Cardiology in the Young

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Urine biomarkers, acute kidney injury, and fluid overload in neonatal cardiac surgery

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Original Article

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Abstract

Background: Cardiac surgery-associated acute kidney injury (CS-AKI) and fluid overload (FO) are common among neonates who undergo cardiopulmonary bypass, and increase mortality risk. Current diagnostic criteria may delay diagnosis. Thus, there is a need to identify urine biomarkers that permit earlier and more accurate diagnosis. *Methods:* This single-centre ancillary prospective cohort study describes age- and disease-specific ranges of 14 urine biomarkers at perioperative time points and explores associations with CS-AKI and FO. Neonates (≤28 days) undergoing cardiac surgery were included. Preterm neonates or those who had pre-operative acute kidney injury were excluded. Urine biomarkers were measured pre-operatively, at 0 to < 8 hours after surgery, and at 8 to 24 hours after surgery. Exploratory outcomes included CS-AKI, defined by the modified Kidney Disease Improving Global Outcomes criteria, and>10% FO, both measured at 48 hours after surgery. *Results:* Overall, α-glutathione S-transferase, β-2 microglobulin, albumin, cystatin C, neutrophil gelatinase-associated lipocalin, osteopontin, uromodulin, clusterin, and vascular endothelial growth factor concentrations peaked in the early post-operative period; over the sampling period, kidney injury molecule-1 increased and trefoil factor-3 decreased. In the early post-operative period, β-2 microglobulin and α-glutathione S-transferase were higher in neonates who developed CS-AKI; and clusterin, cystatin C, neutrophil gelatinase-associated lipocalin, osteopontin, and α-glutathione S-transferase were higher in neonates who developed FO. *Conclusion:* In a small, single-centre cohort, age- and disease-specific urine biomarker concentrations are described. These data identify typical trends and will inform future studies.

Introduction

Cardiac surgery-associated acute kidney injury (CS-AKI) in neonates with congenital heart disease (CHD) is a heterogeneous disease process that both contributes to and is a consequence of fluid overload (FO).[1] CS-AKI and FO are common among neonates who undergo cardiopulmonary bypass where between 42 and 75% of neonates develop CS-AKI[2,3] and between 26 and 65% of neonates develop FO.[4,5] Although concomitant CS-AKI and FO is predictive of the worst outcomes,[6] both CS-AKI[7] and FO[5] have also been independently associated with poor outcomes. Neonates who develop CS-AKI require longer intensive care unit (ICU) and hospital stays, more days of mechanical ventilation, and have a higher mortality rate when compared to neonates without CS-AKI.[2] Neonates who develop FO after cardiac surgery have an increased risk of cardiac arrest, longer ICU stays, and an increased risk of mortality.[5] Despite this increased risk of poor outcomes, diagnosis of CS-AKI and FO is often delayed, and treatment remains supportive.[3]

The diagnosis of CS-AKI has historically relied on traditional definitions of AKI based on serum creatinine (SCr) and urine output.[8] There are major limitations of this definition in neonates after cardiac surgery including difficulty accurately characterising urine output,[2,9] such as in the setting of prophylactic peritoneal dialysis, a delay between the onset of renal injury and a change in SCr level[10] and variability in SCr levels based on sex, muscle mass, dilution from fluid accumulation, and maternal levels in the first week of life.[11] Thus, there is a need to identify alternative biomarkers that permit earlier and more accurate diagnosis of CS-AKI and FO.

Prior studies in adults and children after cardiac surgery have demonstrated an association between urine biomarkers and CS-AKI.[3,12] However, studies in neonates have yielded mixed results[2] partly due to incomplete characterisation of normal age- and disease-specific urine biomarker concentration ranges.[13] One study of critically ill infants after cardiac surgery investigated the association between urine biomarkers and CS-AKI/FO phenotypes,[14] but this relationship has not been explored exclusively in the neonatal population undergoing cardiac surgery. In this manuscript, we describe age- and disease-specific ranges of 14 urine biomarkers (cystatin C [uCysC], neutrophil gelatinase-associated lipocalin [uNGAL], kidney injury

molecule-1 [uKIM1], albumin [uAlb], β-2 microglobulin [uβ2M], calbindin [uCal], clusterin [uClust], epidermal growth factor [uEGF], osteopontin [uOPN], osteoactivin [uOsteo], trefoil factor-3 [uTFF3], uromodulin [uUMOD], vascular endothelial growth factor [uVEGF], and α-glutathione S-transferase [uαGST]) at multiple perioperative time points in neonates with CHD undergoing cardiac surgery on cardiopulmonary bypass and explore their association with CS-AKI, FO, and CS-AKI/FO phenotypes.

