Using Serum Cystatin C to Predict Acute Kidney Injury following Infant Cardiac Surgery

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Abstract

Background: Acute kidney injury (AKI) following cardiopulmonary bypass (CPB) is associated with increased morbidity and mortality. Serum Cystatin C (CysC) is a novel biomarker synthesized by all nucleated cells that may act as an early indicator of AKI following infant CPB.

Methods: Prospective observational study of infants (< 1 year) requiring CPB during cardiac surgery. CysC was measured at baseline and 12, 24, 48, and 72 hours following CPB initiation. Each post-op percent difference in CysC (e.g. $\%CysC_{12hr}$) from baseline was calculated. Clinical variables along with urine output (UOP) and serum creatinine (SCr) were followed. Subjects were divided into two groups: AKI and non-AKI based upon the Kidney Disease Improving Global Outcomes (KDIGO) classification.

Results: AKI occurred in 41.9% (18) of the 43 infants enrolled. Patient demographics and baseline CysC levels were similar between groups. CysC levels were 0.97 ± 0.28 mg/L over the study period, and directly correlated with SCr (R = 0.71, p < 0.0001). Although absolute CysC levels were not significant between groups, the $\%CysC_{12hr}$ was significantly greater in the AKI group (AKI: -16%±22% vs. Non-AKI -28%±9% mg/L; p=0.003). However, multivariate analysis demonstrated that a lower UOP (Odds Ratio:0.298; 95% CI:0.073, 0.850; p=0.02) but not $\%CysC_{12hr}$ was independently associated with AKI.

Conclusions: Despite a significant difference in the $\%CysC_{12hr}$, only UOP was independently associated with AKI. Larger studies of a more homogenous population are needed to understand these results and to explore the variability in this biomarker seen across institutions.

Introduction

Acute Kidney Injury (AKI), occurs in approximately 40% of children following pediatric cardiac surgery and is associated with an increased need for ventilator support, inotropes, utilization of extracorporeal membrane oxygenation (ECMO) and mortality [1-7]. The current diagnosis of AKI relies on the Kidney Disease Improving Global Outcomes (KDIGO) definition, which uses changes in either serum creatinine (SCr) or lower urine output (UOP) to delineate both AKI and its severity [8]. Unfortunately, these measures are often unreliable in neonates and young infants. Neonates and infants have varied creatinine absorption that results from immature proximal tubules, variable muscle mass, and a lower percentage of renal perfusion [9,10]. Furthermore, SCr concentrations are elevated at birth, reflecting maternal creatinine levels, which dramatically decline in the first 5 days of life [11]. Most important is that a significant change to SCr and UOP reflects progression of renal injury to loss of renal function, even in neonates, by which time intervention may have limited benefit [11].

Cystatin C (CysC) is a 13,600 Dalton cysteine protease inhibitor synthesized by all nucleated cells in the human body [12]. Unlike SCr, CysC levels are unaffected by age, gender, muscle mass, disease, or maternal creatinine [12]. Several authors have demonstrated that CysC can be used as an early predictor of AKI in adults and children [12-16]. However, there is limited information on the use of CysC as a

predictor of AKI following infant cardiac surgery requiring cardiopulmonary bypass (CPB). This cohort of children are often the most vulnerable to AKI because they require more extensive operations that in some cases require deep hypothermic circulatory arrest or regional cerebral perfusion.

We sought to characterize perioperative CysC levels from infants undergoing cardiac surgery with CPB. We hypothesized that serum CysC levels would accurately predict AKI at an earlier time point than traditional markers.

Material And Methods

Following Institutional Review Board (IRB) approval, infants requiring cardiac surgery at the University of Rochester (UR)-Golisano Children's Hospital between October 5th, 2020 to May 19, 2021 were approached sequentially for study participation. Inclusion criteria included: 1) Age < 1 year at the time of operation; 2) Expected use of CPB during the repair or palliation; and 3) Urgent or elective operation as defined by the Society of Thoracic Surgeons – European Association for Cardiothoracic Surgery Congenital Heart Surgery Mortality (STAT) Categories [17]. Exclusion criteria included: 1) Pre-operative extra-corporeal membrane oxygenation (ECMO); 2) Non-English speaking parents, or 3) Prior inclusion in the current study.

Operative Methods

Following the induction of anesthesia, a median sternotomy was performed. CPB was achieved using either direct aortic cannulation or cannulation of a 3.5 mm graft sewn to the innominate artery and bicaval venous drainage. In all cases the CPB circuit was connected to a Terumo System 1 Heart Lung Machine (Terumo Corporation, Tokyo, Japan) with an Fx05 oxygenator and hardshell reservoir with integrated arterial line filter (Terumo Corporation, Tokyo, Japan). CPB prime volume (200mL) was similar for all subjects and included: mannitol, bicarbonate, 25% albumin, heparin, tranexamic acid, Plasma-Lyte 148 and allogeneic red blood cells (RBC) when necessary to achieve a hematocrit >24%. Dilutional ultrafiltration was performed at the conclusion of surgery for most subjects, with the exception of those who required aortic arch reconstruction and regional perfusion, in which case zero-balance ultrafiltration was utilized.

When necessary, myocardial arrest was performed using either one or two doses of a modified blood cardioplegia. Regional cerebral perfusion was used in all subjects requiring aortic arch reconstruction. Deep hypothermic circulatory arrest was not utilized during the study period.

Post-Operative Care

Both cerebral and somatic Near Infra-Red Spectroscopy (NIRS) were used and the nadir daily values recorded. Post-operative electrolyte, fluid, and blood product transfusion guidelines were utilized for every subject per local standard of care [18]. Post-operatively patients were given two-thirds maintenance intravenous fluids (IVF) until the morning of post-operative day (POD) 1 or until extubation from invasive

mechanical ventilation (whichever is longer). Maintenance IVF consisted of 10% dextrose and one quarter normal saline (D10 + 0.22% NaCl) in infants < 6 months of age, and 5% dextrose with one half normal saline (D5 + 0.45% NaCl) in infants > 6 months of age. Maintenance IVF rate was calculated using the Holliday-Segar method [19]. Patients were transitioned to a full maintenance IVF rate following extubation and transitioned off IV fluids once tolerating enteral feeds. As per local standard of care, diuretic therapy is not administered prior to POD 2, and only if there is adequate intravascular volume and stable hemodynamics.

