

Serum Cystatin C as an Early Marker of Neutrophil Gelatinase-associated Lipocalin-positive Acute Kidney Injury Resulting from Cardiopulmonary Bypass in Infants with Congenital Heart Disease

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ABSTRACT

Objective. Acute kidney injury (AKI) is a common complication resulting from cardiopulmonary bypass in infants. Urinary neutrophil gelatinase-associated lipocalin (NGAL) is a sensitive and specific marker of such injury. In this study, we compared the performance of serum cystatin C (Cys C) and serum creatinine (Cr) as early markers of renal dysfunction in infants undergoing cardiac surgery under bypass.

Study Design, Setting, and Patients. The study was designed as a prospective observational study. The study was conducted in the cardiac intensive care unit (ICU) of a tertiary, academic children's hospital in the United States. Infants (age <1 year) undergoing cardiac surgery under cardiopulmonary bypass were included in the study.

Outcome Measure. Acute kidney injury was defined based on postoperative urinary NGAL.

Results. A total of 17 infants were included in the study, and five of them developed AKI. Serum Cys C and Cr levels were measured postoperatively on days 1, 2, and 3, and compared with baseline levels. On postoperative day 2, infants with AKI showed significant change from baseline in serum Cys C levels compared with non-AKI infants (28% vs. -9%, $P = .03$). The two groups did not show significant differences with respect to rise in serum Cr on any of the 3 postoperative days. Serum Cr on days 1 and 2 showed nonspecific increases in both AKI and non-AKI groups. The area under the receiver operating characteristic curve for day 2 Cys C was 0.87 (95% CI 0.67–1.00) in recognizing NGAL-positive AKI.

Conclusions. Postoperative serum Cys C appears to be a more specific and sensitive biomarker for NGAL-positive AKI resulting from cardiopulmonary bypass surgery in infants undergoing cardiac surgery.

Key Words. AKI; Cardiopulmonary Bypass; Infants; Cystatin C; Creatinine; Urine NGAL

Introduction

Acute kidney injury (AKI) is a well-recognized potential complication following cardiopulmonary bypass (CPB) and circulatory arrest in children undergoing surgery for congenital heart

disease (CHD).¹ It is associated with an increase in in-hospital mortality, longer mechanical ventilation times, and longer duration of inotropic support in these children.^{2,3} Hence, early identification of AKI is essential in these patients. Serum creatinine (Cr) is the conventional biomarker that is commonly used to identify such AKI. But Cr is a marker of glomerular filtration rate (GFR) rather than injury to the kidney itself. Serum concentration does not increase significantly until approximately 50% of renal function is lost.⁴ As a result,

Grants and funding

This study was funded by the Caruth Center for Pediatric Translational Clinical Research, Children's Health, Dallas, TX.

there is a time lag between initiation of AKI and rise in serum Cr that can result in delayed identification. Also, if early rise in serum Cr does occur, it is nonspecific and can simply reflect prerenal state. Hence, it has various limitations in being a good marker for renal function in AKI.

Cystatin C (Cys C) is a cationic nonglycosylated low-molecular-weight inhibitor of cysteine proteases that is produced by all nucleated cells. It was first proposed as a marker for GFR in 1985.^{5,6} There is increasing evidence that serum Cys C can be used as an early biomarker of AKI resulting from CPB.^{7,8} Multiple studies have evaluated its performance in comparison with serum Cr.⁷⁻¹¹ A potential limitation of these studies is that they define AKI based on increase in serum Cr or decrease in Cr-based GFR measurements. Recent evidence suggests that urinary neutrophil gelatinase-associated lipocalin (NGAL) is a more specific and sensitive early marker of AKI in this patient population.^{12,13} The purpose of this study was to evaluate serum Cys C as an early biomarker of renal dysfunction in patients with NGAL-positive AKI sustained during CPB, defining AKI based on urinary NGAL instead of serum Cr. As a secondary objective, we also evaluated the utility of Cr clearance measurement based on an abbreviated 4-hour urine collection in this setting.