Materials and methods

We performed a single-institution ancillary prospective cohort study of neonates with CHD enrolled in the Steroids to Reduce Systemic Inflammation after Infant Heart Surgery (STRESS) trial (NCT03229538)[15,16] who underwent surgery with cardiopulmonary bypass in the first 28 days of life at Duke University Medical Center (Durham, NC, USA). As described in detail elsewhere, the STRESS trial was a multi-site, randomised, double-blind, placebo-controlled trial designed to evaluate the safety and efficacy of perioperative steroids in infants undergoing cardiac surgery on cardiopulmonary bypass.[15,16] All infants approached for enrolment in the STRESS trial at Duke between June 2019 and May 2020 were given the option to opt-in to additional urine sampling. We excluded infants <37 weeks corrected gestational age at time of surgery and those who had pre-operative AKI or renal failure. Informed consent was obtained from all parents or legal guardians. The study was approved by the Duke Institutional Review Board.

We prospectively collected the following demographic and clinical data from the electronic health record (EHR): postnatal age at surgery; weight at surgery (anchor weight); sex; race; ethnicity; cardiac diagnosis; surgery performed, including the Society of Thoracic Surgeons – European Association for Cardio-Thoracic Surgery Congenital Heart Surgery Mortality (STAT) category,[17] cardiopulmonary bypass time, cross-clamp time, and use of sustained all-region (STAR) perfusion;[18] lowest intraoperative temperature; need for extracorporeal membrane oxygenation (ECMO) or repeat surgery; receipt of diuretic in the first 24 hours after surgery; lab values (e.g., creatinine and lactate) during the first 7 days after surgery; and total intake, output, and overall fluid balance over the first 72 hours after surgery.

Our primary outcome was the development of CS-AKI. We defined CS-AKI based on the modified neonatal Kidney Disease Improving Global Outcomes (KDIGO) criteria.[19] Baseline creatinine was considered the last creatinine obtained prior to undergoing cardiopulmonary bypass. Maximum creatinine in the first 48 post-operative hours was used to determine CS-AKI.[19]

Our secondary outcome was the development of FO. FO was defined as at least 10% positive cumulative fluid balance at 48 hours since this degree of cumulative FO during this time frame has been associated with increased post-operative morbidity.[20] Fluid balance was calculated using the cumulative fluid input and output methodology. Percent fluid balance was calculated by equation 1:[21]

$$\text{Percent fluid balance} = (\text{Total Intake [mL]} - \text{Total Output [mL]})/(\text{Anchor Weight [kg]}) * 100 \quad (1)$$

where anchor weight was the dosing weight at time of surgery. Cumulative fluid balance defined continuously and the presence of FO defined categorically were evaluated at 24, 48, and 72 hours after surgery.

In an exploratory analysis, we investigated the association between urine biomarker concentrations and CS-AKI phenotype, defined as a combination of CS-AKI and FO (e.g., AKI-/FO-, AKI+/FO-, AKI-/FO+, and AKI+/FO+).[22]

Urine biomarker collection

Spot urine samples were collected directly from the metered collection column of the foley catheter at three perioperative time points: (1) pre-operative or before bypass; (2) in the early post-operative period, defined as 0 to <8 hours after separation from bypass; and (3) in the late post-operative period, defined as 8 to 24 hours after separation from bypass. Urine samples were centrifuged at room temperature at 2000 × g for 10 minutes, and the supernatants were stored in cryovials at −80°C. No additives or protease inhibitors were added. Urine samples were shipped to the Institute of Drug Safety Sciences at the University of North Carolina Eshelman School of Pharmacy where they were run through the Meso Scale Discovery Human Kidney Injury Multiplexed ELISA panels (Supplementary Item 1).[23] Control samples with high, medium, and low levels of each analyte were measured using a minimum of 2 replicates on 11 runs over 5 days. All samples were run in duplicate.

