Children's Hospital for surgical correction of congenital heart disease between January 2004 and November 2004 were prospectively enrolled. Exclusion criteria included pre-existing renal insufficiency, diabetes mellitus, peripheral vascular disease, and use of nephrotoxic drugs before or during the study period. We therefore studied a homogeneous population of children with possibly no major confounding variables, in whom the only obvious renal insult would be the result of ischemia–reperfusion injury after CPB.

Procedures

All children received at least 80% of their maintenance fluid requirements during the first 24 h after surgery and 100% maintenance subsequently to minimize post-operative volume depletion. We obtained spot urine samples at baseline and at frequent intervals for 5 days after CPB. Urine samples were obtained every 2 h for the first 12 h and then once every 12 h. When the CPB time exceeded 2 h, the first postoperative urine sample was obtained at the end of CPB, and this sample was regarded as the 2 h sample. We centrifuged samples at 2000 *g* for 5 min and stored the supernatants in equal volumes at -80°C. Serum creatinine was measured at baseline and routinely monitored at least twice a day in the immediate post-operative period, and at least daily after postoperative day 3.

The primary outcome variable was development of AKI, defined as a 50% or greater increase in serum creatinine from baseline. In patients who developed AKI, we also recorded the number of days the serum creatinine remained elevated by $>50\%$ as a surrogate for severity of AKI. Other variables obtained included age, sex, ethnic origin, CPB time, previous heart surgery, urine output, and urine creatinine.

Enzyme-linked immunosorbent assay for NGAL and IL-18 quantitation

The technique for the NGAL enzyme-linked immunosorbent assay as well as the urinary NGAL levels in this cohort of patients has been described in detail.[12] IL-18 was measured in human urine using a human IL-18 enzyme-linked immunosorbent assay kit (Medical and Biologic Laboratories, Nagoya, Japan) that specifically detects the mature form of IL-18.[13,17] The crossreactivity of the kit for pro–IL-18 is extremely low.[15] The coefficient of variation of inter-assay and intra-assay reproducibility for IL-18 concentration ranges from 5 to 10% in our laboratory and corresponds to that reported by the kit manufacturer. The measurements were made in duplicate and in a blinded fashion.

Statistical analysis

SAS version 8.2. and SPSS version 13.0 were used for analyses. To compare continuous variables, we used a two-sample *t*-test or Mann–Whitney rank sum test, and to compare categorical variables we used the χ^2 or Fisher's exact test, as indicated. Univariate and multivariate stepwise multiple linear regression analyses were undertaken to assess predictors for severity of AKI. To satisfy the assumptions of linear regression, logarithmic transformations were applied to NGAL and IL-18. Severity of AKI was denoted by the number of days the patient had serum creatinine of $>50\%$ above baseline. Potential-independent predictor variables included age, gender, ethnic origin, CPB time, previous heart surgery, urine NGAL, and urine IL-18 after CPB. Sensitivity and specificity of IL-18 and NGAL were calculated and the accuracy for predicting AKI at different time points was assessed by receiver operating characteristic curves. We judged $P<0.05$ to be significant and all the statistical tests were two-tailed.

Ethical considerations

This investigation was approved by the institutional review board of the Cincinnati Children's Hospital Medical Center. Written informed consent from the legal guardian of every child was obtained before enrolment.

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