Strict measurements of all intake and output were tracked per intensive care unit (ICU) protocol with the aid of an indwelling urinary catheter. Assessment for "fluid overload" was determined at ICU admission, and on POD 0, 1, 2, and 3 by dividing each subject's net fluid balance (milliliters) by their weight (grams), and multiplying by 100 (i.e., net balance(mL)/weight(gm) x 100). Subject characteristics and details regarding cardiac morphology, surgical procedure and intra-and post-operative data were collected from the electronic medical record.

Pre-operative and post-operative factors potentially impacting renal function were assessed and included: 1) Nephrotoxic agent exposure within 7 days prior to surgery of 3 medications as outlined by Goldstein, et al, along with all non-steroidal anti-inflammatory drugs and intravenous contrast agents [20]; 2) Chronic kidney injury defined as any evidence of structural nephro-ureteral abnormality or a prior AKI episode without normalization to baseline SCr in those 3 months of age, or a SCr > 0.4 mg/dL for the past 3 months; 3) Renal angina index was defined using a calculation based on inotropic support, fluid overload and creatinine change as outlined by Menon, et al [21]; 4) Shock defined as arterial lactate > 4 mmol/L on two sequential blood gases after the initial post-operative peak and nadir, or 2 or more signs of end-organ injury; 5) "urgent surgery" defined as non-elective cases that required urgent admission or continued inpatient stay to undergo surgery before discharge home; 6) Severe bleeding was defined as a) bleeding that leads to 1 or more organ dysfunction, or b) bleeding that leads to hemodynamic instability (>20% increase in HR or >20% decrease in BP)[22], or c) requirement for surgical exploration for cardiac tamponade or bleeding in the first 24 hours; 7) Necrotizing enterocolitis (NEC) was defined as stage IIA NEC or greater as outlined by the modified Bell staging criteria [23]; 8) The highest vasoactive inotropic score (VIS) on POD 0-3 was calculated as outlined by Gaies, et al. [24].

Laboratory Measures

The hospital laboratory was utilized for all standard laboratory analyses, including hemoglobin, lactate, blood urea nitrogen (BUN), and SCr per local standard of care. Baseline measurements were routinely taken at either the morning of, or 1-3 days prior to surgery. Post-operative measurements were obtained at ICU admission and in the morning during each post-operative day.

Baseline measurements for serum CysC were obtained at time of intra-operative central line placement prior to surgical stimulation, and at 12, 24, 48, and 72 hours following CPB initiation. 1.3 mL of blood was collected in a plasma separator tube, centrifuged at 1100 x g for 10 minutes at room temperature (20°C) and serum supernatant aliquoted into a 2 mL conical microcentrifuge tube within 1 hour of collection and

stored at -80°C. The International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) calibration was used for Cystatin C concentration, which was reported in mg/L, as previously outlined by Schwartz, et al [25]. Samples were thawed and analyzed using a commercially available kit for human cystatin C (e.g. N latex CysC Kit standardized to ERM-DA471/IFCC reference material; Siemens Healthineers, Erlangen, Germany) and the Siemens BN II nephelometric analyzer (Siemens Healthcare Diagnostics, Newark, DE) via a latex-enhanced immunoassay using a 1:100 dilution in phosphate buffered saline performed with a six-point calibration generated from multiple dilutions of a human cystatin C calibrator [26]. The lower limit of detection for CysC was 0.27 mg/L. The intra-assay and inter-assay coefficients of variation were below 5%.

Outcome

Subjects were divided in 2 groups for primary analysis (AKI and non-AKI within POD 0-3) based on meeting the criteria for AKI as per KDIGO criteria:

1) Stage 1 AKI: SCr ≥ 1.5-1.9 times baseline, or SCr increase ≥ 0.3 mg/dl within 48 hours, or a urine output < 0.5mL/kg/hr for 6-12 hours;

2) Stage 2 AKI: SCr ≥ 2 – 2.9 times baseline or urine output < 0.5 mL/kg/hr for ≥ 12 hours;

3) Stage 3 AKI: SCr 3 times baseline, or increase in SCr ≥ 4.0 mg/dL or initiation of renal replacement therapy, or a urine output < 0.3 mg/kg/hr for ≥ 24 hours, or anuria for ≥ 12 hours [8,27].

Statistics

Data are presented as mean ± standard deviation, frequency and percentage, or median with inter-quartile range (IQR). The percent change in post-operative CysC, SCr, and BUN from baseline (%CysC, %SCr, %BUN respectively) were calculated (e.g. $\%CysC_{12hr} = (CysC_{12hr} - CysC_{baseline})/CysC_{baseline} \times 100$) for each post-operative time point as previously described [14]. Continuous variables were evaluated using the Shapiro-Wilk test for normality. Significance was determined using either a 2-tailed Student's t-test or Mann-Whitney when comparing two groups. To examine differences of SCr, BUN, CysC, and urine output over sequential time points, a repeated measures test was performed. Comparison of categorical variables were evaluated with Fisher's exact test. Linear regression was used to identify the relationship between CysC and other markers for AKI, as well as change in fluid status. Receiver Operating Characteristic (ROC) curves were constructed to identify variables that could predict AKI during POD 0 and the area under the curve (AUC) quantified. The two variables with the highest predictive ability were combined using a binary logistic regression to identify if together they increased the predictive ability for AKI. A backward stepwise logistical regression method identified variables associated with AKI, such that variables were removed until all p values in the model were significant. All statistics were completed using GraphPad Prism version 5.0b (GraphPad Software, San Diego CA) and SPSS 28 where a *P* value of < 0.05 was considered statistically significant.