Definitions

Vasoactive-inotropic score (VIS) was calculated based on inotrope and vasoactive dose, where moderate support equates to a VIS of 15.[23] The definition of low cardiac output syndrome was derived from the Prophylactic Intravenous Use of Milrinone After Cardiac Operation in Pediatrics (PRIMACORP) study and was limited to the lab criteria of >30% difference in arterial and mixed venous saturation or a metabolic acidosis with lactate >5 in the first 24 hours after surgery or an increase in lactate of >2 on 2 successive blood gases within 4 hours apart.[24]

Statistical analysis

The distribution of continuous variables was described using medians (25th–75th percentiles), and the distribution of binary or categorical variables was described with counts (percentages). Summary statistics were used to describe the neonates in the cohort. Neonates were categorised by CS-AKI stage (0–3) and as a binary variable (any versus no CS-AKI). Due to the lack of available data on the predictive ability of urine biomarkers in this population and the nature of this pilot study, no formal sample size calculations were performed. Changes in urine biomarkers over time and associations with CS-AKI and FO were graphically explored. All analyses were conducted in STATA SE (version 16.1, Stata Corps, College Station, TX), and graphs were created using the "ggplot2" package in R (version 3.2.0) and RStudio (version 2023.3.1).

Results

In total, 13 neonates met inclusion criteria. Neonate demographics are shown in Table 1. A total of 23 urine samples were collected: 6 in the pre-operative period; 11 in the early post-operative period; and 6 in the late post-operative period. Gestational age at birth was a median (25th–75th percentile) of 39 weeks (38–39 weeks). At surgery, postnatal age was 3 days (2–5 days), and weight was 3.4 kg (3.2–3.7 kg). Seven neonates (54%) were male. No infants received prophylactic peritoneal dialysis or other kidney-supportive

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Table 1. Neonate demographics and clinical characteristics

Characteristic	All neonates $n = 13$ (%)	No CS-AKI $n = 4$ (%)	Any CS-AKI $n = 9$ (%)
Gestational age at birth (weeks)	39 (38–39)	39 (39–39)	38 (37–39)
Age at surgery (days)	3 (2–5)	2 (1.5–4)	4 (3–5)
Weight at surgery (kg)	3.4 (3.2–3.7)	3.54 (3.09–4.26)	3.3 (3.2–3.7)
Male	7 (54)	3 (75)	4 (44)
Ethnicity/race			
White	10 (77)	4 (100)	6 (67)
Black	3 (23)	0	3 (33)
Arch repair	5 (38)	3 (75)	2 (22)
Baseline creatinine	0.5 (0.5–0.7)	0.8 (0.7–0.85)	0.5 (0.4–0.5)
STAT category			
3	5 (38)	2 (50)	3 (33)
4	3 (23)	1 (25)	2 (22)
5	5 (38)	1 (25)	4 (44)
CPB time (mins)	188 (164–289)	132 (98.5–170.5)	195 (173–225)
Cross-clamp time (mins)	47 (34–117)	23.5 (0–84)	91 (37–117)
Sustained all region perfusion	4 (31)	1 (25)	3 (33)
Lowest intraoperative temp (°C)	28 (27.8–31.4)	29.7 (27.9–31.7)	28 (27.8–31.4)
Low cardiac output syndrome	9 (75)	3 (75)	6 (75)
Vasoactive-inotropic[a] score	10.2 (7.8–11.8)	11 (7.5–13.5)	9.9 (7.8–11.6)
Cumulative fluid balance (mL/kg)			
24 h	54 (38–107)	47 (22–88)	54 (39–107)
48 h	−14 (−47 to 103)	−26 (−58 to 64)	−14 (−34 to 103)
72 h	−68 (−136 to 45)	−60 (−102 to −20)	−78 (−153 to 59)
Fluid overload			
24 h	13 (100)	4 (100)	9 (100)
48 h	5 (38)	1 (25)	4 (44)
72 h	5 (38)	1 (25)	4 (44)