Methods

This study was performed at the cardiac intensive care unit (ICU) in Children's Medical Center Dallas (CMCD) over a 1-year period from July 2011 through June 2012. It was designed as a prospective observational cohort study and was approved by the local Institutional Review Board. We compared children with and without AKI, with respect to serum Cys C and serum Cr. AKI was defined as post-CPB urinary NGAL > 150 ng/mL. The study was limited to term infants under 12 months of age as this is the age group with the highest incidence of AKI secondary to CPB. Children undergoing cardiac surgery under CPB support were enrolled in the study after obtaining informed consent and Health Insurance Portability and Accountability Act of 1996 authorization. Those with exposure to nephrotoxic medications and intravenous contrast and/or evidence of AKI prior to CPB, defined as meeting "Risk," "Injury," or "Failure," criteria of pRIFLE during the preceding 3 days before surgery, were excluded to avoid the confounding effect on postoperative serum Cys C level, serum Cr, and Cr clearance.¹⁴

In addition, children with evidence of chronic kidney disease (CKD), based on either the estimated Cr clearance using Schwartz's formula or measured Cr clearance in a 24-hour urine collection, were excluded to avoid the confounding effect of CKD on postoperative serum Cys C levels, serum Cr, and Cr clearance. CKD was defined as estimated GFR less than 2 standard deviations below age-based mean GFR.

Patient demographic data, including age, gender, weight, type of cardiac lesion, anthropometric data, and surgical information, were obtained. Surgical complexity was ranked according to the Risk Adjustment for Congenital Heart Surgery 1 (RACHS-1) scoring system.¹⁵ Intraoperative data obtained included duration of CPB, cross-clamp time, and circulatory arrest time. Postoperative hourly urine output and need for renal replacement therapy (RRT) were monitored.

Urine and serum samples were obtained prior to surgery to measure the baseline urinary NGAL, serum Cr, and serum Cys C. A fresh urine sample was obtained within 4–6 hours after coming off of CPB to measure postoperative urinary NGAL.¹² Serum and urine samples were obtained on postoperative days (POD) 1, 2, and 3 to measure serum Cys C, serum Cr, and 4-hour urine Cr clearance on those days.

Urine NGAL Measurement

Two mL of the urine sample collected for NGAL measurement was sent to the core chemistry lab at CMCD immediately after collection. The sample was centrifuged at 1840 *g* for 10 minutes and 1.5 mL of supernatant was obtained and frozen within 1 hour of collection and stored at -70°C . Samples were sent in batches to the Biomarker Laboratory (Cincinnati Children's Research Foundation, Cincinnati, OH) for analysis. They were analyzed by enzyme-linked immunosorbent assay (ELISA) using NGAL Rapid ELISA Kit (Kit 037, Bioporto Diagnostics, Gentofte, Denmark) and reported in ng/mL. The cutoff used was 150 ng/mL and AKI was diagnosed if it was >150 ng/mL.¹³

Serum Cys C Measurement

One mL of blood sample collected for serum Cys C measurement was sent to the core chemistry lab at CMCD. Serum (0.5 mL) was obtained from this sample and frozen within 1 hour of collection and stored at -20°C . It was then shipped to an outside lab (Mayo Medical Laboratories, Rochester, MN). The sample was analyzed by latex particle-

enhanced immunonephelometry using BN II System (Dade Behring, Deerfield, IL, USA) and reported in mg/L.¹⁶ Serum Cys C-based GFR was calculated using the formula $\log(\text{GFR}) = 1.962 + (1.123 \times \log [1/\text{CysC}])$.^{17,18} Cr-based GFR was calculated using the Schwartz formula.^{19,20}

Statistical Analysis

The cohort of patients was grouped into AKI and non-AKI groups based on postoperative urinary NGAL measurements. The two groups were compared with respect to baseline characteristics. Serum Cys C and Cr between the two groups were compared with respect to change from baseline and estimated GFR on each of PODs 1, 2, and 3. Also, sensitivity and specificity of Cys C and Cr in identifying NGAL-positive AKI were calculated. Receiver operating curves were constructed and area under the curves compared. Acute kidney injury and non-AKI groups were also compared with respect to hourly urine output and abbreviated 4-hour Cr clearance on PODs 1, 2, and 3. Two-tailed Wilcoxon rank sum tests and paired *t*-tests were used for continuous variables and Fisher's exact tests for categorical variables. All statistical analyses were performed using R version 3.1.0.^{21,22}