Results

Forty-three children were included in the current prospective observational study (Figure S1 data supplement). AKI was identified in 18 (41.9%) subjects, often on POD 1, and was most often limited to Stage 1 (Table S1 data supplement). Baseline demographics were similar between the AKI and non-AKI groups. Only the use of antegrade cerebral regional perfusion was significantly associated with post-operative AKI (Table 1). Difference in timing of initiation of diuretic therapy on POD 2 was not statistically significant, although the AKI group was more likely to have diuretics withheld (Table 2). Peak arterial lactate was significantly greater within the AKI group (Table 3). There were no differences in the rate of complications, but the AKI group had a significantly longer intensive care unit length of stay (Table 3). Although there was no difference in intra-operative volume administration or fluid balance, post-operative net fluid balance on POD 0-2 was significantly higher, along with increased mediastinal tube (MT) output in the AKI group (Table 4). There were two operative mortalities, both in the AKI group (description of mortalities are found within the data supplement). One of those subjects required continuous renal replacement therapy on POD 23. No other subjects required dialysis.

From two hundred and five CysC values, the mean serum CysC measured over the study period was 0.97±0.28 mg/L and was normally distributed (Figure 1a). CysC was unable to be calculated from one 12-hour, two 24-hour, two 48-hour, and five 72-hour samples due to either patient demise, sample hemolysis, or removal of central venous access. There was a strong direct correlation between CysC and SCr measurements ($R = 0.71$, $p < 0.0001$), a weaker correlation between CysC and BUN, and a poor correlation with CysC and UOP (Figures 1b, 1c, and 1d). There were no baseline differences in SCr, BUN, or CysC between the AKI and Non-AKI groups. CysC values decreased in 90% (38) of subjects at 12-hours from baseline measurements, and the majority of CysC levels remained below baseline at each successive time point (Figure 2a & 3a). There were no significant differences in the absolute CysC levels between groups during the post-operative period. In contrast, SCr was significantly greater on POD 1 and 2 while BUN values were significantly greater on POD 2 (Figures 2b and 2c). Urine output was significantly lower on POD 0,1 and 2 within the AKI group (Figure 2d). Calculated GFR from CysC and SCr, as outlined by Schwartz, et al., was not significantly different between groups (Figures S2a and S2c, Data supplement) [28].

While absolute values for CysC were not significantly different, the percent change of CysC from baseline was significantly greater within the AKI group at 12 ($p = 0.003$), 24 ($p=0.02$), and 48-hours ($p=0.02$) (Figure 3a). Similarly, the percent change in GFR as measured by CysC was significantly lower at 12, 24, and 48 hours following CPB within the AKI group (Data supplement Figure S2b). The percent change in SCr was significantly greater on POD 1 and 2, along with the percent change in BUN on POD 2 within the AKI group (Figures 3b and 3c). The percent change in the GFR as measured by SCr was significantly lower on POD 1 and 2 (Data supplement Figure S2d). There were no direct correlations between the percent change in post-operative CysC and CPB hemofiltration, or parameters of intra-operative and post-operative fluid balance (Data Supplement Figures S3a-d).

A ROC curve was constructed to understand if the percent change in 12-hour CysC was able to earlier discriminate AKI than using the standard markers (SCr, BUN, UOP) (Figure 4). Although absolute values of SCr and CysC were not significantly predictive, the percent difference in CysC at 12 hours trended toward significance (AUC:0.67 p=0.053). Urine output during POD 0 was the greatest predictor of AKI (AUC: 0.71, p=0.021). The combination of UOP and the $\%CysC_{12hr}$ increased the AUC (0.76), as well as the significance (Figure S4). Multivariate analysis demonstrated that the percent change in CysC at 12 hours was not independently associated with AKI, but that the use of antegrade cerebral perfusion, and decreased urine output on POD 0 were independently associated with the development of AKI (Table 5). By either POD 7 or hospital discharge (whichever was earlier), only 17 % (n=3) of the AKI group and 24.0% (n=6) of the non-AKI group, had a SCr value that was above baseline measurements (Table S2 data supplement).

Discussion

Our study of infants undergoing CPB for cardiac surgery did not show a specific level of CysC that defined AKI, however the percentage change of CysC was associated with AKI. The most predictive early indication of AKI was UOP. The combination of percentage CysC change and decreased UOP on POD 0 increased the ability to predict AKI. In multivariate analysis, only decreased UOP on POD 0 and the use of antegrade regional cerebral perfusion were independently associated with AKI.

The definition of pediatric AKI has varied; defined by the Acute Kidney Injury Network (AKIN) criteria, p-ediatric-modified Risk Injury Failure Loss End-stage kidney disease (pRIFLE) class, and most recently the KDIGO guidelines[8,27,29,30]. Although the KDIGO criteria is now the most commonly accepted, each definition has its advantages, and though they differ in diagnostic criteria, all have demonstrated strong ability to predict increasing mortality and ICU length of stay with progressive severity of AKI staging [31]. Interestingly, our rate of AKI using the KIDGO guidelines within infants, who often are the most vulnerable to injury, was similar to other groups who included older children using less sensitive criteria that did not include urine output [1-3,5,6]. This may reflect both our CPB strategy as well as limited early use of diuretics.

Similar to our results, other studies in adults and children have shown that CysC levels decline below baseline during the early post-operative period [2,3,16,32,33]. Hassinger et al. demonstrated that post-operative CysC declines in proportion to volume of hemofiltration during CPB and remains below pre-operative baseline values in children without AKI, whereas values rise above baseline in children with AKI [34]. Adult studies have also found CysC values to fall below baseline post-operatively [16,33]. The magnitude in the change of CysC may be related to variable practices in intra-operative fluid management which have commonly not been investigated. Our practice of using zero-balance ultrafiltration for aortic arch reconstructions, and dilutional ultrafiltration for all other cases, may have impacted the drop in CysC measurements seen in our patient cohort. We found that although a greater net positive fluid balance correlated with AKI on POD 0, 1, and 2, this did not directly correlate with a

change in CysC values suggesting multiple parameters are likely involved in the variability of CysC measurements.