Data are presented as counts (percentages) or medians (25^{th}–75^{th} percentiles). CS-AKI = cardiac surgery-associated acute kidney injury; STAT = Society of Thoracic Surgeons; CPB = cardiopulmonary bypass.
[a]Maximum value calculated in the first 24 hours after bypass.

therapies, and no infants died. Nine (69%) neonates were diagnosed with CS-AKI. All 13 (100%) neonates were fluid overloaded at 24 hours, with 5 (38%) neonates remaining fluid overloaded at 48 hours. Unadjusted urine biomarkers in all neonates at the three perioperative time points are shown in Supplementary Table 1, and trends are graphically shown in Figure 1. Overall, uαGST, uβ2m, uAlb, uCysC, uNGAL, uOPN, uUMOD, uClust, and uVEGF concentrations peaked in the early post-operative period before returning to pre-operative baseline or lower. uKIM1 increased across all three time points, whereas uTFF3 decreased over the study period. The remaining biomarkers, uCal, uClust, uEGF, and uOsteo did not have clear trends in relation to bypass.

Of the nine neonates who were diagnosed with CS-AKI, all (100%) had stage 1 CS-AKI. Unadjusted urine biomarkers by CS-AKI at the three perioperative time points are reported in Table 2. Early post-operative uCysC, uβ2m, uUMOD, and uVEGF were higher in neonates who developed CS-AKI, although the ranges were wide.

Of the patients with CS-AKI, four (44%) developed FO at 48 hours, and of those with no AKI, one (25%) developed FO at 48 hours. Table 2 shows unadjusted urine biomarkers by FO status at the three perioperative time points. Similar to results seen with CS-AKI, uCysC, uβ2m, and uVEGF were higher in neonates who developed FO at 48 hours in the early post-operative period. Additionally, uNGAL and uαGST were higher in the early post-operative period and uKIM1, uClust, and uTFF3 were higher in the early and last post-operative periods in neonates who developed FO at 48 hours.

In an exploratory analysis of CS-AKI phenotypes, three infants (23%) were CS-AKI−/FO−, five infants (38%) were CS-AKI+/ FO−, one infant (8%) was CS-AKI−/ FO+, and four infants (31%) were

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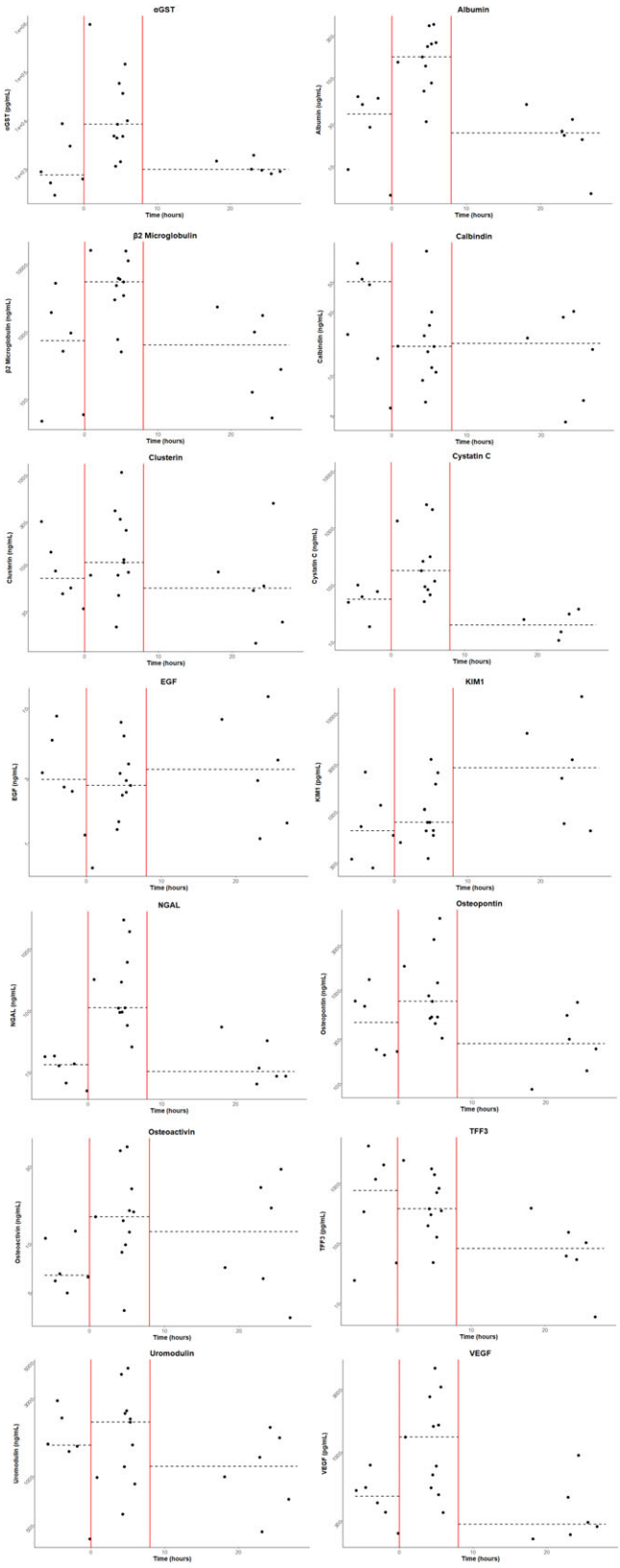