Results

A total of 20 patients were enrolled in the study. Three of the 20 patients were excluded due to absence of postoperative urinary NGAL data. Of the 17 patients included in the final analysis, five developed AKI based on postoperative urinary NGAL > 150 ng/mL. Median age of the cohort was 76 days (range 5, 272 days) and median weight

was 3.5 kg (range 2.5 to 8.9 kg). Median surgical complexity ranking based on risk adjustment for congenital heart disease (RACHS-1) was 3 (range 1, 6). There was male preponderance, with 12 males and 5 females in this cohort. Eight patients had lesions with single ventricle physiology. The median CPB time was 108 minutes (range 21, 163 minutes) and the median aortic cross-clamp time was 74 minutes (range 0, 103 minutes). Only three patients had circulatory arrest (3, 9, and 16 minutes), and two of them developed AKI.

Table 1 compares baseline characteristics between AKI and non-AKI groups. Children in the AKI group were younger compared with non-AKI group (median age 5 vs. 99 days, *P* = .007). They also had more complex surgeries (median RACHS-1 ranking 4 vs. 2.5, *P* = .04). AKI group had longer durations of CPB (130 vs. 84 minutes, *P* = .01) and cross-clamp times (92 vs. 66 minutes, *P* = .06), and required other vasopressor/inotropic support in addition to milrinone. Baseline serum Cr was higher and Cr-based estimated baseline GFR lower in the AKI group compared with non-AKI group. But baseline Cys C and Cys C-based estimated GFR were similar in the two groups. Baseline urinary NGAL and urine output were within normal limits in both the groups prior to surgery.

The lowest postoperative urinary NGAL was 263 ng/mL in the AKI group. Postoperative changes in serum Cys C and Cr for the two groups are shown in Figure 1. The AKI group did not show statistically significant (at $\alpha = .05$) postoperative increase in serum Cr or decrease in Cr-based GFR compared with baseline on any of the initial 3 PODs (Table 2). Significant postoperative increase

Table 1. Baseline Characteristics—Non-AKI vs. AKI

	Non-AKI Group (n = 12) Median (Range)	AKI Group (n = 5) Median (Range)	<i>P</i> Value*
Age (days)	99 (5, 272)	5 (4, 16)	.007
Gender—Male:Female (Expressed as Proportion)	60:40	75:25	.60
RACHS-1 ranking	2.5 (1, 6)	4 (3, 6)	.04
CPB time (minutes)	84 (21, 163)	130 (115, 157)	.01
Cross-clamp time (minutes)	66 (0, 98)	92 (72, 103)	.06
Baseline Cr (mg/dL)	0.4 (0.3, 0.6)	0.6 (0.4, 0.8)	.02
Baseline Cr-based GFR [†] (mL/min/1.73 m ²)	66 (38, 93)	36 (26, 57)	.004
Baseline CysC (mg/L)	1.2 (0.7, 1.8)	1.2 (1.0, 1.8)	.79
Baseline CysC-based GFR [‡] (mL/min/1.73 m ²)	68 (48, 137)	75 (47, 92)	.96
Baseline NGAL (ng/mL)	13 (2, 43)	19 (8, 26)	.68
Baseline hourly urine output (mL/kg/hour)	2.5 (1.2, 4.3)	3.5 (1.8, 6.6)	.54

*Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables. Bold values indicate statistical significance *P* < .05.

[†]GFR estimated from serum Cr using Schwartz formula.

[‡]GFR estimated from serum CysC using the formula $\log(\text{GFR}) = 1.962 + (1.123 \times \log [1/\text{CysC}])$.

AKI, acute kidney injury; Cr, creatinine; CPB, cardiopulmonary bypass; Cys C, Cystatin C; GFR, glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin.