We demonstrated only a modest ability to predict AKI in infants 12 hours after CPB initiation using the percent change in CysC. The literature regarding the use of CysC to predict AKI is mixed. Although some studies have demonstrated that CysC was predictive of AKI at 2, 6, and 12 hours in children after surgery, other groups have found the opposite [2,35-37]. In a large meta-analysis in adults, the discriminatory capacity for serum CysC in predicting AKI within 24 hours of cardiac surgery was modest at best [38]. A similar pilot study by Zheng and colleagues showed that serum CysC had a positive predictive value of only 62% in detecting AKI in children under 3 years of age after CPB [2]. Greenberg, et al. examined 408 children after cardiac surgery and found serum CysC to be a poor discriminator of AKI progression [3]. Thus, the accuracy of CysC to predict AKI appears variable, with conflicting results.

There are several reasons for the heterogeneity among studies and why CysC may not be a reliable early marker of renal injury following CPB. The pathophysiology of AKI after cardiac surgery when using CPB is multi-factorial. Cardiac morphology and physiology is altered at different time points (pre-, intra-, and post-operative) and changes in systemic vasoconstriction, cardiac output, hemodynamics, inflammation and neuroendocrine systems likely contribute to renal injury [39]. The causative factors of renal injury during CPB alone are multi-factorial, including non-pulsatile CPB flow, endogenous and exogenous nephrotoxins, microemboli from platelet and blood cell aggregates, fluid overload, and the systemic inflammatory response resulting in cellular and cytotoxic injury, all contributing to tubular injury [40]. Glomerular injury follows tubular injury, and given that CysC is a marker of glomerular filtration, elevation in CysC may thus be delayed.

We focused on infants requiring cardiac surgery in conjunction with CPB as this population is at high risk for renal injury and has not been extensively studied in predicting AKI with CysC. Unique to our study, we included urine output in defining AKI as per the KDIGO guidelines, to maximize the validity and accuracy in determining our incidence of AKI. To help answer the question of whether fluid overload effects serum CysC concentrations, we looked at the correlation between CysC values and percent change in CysC with relation to intra-operative net fluid intake, intra-operative and post-operative net fluid balance, and amount of hemofiltration used after bypass, and found no significant correlation.

This study has several limitations including a small sample size with a heterogenous group of infants receiving a wide range of surgeries across all surgical severity levels, and as such had a wide-ranging clinical course. Several CysC levels were unavailable at each time point. Daily weights are not obtained routinely for critically unstable patients in the immediate post-operative period per local standard of care and were therefore not available for determination of fluid balance. CysC levels were drawn at intervals in relationship to CPB initiation, but serum creatinine and BUN levels were obtained at intervals according to local standard of care.

Conclusion

We demonstrate that percent changes in CysC at 12 hours are modestly predictive of AKI from infants requiring CPB during cardiac surgery, highlighting the importance of characterizing change from baseline, similar to SCr. Earlier indication of AKI would allow the clinician to tailor medical management to ameliorate renal injury and promote renal recovery. Given the complexities of post-operative congenital cardiac surgical management (e.g., intravascular volume status and fluid shifts, different aortic cross-clamp times and ultrafiltration practices, alterations in hemodynamics and cardiac function, varying volume of distribution in infants, and medication utilization that often includes requirement of nephrotoxic medications), biomarkers that are specific and sensitive for renal injury are needed. This study highlights the difficulty in finding a generalizable serum biomarker across all age groups, disease conditions and institutional practices. It is likely that each center's intra-operative and CPB fluid management protocols may affect serum CysC levels and limit this measure's applicability across institutions. This pilot data highlights the need for larger studies controlling for intra-operative and surgical variables examining biomarkers on infants undergoing higher risk surgeries where AKI is common.

Declarations

Compliance with Ethical Standards:

- Funding: Partial funding for the study was provided by the Clausen and Bradford Fellowship Award. University of Rochester Medical Center. Abadeer M, Cholette J. October 16, 2020. Status: Awarded. Amount: $25,000.
- Disclosure of potential conflicts of interest: Individual authors have no financial disclosures or conflicts of interest.
- Research involving Human Participants: study approval was obtained from the Research Subjects Review Board to ensure that rights and welfare of human subjects is protected
- Informed consent: informed written consent was obtained from all parent guardians along with written assent or script assent from pediatric subjects when age-appropriate.

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Tables

Table 1. Pre- and Intra-Operative Details

Demographics	No AKI (n=25)	AKI (n=18)	P-Value
Age (months)	3.7 [1.0,6.8]	2.7 [0.5,7.7]	0.692
Neonate (<30 days)	28.0% (7)	38.9% (7)	0.521
Prematurity (<37 weeks gestational age)	16% (4)	11% (2)	>0.999
Male Gender	68.0% (17)	66.7% (12)	>0.999
Caucasian Race	80.0% (20)	88.9% (16)	0.680
Non-Hispanic Ethnicity	100.0% (25)	100.0% (18)	>0.999
Weight (kg)	5.4 [3.6,6.4]	5.1 [3.4,7.4]	0.841
Height (cm)	58 [52,64]	55 [50,69]	0.756
Pre-Operative Risks			
Single ventricle physiology	12% (3)	16.7% (3)	0.683
Cyanotic	36.0% (9)	33.3% (6)	>0.999
Prostaglandin infusion	16.0% (4)	44.4% (8)	0.083
Extra-Cardiac Abnormality	44.0% (11)	33.3% (6)	0.541
Chromosomal Abnormality	36.0% (9)	27.8% (5)	0.744
Mechanical Ventilation	12.0% (3)	22.2% (4)	0.427
Shock	4.0% (1)	22.2% (4)	0.144
Nephrotoxin	12.0% (3)	11.1% (2)	>0.999
CKD	12.0% (3)	5.6% (1)	0.628
Urgent Surgical Repair	44.0 % (9)	61.1 % (11)	0.130
Primary Operative Procedure			
Tetralogy of Fallot repair	20.0% (5)	5.6 % (1)	0.375
VSD repair	28.0 % (7)	16.7 % (3)	0.480
TAPVR	8.0 % (2)	5.6 % (1)	>0.999
Aortic Arch Repair VSD repair	12.0% (3)	16.7% (3)	0.683
Bidirectional Glenn procedure	8.0% (2)	16.7% (3)	>0.999
Aortic Arch Repair with PA Band	0.0% (0)	11.1% (3)	0.066
Norwood	0.0% (0)	11.1% (2)	0.169
RVOT Procedure	0.0% (0)	11.1% (2)	0.169