Figure 1. Urine biomarkers over time in relation to bypass. Red vertical lines denote sampling time periods (pre-operative, early post-operative, and late post-operative). *Y*-axis is depicted as log scale. Dashed horizontal lines represent the median value of the biomarker during the sampling time period. αGST = α-glutathione S-transferase; Alb = albumin; β2M = β-2 microglobulin; Cal = calbindin; Clust = clusterin; CysC = cystatin C; EGF = epidermal growth factor; KIM1 = kidney injury molecule-1; NGAL = neutrophil gelatinase-associated lipocalin; Osteo = osteoactivin; OPN = osteopontin; TFF3 = trefoil factor-3; UMOD = uromodulin; VEGF = vascular endothelial growth factor.

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Table 2. Unadjusted biomarkers by CS-AKI and FO at 48 hours at perioperative time points (presented as medians [25th–75th percentile])

	No CS-AKI	CS-AKI	No FO	FO
Biomarker	*N* = 4	*N* = 9	*N* = 8	*N* = 5
***α*GST** (pg/mL)				
Pre-operative	2906.1 (513.7–8484.1)	617.0 (287.3–863.2)	1761.8 (565.4–5695.3)	575.3 (287.3–863.2)
Early post-operative	7036.4 (2846.0–22755.8)	8179.9 (4630.4–141441.8)	4487.5 (4283.3–9789.5)	99194.7 (29178.1–532757.7)
Late post-operative	943.5 (792.8–1901.0)	978.4 (875.2–1441.4)	943.5 (875.2–978.4)	1441.4
Alb (μg/mL)				
Pre-operative	58.2 (27.5–60.6)	9.1 (4.7–49.6)	42.9 (16.1–59.4)	29.4 (29.4–49.6)
Early post-operative	244 (187–317)	150 (70.2–224)	135 (70.2–239)	305 (187–392)
Late post-operative	22.2 (20.0–33.6)	24.7 (4.9–49.8)	22.2 (20.0–24.7)	49.8
***β*2M** (ng/mL)				
Pre-operative	945.0 (510.1–1903.5)	58.5 (47.3–5146.6)	727.5 (284.4–1414.2)	2596.9 (47.3–5146.6)
Early post-operative	3072.9 (630.7–8211.1)	5928.7 (3404.7–15400)	4778.8 (2925.7–6130.3)	10,700 (3214.5–15600)
Late post-operative	984.2 (52.9–1732.4)	274.5 (125.6–2288.7)	274.5 (125.6–984.2)	2288.7
Cal (ng/mL)				
Pre-operative	47.7 (13.3–69.1)	20.2 (5.7–52.4)	38.5 (9.5–58.4)	36.3 (20.3–52.4)
Early post-operative	10.9 (8.4–17.5)	16.5 (15.0–29.7)	11.4 (9.2–29.7)	16.5 (15.7–20.1)
Late post-operative	6.5 (4.5–30.1)	19.0 (15.6–27.1)	15.6 (6.6–27.1)	19.0
Clust (ng/mL)				