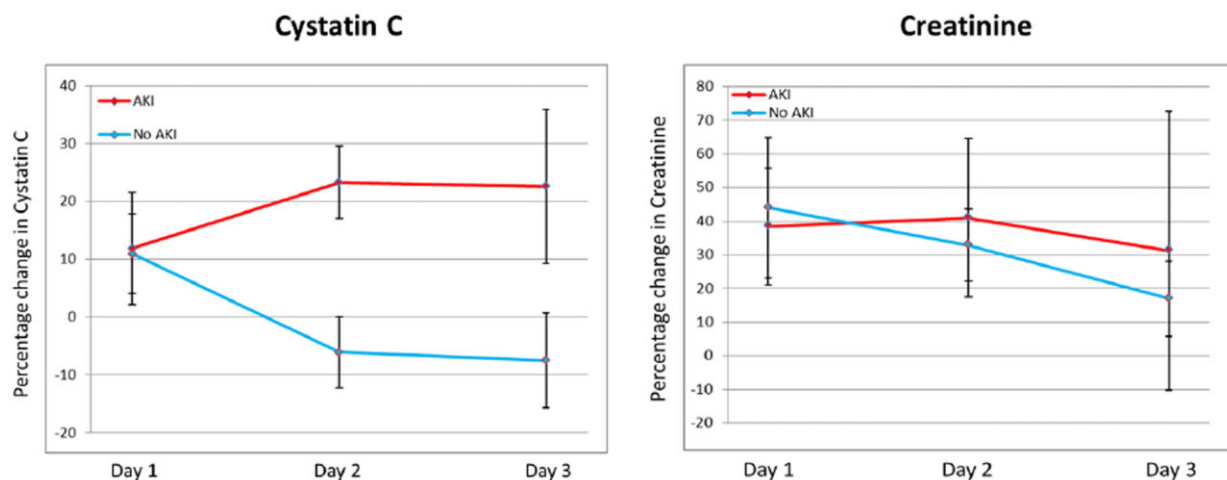


Figure 1. The plot to the left shows percentage change from baseline in Cys C levels on days 1, 2, and 3 for AKI and non-AKI groups. The plot on the right shows percentage change from baseline in Cr levels on days 1, 2, and 3 for AKI and non-AKI groups. The plotted points represent mean values and the error bars show standard error. AKI, acute kidney injury; Cr, creatinine; Cys C, Cystatin C.

Table 2. Creatinine and Cystatin C—Postoperative Changes from Baseline

	Non-AKI Group Mean Difference (P Value*)	AKI Group Mean Difference (P Value*)
Increase in Cr[†]		
Postoperative day 1	0.15 (.04)	0.24 (.05)
Postoperative day 2	0.13 (.009)	0.28 (.15)
Postoperative day 3	0.06 (.15)	0.28 (.40)
Increase in CysC[‡]		
Postoperative day 1	0.17 (.17)	0.10 (.47)
Postoperative day 2	0.06 (.44)	0.32 (.02)
Postoperative day 3	0.11 (.33)	0.40 (.20)
Decrease in Cr-based GFR^{§,¶}		
Postoperative day 1	11 (.20)	6 (.40)
Postoperative day 2	13 (.02)	5 (.50)
Postoperative day 3	5 (.37)	28 (.48)
Decrease in Cys-based GFR^{§,¶}		
Postoperative day 1	1 (.65)	9 (.24)
Postoperative day 2	-11 (.04)	14 (.04)
Postoperative day 3	-14 (.07)	8 (.23)

*Paired t-test.
[†]Cr in mg/dL.
[‡]CysC in mg/L.
[§]GFR in mL/min/1.73 m².
[¶]GFR estimated from serum Cr using Schwartz formula.
[¶]GFR estimated from serum CysC using the formula log (GFR) = 1.962 + (1.123 × log [1/CysC]).
 AKI, acute kidney injury; Cr, creatinine; Cys C, Cystatin C; GFR, glomerular filtration rate. Bold values indicate statistical significance P < .05.

in serum Cys C and decrease in Cys C-based GFR were noted on POD 2 in the AKI group (Table 2). Interestingly, significant postoperative elevation in serum Cr was noted in the non-AKI group. Serum Cys C did not show such elevation in this group. The AKI and non-AKI groups differed with respect

to serum Cys C on PODs 2 (P = .03) and 3 (P = .09), but not with respect to serum Cr (P = .75 and .71, respectively) (Figure 2).

Figure 3 shows comparison between non-AKI and AKI groups with respect to urine output and 4-hour abbreviated Cr clearance. Urine output was a good marker of AKI on POD 1, with all patients in the non-AKI group having a urine output >1 mL/kg/hour and most patients in the AKI group <1 mL/kg/hour (P = .002). But urine output normalizes in the AKI group on PODs 2 and 3 (P = .44 and .72, respectively). Similarly, abbreviated Cr clearance based on a 4-hour urine collection showed significant difference between the two groups on POD 1 (P = .002) and no difference on days 2 and 3 (P = .64 and .54, respectively).