DORV repair	12.0% (3)	0.0% (0)	0.253
Other	12.0% (3)	11.1 % (2)	>0.999
STAT category 4 or 5	44.0% (11)	61.1% (11)	0.358
Operative Details			
CPB time (min)	101 [84,133]	110 [94,160]	0.233
Aortic cross clamp (min)	55 [41, 80]	53 [40, 72]	0.648
Regional perfusion (%)	12% (3)	44% (8)	0.031
Regional perfusion (min)	33 [31,36]	31 [27,78]	0.812
Nadir Intra-operative Temp (°C)	30.7 [29.2,33.8]	28.4 [23.6,34]	0.328

All data presented as median [IQR] or percentage with frequency

Abbreviations

SV: single ventricle; PGE: prostaglandin; CKD: chronic kidney disease; STAT: The Society of Thoracic Surgeons-European Association for Cardio-Thoracic Surgery score; VSD: ventricular septal defect; TAPVR: total anomalous pulmonary venous return; PA: pulmonary artery; RVOT: right ventricular outflow tract; DORV: double outlet right ventricle; CPB: cardiopulmonary bypass.

Table 2 Post-Operative Variables

	Non-AKI (n=25)	AKI (n=18)	P-Value
Renal NIRS			
Baseline	81 [76,89]	76 [60,88]	0.250
POD 0	83 [76.0, 88.0]	80.5 [69.3, 86.3]	0.297
POD 0 % change from baseline	*1.2 [-8.5,10.8]*	*1.4 [-5.6,9.8]*	*0.973*
POD 1	80 [70.7, 86]	77.0 [71.0, 82.0]	0.389
POD 1 % change from baseline	*-4.3 [-13.6,4.3]*	*-1.9 [-10.7,3.7]*	*0.625*
Renal Angina Index[a]			
POD 0	5 [5,10]	8 [5,10]	0.508
POD 1	10 [7.5,20]	20 [10,20]	0.102
POD 2	8 [3,20]	10 [6,30]	0.265
POD 3	5 [2,10]	10 [2.5,30]	0.206
Inotrope Utilization			
Peak Post-Operative VIS[b]	10 [10,10]	10 [9,13]	0.584
Inotrope duration (days)	1 [1,3]	1 [1,3.3]	0.817
Milrinone duration (days)	3 [1,4.5]	3.5 [1,10.3]	0.352
Nephrotoxin exposure	4% (1)	5.6% (1)	>0.999
Diuresis			
Initiation of Diuretics			
POD 1	0.0% (0)	5.6% (1)	0.419
POD 2	88.0% (22)	58.8% (10)	0.062
POD 3	88.0% (22)	88.2% (15)	>0.999
1st POD with negative fluid balance	2 [2,3]	3 [2,3.3]	0.139
Dexmedetomidine			
POD 0	68.0% (17)	55.6% (10)	0.526

POD 1	60.0% (15)	52.9% (9)	0.756
POD 2	36.0% (9)	35.3% (6)	>0.999

All data presented as median [IQR] or percentage with frequency

Abbreviations

POD: post-operative day; NIRS: near-infrared spectroscopy; VIS: Vasoactive Inotropic Score.

[a]calculated by assigning two scores and multiplying them together, giving a range of 1-40:

1) AKI Risk score: 1 point for ICU level care or 5 points if on vasoactive support and mechanical ventilation.

2) AKI Injury score: an injury score of 1,2,3,4, or 8 points is assigned based on degree of fluid overload or creatinine elevation as outlined in figure 1 by Menon, et al. [21]

[b]calculated as outlined by Gaies, et al, using vasoactive drip rates in units of mcg/kg/min as follows: [dopamine rate] + [10 x milrinone rate] + [100 x epinephrine rate], etc. = VIS. [24]

Table 3 Post-Operative Laboratory Measures and Outcomes

Laboratory Measures	Non-AKI (n=25)	AKI (n=18)	P-Value
Peak Arterial Lactate (mmol/L)	_	_	_
POD 0	2.9 [2.2,3.7]	4.9 [2.6,6.2]	0.008
POD 1	1.8 [1.1,2.5]	2.4 [1.4,3.3]	0.166
Hemoglobin (g/dL)	_	_	_
Baseline	12 [10.6,12.5]	11.2 [10.3,11.7]	0.205
POD 0	12.4 [11.4,13.5]	11.9 [11.4,12.6]	0.235
POD 1	12.0 [11,13.2]	12.4 [11.4,13.8]	0.489
POD 2	11.9 [11,13.2]	11.9 [11.4,12.9]	0.756
Complications			
Delayed chest closure	12.0% (3)	22.2% (4)	0.427
CPR (%)	0.0% (0)	11.1% (2)	0.169
Severe Bleeding (%)	0.0% (0)	11.1% (2)	0.169
Shock (%)	8.0% (2)	27.8% (5)	0.110
NEC	0.0% (0)	5.6% (1)	0.419
Infection	8.0% (2)	22.2% (4)	0.218
Outcomes			
Mechanical Vent (days)	2 [1,5]	3 [1,23]	0.192
Hospital LOS (days, median)	12 [7,15]	15 [7,38]	0.076
ICU LOS (days, median)	5 [2,10]	11 [4,31]	0.044
Mortality (%)	0.0% (0)	11.1% (2)	0.169

Abbreviations

POD: post-operative day; CPR: cardiopulmonary resuscitation; NEC: necrotizing enterocolitis; LOS: length of stay; ICU: intensive care unit.