Performances of Cys C-based and Cr-based estimations of GFR to diagnose AKI were assessed using urinary NGAL as the gold-standard test. Decrease in GFR of ≥25% from baseline was used as the cutoff for both Cr-based estimates and Cys C-based estimates. Cys C showed good specificity on each of the three PODs (0.78, 0.89, and 0.89, respectively). But the sensitivity was poor (0.20, 0.40, and 0.50, respectively). In contrast, Cr showed poor specificity (0.58, 0.50, and 0.75, respectively) but better sensitivity (0.80, 0.60, and 0.40, respectively) on each of the three PODs. When the highest decrease in GFR between days 2 and 3 was used, Cys C performed better compared with Cr with respect to both sensitivity (0.80 vs. 0.60) as well as specificity (0.89 vs. 0.50). Positive predictive value was 0.80 for Cys C compared

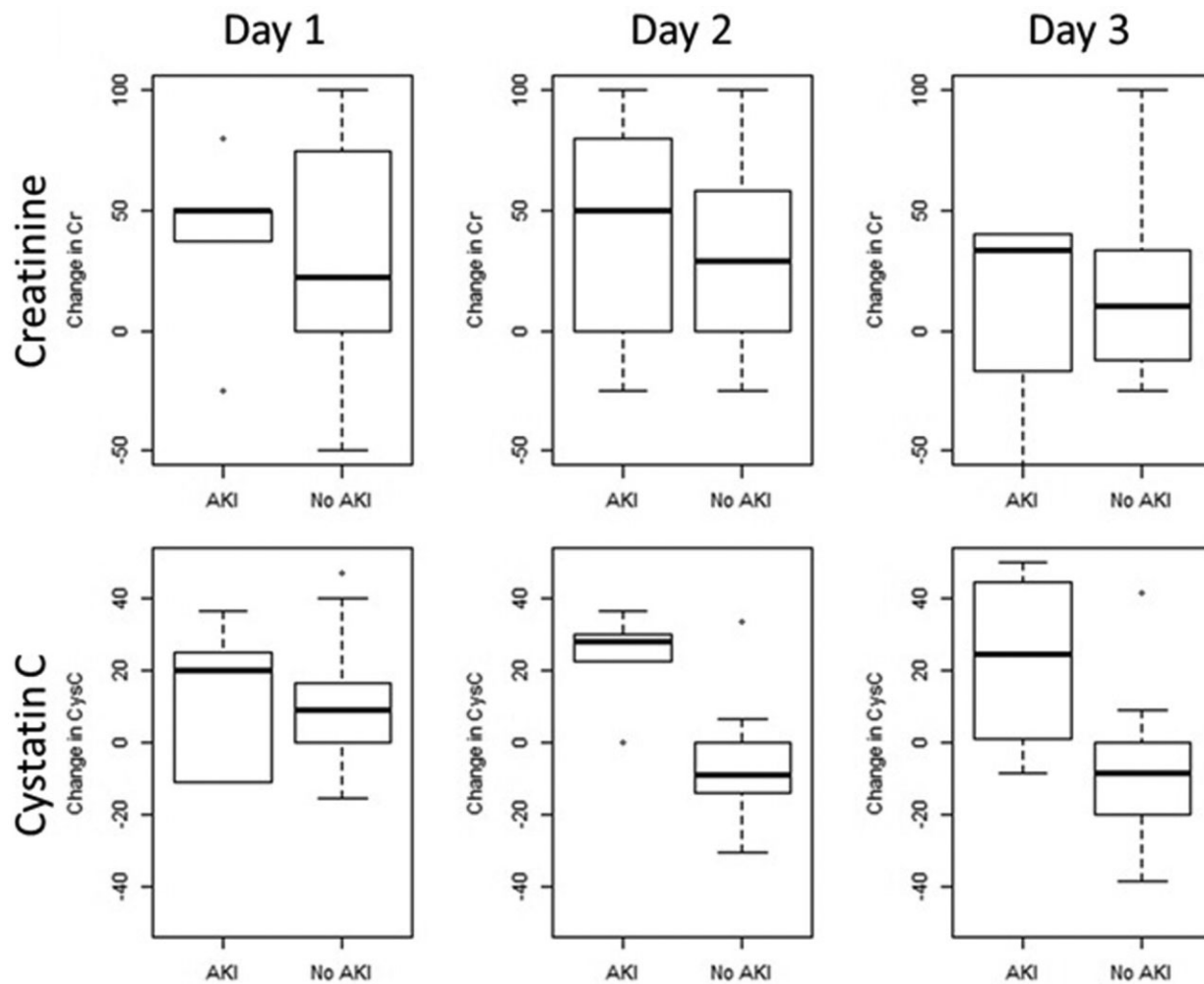


Figure 2. The figure shows box plots comparing infants with and without AKI. Top row compares the change in serum Cr from baseline and bottom row compares change in serum Cys C levels from baseline. First column shows changes noted on day 1, second column shows changes noted on day 2, and third column shows changes noted on day 3. AKI, acute kidney injury; Cr, creatinine; Cys C, Cystatin C.

with 0.33 for Cr, and negative predictive value was 0.89 for Cys C compared with 0.75 for Cr. Figure 4 shows receiver operating characteristic curves for Cys C and Cr for PODs 1, 2, and 3. The areas under the curve for day 2 were 0.87 and 0.58, respectively, for Cys C and Cr. A cutoff value of about 15% increase from baseline for Cys C on POD 2 provided the best sensitivity and specificity (0.80 and 0.89, respectively).

Discussion

In the recent literature, serum Cys C has shown promise as a potential early biomarker of AKI and measure of GFR in children sustaining AKI due to CPB. In this study, we compared Cys C with Cr in recognizing NGAL-positive AKI. We found that

Cys C is a more specific marker of AKI compared with Cr. Cys C levels on POD 2 had the best discriminatory ability with an area under the receiver operating characteristic curve of 0.87. In contrast, there was significant elevation of Cr in most patients after CPB, irrespective of whether they developed AKI or not, and poor correlation with severity of AKI as measured by post-CPB urinary NGAL levels.

In our cohort, we noticed a significant difference in baseline Cr as well as Cr-based estimates of GFR between the AKI and non-AKI groups. AKI group had higher Cr at baseline (preoperatively) even though there was no evidence of AKI at that time as evidenced by normal preoperative urinary NGAL levels. This is likely reflective of the age difference between the two groups. Because all the children