All data presented as median with IQR or percentage with frequency

Table 4. Intra- and Post-Operative Fluid Balance

_	Non-AKI (n=25)	AKI (n=18)	P-Value
Intra-Operative Fluid Balance			
CPB prime volume (cc/kg)	36.8 [31.7,56.9]	42.7 [24.6, 59.6]	0.976
Red blood cell transfusion (cc/kg)	11.9 [6.4,18.1]	14.2 [7.5,24.4]	0.473
Intra-operative urine output (cc/kg)	11.2 [7.1,21.7]	9.9 [2.5,17.1]	0.266
Intra-operative total fluid (cc/kg)	109.7 [81.4,141.4]	125.8 [87.5,170.4]	0.220
Net intra-operative fluid balance (cc/kg)	8.0 [-4.0,25.6]	16.2 [-8.5,33.8]	0.968
Net intra-operative fluid balance (%)	0.8 [-0.4,2.6]	1.6 [-0.9,3.4]	0.968
Post-Operative Fluid Balance			
POD 0			
Mediastinal chest tube output (cc/kg/hr)	1.1 [0.9,1.5]	1.4 [0.9,1.8]	0.529
Net fluid balance (%)	1.0 [0.4,1.9]	1.8 [1.2,3.0]	0.038
POD 1			
Cell saver transfusion (cc/kg)	9.9 [0,10.3]	4.9 [0,11.6]	0.814
Red blood cell transfusion (cc/kg)	0 [0,0]	0 [0,0]	0.502
Mediastinal chest tube output (cc/kg/hr)	0.7 [0.6,1.2]	0.7 [0.5,1.3]	0.954
Net fluid balance (%)	7.2 [4.6,10.3]	9.6 [7.1,13.4]	0.029
POD 2			
Red blood cell transfusion (cc/kg)	0 [0,0]	0 [0,0]	0.751
Mediastinal chest tube output (cc/kg/hr)	0.6 [0.5,0.9]	0.9 [0.5,1.5]	0.036
Net fluid balance (%)	7.5 [2.9,11.0]	9.5 [7.6,15.8]	0.015
POD 3			
Red blood cell transfusion (cc/kg)	0 [0,0]	0 [0,0]	0.419
Mediastinal chest tube output (cc/kg/hr)	0.4 [0.3,0.8]	0.7 [0.4,1.1]	0.064
Net fluid balance (%)	7.3 [0.7,11.3]	10.2 [-1.7,14.0]	0.364

Table 5. Multivariate analysis to predict AKI

Variable	Odds Ratio	95 % CI	P value
Age	-	-	0.883
Male Gender	-	-	0.488
Regional Perfusion	1.057	1.006, 1.110	0.027
Change in CysC	-	-	0.187
Change in SCr	-	-	0.439
Change in BUN	-	-	0.943
UOP	0.248	0.073, 0.850	0.026

Abbreviations

CysC: cystatin C; SCr: serum creatinine; BUN: blood urea nitrogen.