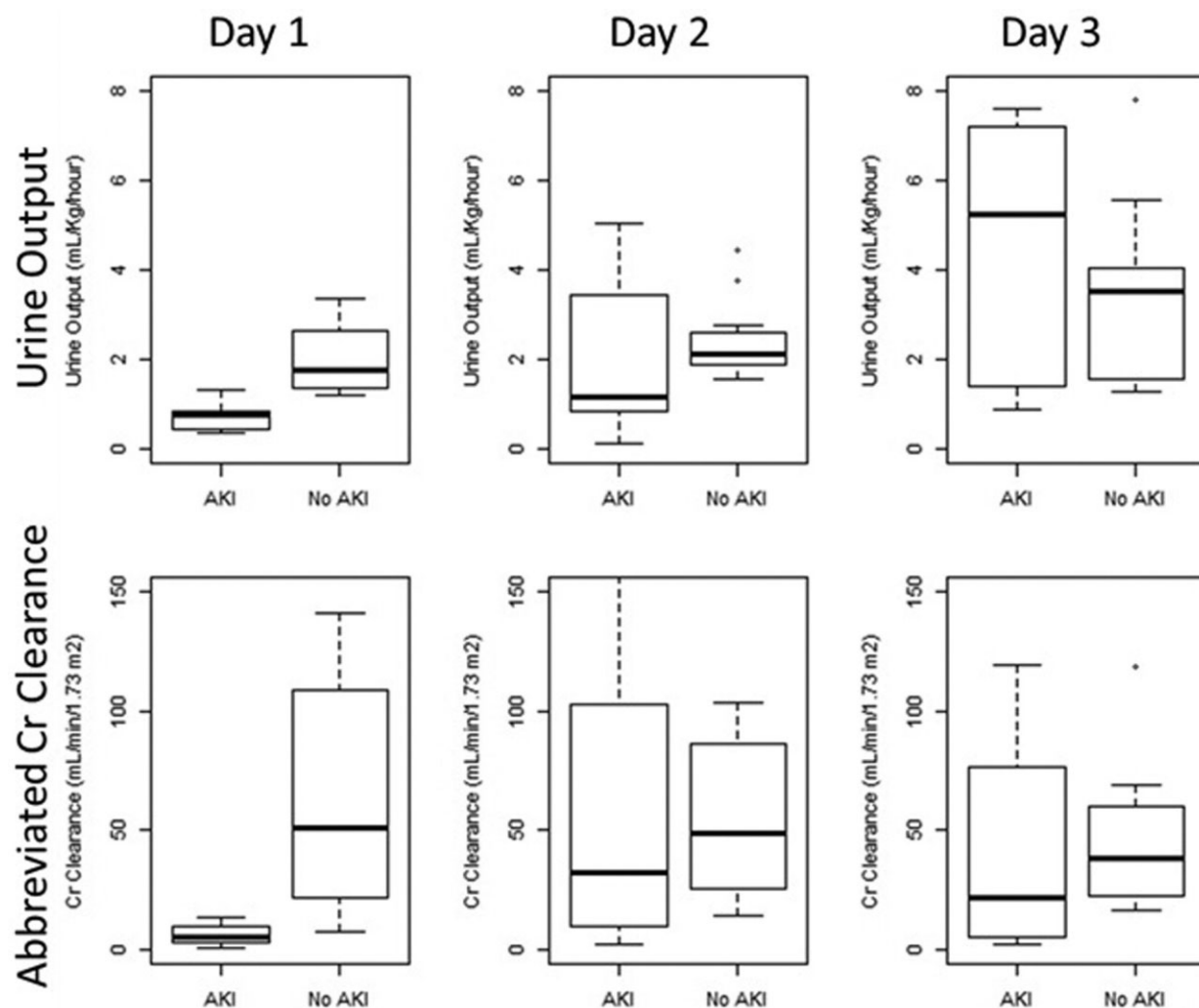


Figure 3. The figure shows boxplots comparing infants with and without AKI. Top row compares the hourly urine output and bottom row compares measured Cr clearance based on a 4-hour abbreviated urine collection. First column shows changes noted on day 1, second column shows changes noted on day 2, and third column shows changes noted on day 3. AKI, acute kidney injury; Cr, creatinine.

with AKI were neonates, their serum Cr levels were higher. Serum Cys C did not show such difference between the two groups at baseline, prior to going on CPB. Presumably this is indicative of the fact that Cys C is a more reliable marker of AKI, especially in the neonatal age group.

Serum Cr showed a postoperative rise in both AKI and non-AKI groups, with a mean increase of around 40% from baseline on day 1 and of about 30–40% on day 2 in both groups. The reason for this nonspecific rise is unclear but may be reflective of prerenal state associated with CPB rather than true AKI. This further supports the fact that serum Cr has poor specificity in identifying true AKI, especially during the early stages. Serum Cys C, on the other hand, did not show any significant

rise postoperatively in the non-AKI group, on any of the 3 days. But, in the AKI group, there was significant rise on POD 2. We failed to notice a difference in Cys C levels in the AKI group on POD 1, despite previous studies showing a rise in Cys C levels in AKI patients as early as 8–12 hours postinjury. The dilutional effect of CPB could potentially explain this observation in our cohort. Similarly, we failed to notice a difference in Cys C levels on POD 3. This is likely suggestive of improvement in renal function, with a return of the Cys C levels back toward baseline on day 3. Of the five patients with AKI, only one had significant renal dysfunction requiring RRT. This patient's severe AKI was identified by a very high urinary NGAL post-CPB (>1500 ng/mL). Also,

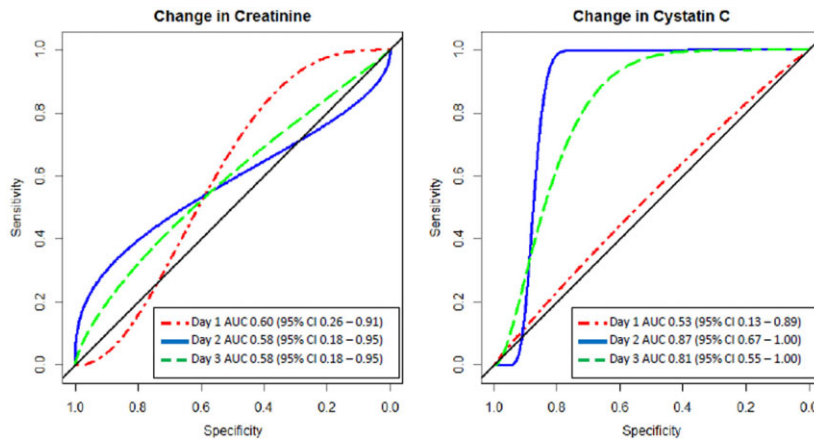


Figure 4. The figure on the left shows receiver operating characteristic curves for serum Cr on days 1, 2, and 3 to identify NGAL-positive AKI. The figure on the right shows receiver operating characteristic curves for serum Cys C. Areas under the curves with 95% confidence intervals for each of them are shown in the inset boxes.