Figures

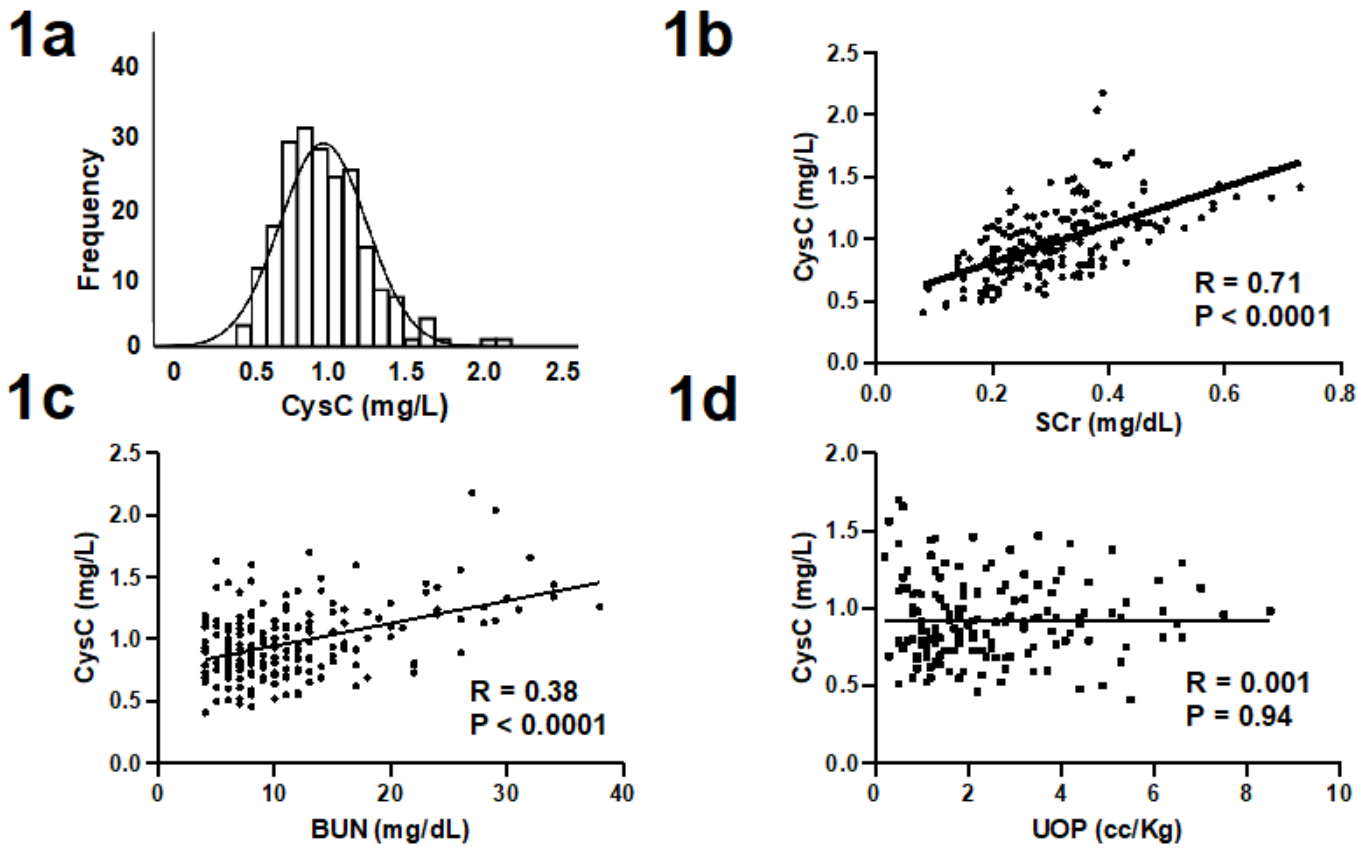

Figure 1

a) Histogram of Baseline, and all post-operative serum Cystatin-C (CysC) values from a cohort of 43 infants requiring cardiac surgery with cardiopulmonary bypass, **1b)** Linear regression (solid line) and 95% confidence intervals (dashed line) between baseline and post-operative serum creatinine (SCr) and CysC values, **1c)** Linear regression(solid line) and 95% confidence intervals (dashed line) between baseline and post-operative CysC and blood urea nitrogen (BUN) values, **1d)** Linear regression(solid line) and 95% confidence intervals (dashed line) between post-operative serum CysC values and urine output (UOP)

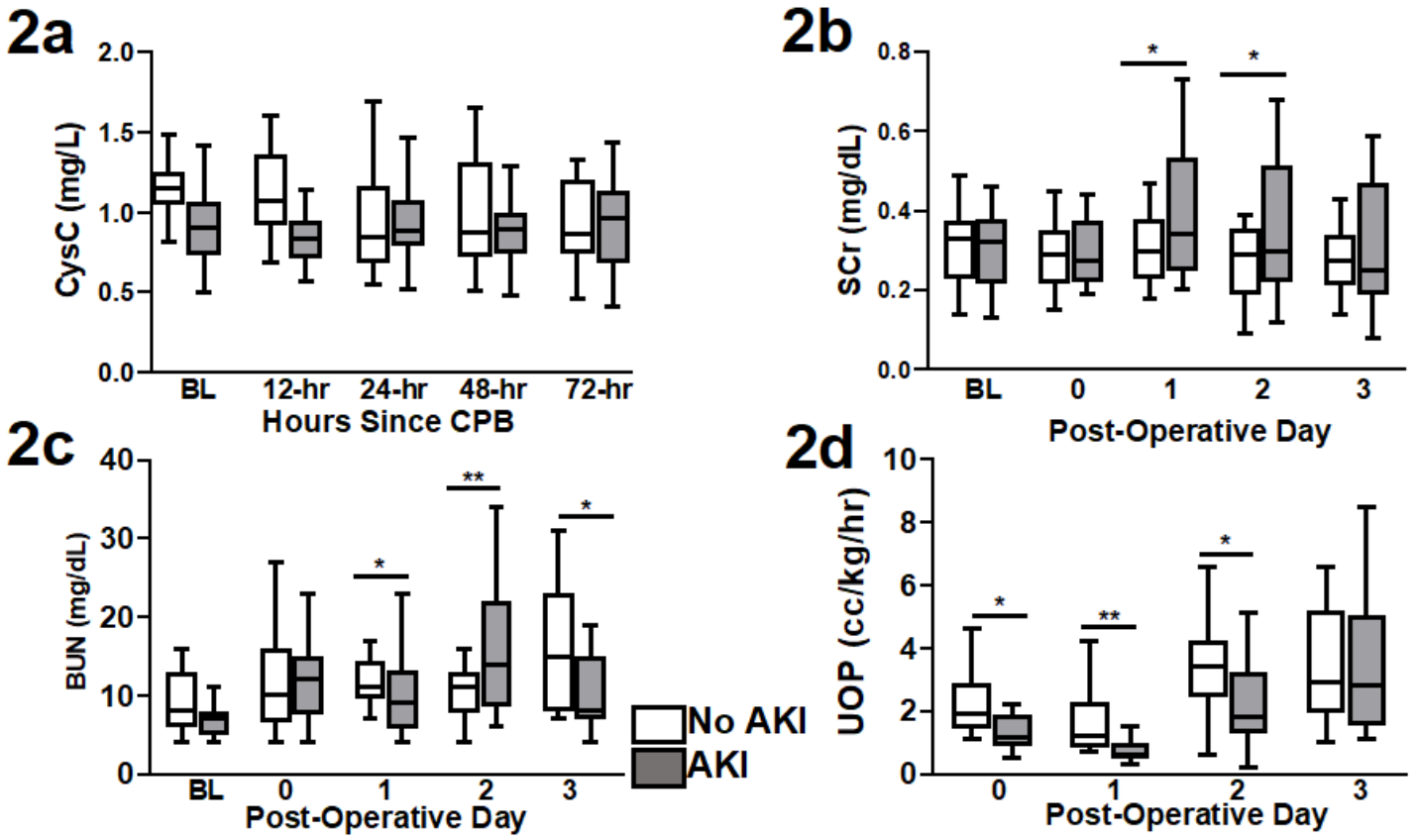

Figure 2

Box and whisker Plots for infants that developed acute kidney injury (grey) and those who did not (white): **2a**) Serum Cystatin C (CysC), 2b) Serum Creatinine (SCr), 2c) Blood Urea Nitrogen (BUN), and 2d) Urine output (UOP) at each time point