his serum Cys C was 2.2 mg/L on POD 2 and continued to rise to 2.7 mg/L on POD 3. Serum Cr also showed progressive worsening from days 1 to 3 in this patient. Another patient with more severe AKI (post-CPB NGAL > 500 ng/mL) also showed a progressively worsening serum Cys C, peaking at 2.5 mg/L on day 3. The other three patients with milder AKI (post-CPB NGAL < 500 ng/mL) had short, relatively benign courses in the cardiac ICU.

Previous studies in the recent literature have evaluated serum Cys C as an early marker of AKI, as well as its severity, in children sustaining AKI secondary to CPB.⁷⁻¹¹ They have shown that it is a good marker. But all of these studies are limited by the fact that AKI was defined using either serum Cr or Cr-based estimations of GFR. In this study, we defined AKI based on urinary NGAL levels. The etiology for AKI in postcardiac surgery children can be multifactorial. Urinary NGAL levels at 4–6 hours post-CPB have been shown to detect AKI resulting from the CPB, with excellent sensitivity and specificity.¹² As we were specifically interested in identifying AKI resulting from CPB and no other etiologies, using urinary NGAL instead of serum Cr helped us in making a more rational comparison between Cys C and Cr in identifying such injury. Another major limitation of the previous studies is the wide age range of the cohorts. Serum Cys C levels are higher in the infants, especially during the neonatal period.²³ But this is the age group at higher risk for developing AKI. Hence, the higher Cys C levels seen in the AKI groups in previous studies may simply reflect the fact that the AKI group had younger

children compared with the non-AKI group. In our study, to overcome this limitation, we restricted the age for inclusion to less than 1 year. Also, we evaluated the change from baseline instead of absolute value of Cys C, thus minimizing the effect of intersubject variability.

In our study, we also found that urine output on POD 1 is a very sensitive and specific marker for AKI. But on subsequent days it loses its sensitivity. This could potentially be due to aggressive usage of diuretics during the postoperative period in these infants. Also, abbreviated 4-hour urine collection to measure Cr collection could be a potentially useful way to assess renal function in these children.

One of the major limitations of our study is the small sample size. But in spite of this limitation, we were able to show significant differences between the performances of Cys C and Cr in recognizing NGAL-positive AKI. This sample size yielded a power of 75% ($\beta = .25$) for the estimation of area under the receiver operating characteristic curve for Cys C on POD 2. Being a single-center study is another potential limitation. But, on the other hand, the effect of institutional bias is limited as all the patients are from a single institution. Also, our results are consistent with previous studies that have shown similar advantages to using serum Cys C rather than serum Cr.

Conclusions

In our small cohort of infants undergoing cardiac surgery under CPB, we found that serum Cys C is an early and specific biomarker for identification

of NGAL-positive AKI. It showed best performance characteristics as a marker of AKI on POD 2. Serum Cr, on the other hand, is a sensitive, but very nonspecific marker of such AKI. Future studies should focus on looking at the utility of Cys C in identifying non-CPB AKI in children with critical illness.

Authors Contributions

Carrie Herbert—Data acquisition and analysis, drafting of the article, and approval of the submitted final version.

Mehul Patel—Data acquisition and analysis, drafting of the article, and approval of the submitted final version.

Alan Nugent—Research design and interpretation, critical revision of the article, and approval of the final version.

Vivian Dimas—Research design and interpretation, critical revision of the article, and approval of the final version.

Kristine J. Guleserian—Data acquisition, critical revision of the article, and approval of the final version.

Raymond Quigley—Research design and interpretation, critical revision of the article, and approval of final version.

Vinai Modem—Research design, data acquisition, analysis and interpretation, drafting of the article and approval.

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Conflict of interest: None.

Accepted in final form: January 18, 2015.

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