*Denotes P value < 0.05 **Denotes P value of < 0.01

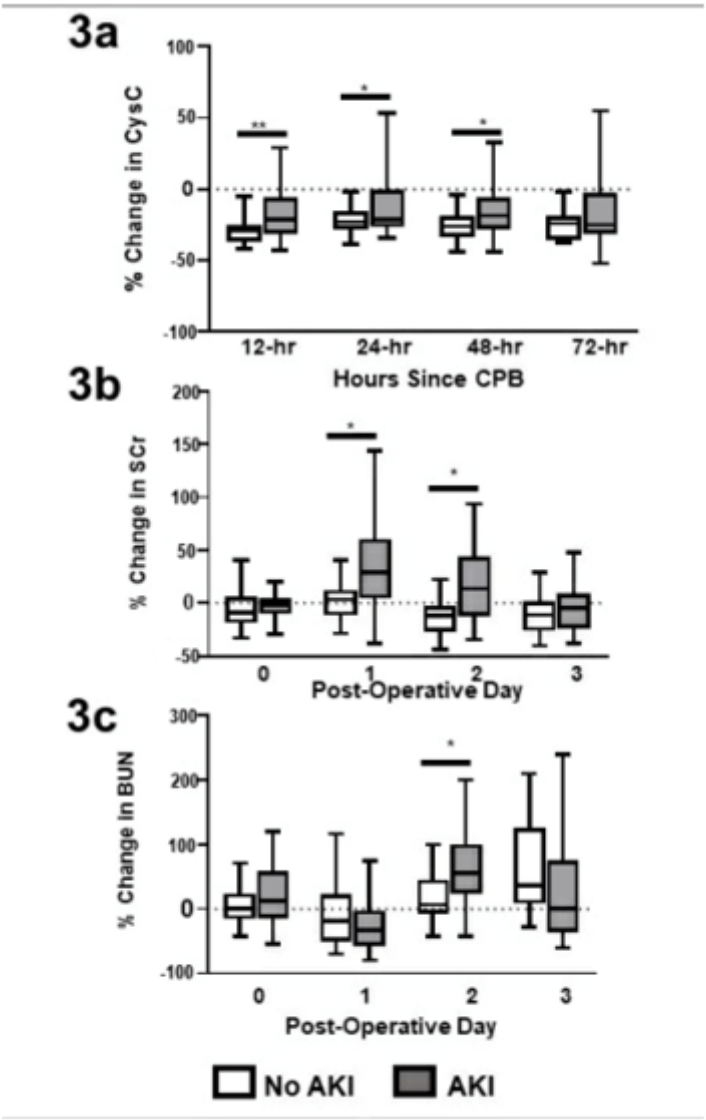

Figure 3

Box and Whisker Plots of infants that developed acute kidney injury (grey) and those who did not (White): **3a)** Percent change in Serum Cystatin C (CysC), 3b) Percent change in Serum Creatinine (SCr), and 3c) Percent change in Serum Blood Urea Nitrogen (BUN) at each time point

*Denotes P value < 0.05 **Denotes P value of < 0.01

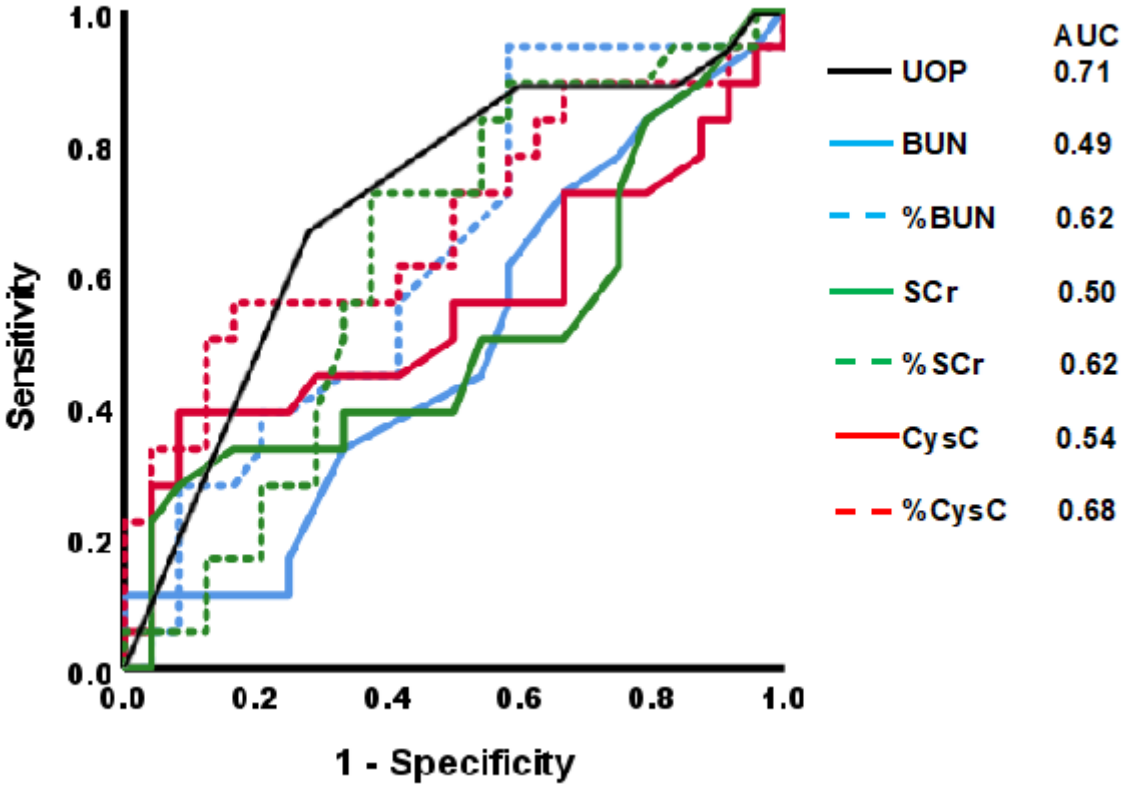

Figure 4

Receiver operating characteristic curve demonstrating the Area Under the Curve (AUC) for renal markers on post-operative day 0 to predict acute kidney injury

Abbreviations: UOP-Urine output, BUN-Blood Urea Nitrogen, SCr-Serum Creatinine, CysC-Cystatin

Supplementary Files

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