Pre-operative	54.8 (46.9–137.2)	84.3 (32.0–302.2)	50.8 (39.4–96.0)	193.2 (84.3–302.2)
Early post-operative	97.2 (78.6–589.2)	105.1 (45.2–320.0)	81.5 (45.2–113.0)	280.2 (157.92–692.7)
Late post-operative	57.3 (13.1–480.6)	51.1 (22.6–82.1)	51.1 (22.6–57.3)	82.1
CysC (ng/mL)				
Pre-operative	75.7 (18.3–98.8)	48.8 (0.1–61.2)	47.0 (9.2–87.2)	55.0 (48.8–61.2)
Early post-operative	99.0 (66.0–213.9)	258.9 (92.1–2089.6)	116.2 (66.1–258.9)	1709.0 (705.0–2320.4)
Late post-operative	30.7 (14.9–37.4)	10.6 (0.1–24.3)	14.9 (10.6–30.7)	24.3
EGF (ng/mL)				
Pre-operative	2.6 (2.4–5.7)	3.3 (1.1–8.7)	2.5 (1.8–4.2)	6.0 (3.3–8.7)
Early post-operative	3.0 (2.5–4.7)	2.2 (1.3–3.8)	2.7 (1.4–3.3)	3.0 (1.4–5.0)
Late post-operative	4.9 (1.1–12.1)	2.9 (1.4–8.2)	2.9 (1.4–4.1)	8.2

(*Continued*)

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Table 2. (*Continued*)

Biomarker	No CS-AKI $N = 4$	CS-AKI $N = 9$	No FO $N = 8$	FO $N = 5$
KIM1 (pg/mL)				
Pre-operative	703.3 (266.2–1144.7)	570.9 (326.5–2494.7)	637.1 (418.6–924.0)	1410.6 (326.5–2494.7)
Early post-operative	1613 (709.8–2988.8)	634.09 (481.2–1045.1)	642.4 (570.3–1045.1)	1327.9 (628.2–2623.9)
Late post-operative	3337.8 (754.6–14682.6)	2165.5 (634.7–6183.0)	2165.5 (754.6–3337.8)	6183.0
NGAL (ng/mL)				
Pre-operative	13.5 (6.6–18.0)	12.6 (4.9–17.6)	10.1 (5.8–15.8)	15.1 (12.6–17.6)
Early post-operative	190.1 (67.7–444.3)	108.9 (92.7–1887.3)	93.7 (56.6–286.1)	1101.9 (213.4–2404.2)
Late post-operative	11.5 (8.5–32.1)	8.5 (6.3–53.3)	8.5 (8.5–11.5)	53.3
Osteo (ng/mL)				
Pre-operative	5.9 (5.0–11.9)	6.5 (6.2–10.8)	6.0 (5.4–9.1)	8.6 (6.5–10.8)
Early post-operative	15.8 (14.8–27.7)	11.7 (8.8–21.8)	13.8 (8.9–15.9)	18.2 (12.2–30.6)
Late post-operative	16.6 (6.1–28.6)	7.1 (3.5–22.2)	16.6 (6.1–22.2)	7.1
OPN (ng/mL)				
Pre-operative	230.2 (200.5–664.6)	758.7 (219.4–1284.7)	224.9 (210.0–447.4)	1021.7 (758.7–1284.7)
Early post-operative	473.4 (369.3–847.2)	864.8 (512.0–3447.0)	512.0 (495.5–864.8)	2620.1 (1114.2–4621.1)
Late post-operative	296.7 (136.7–735.6)	232.5 (86.4–534.6)	296.6 (232.5–534.6)	86.4
TFF3 (pg/mL)				
Pre-operative	1159.6 (332.2–1995.1)	46.5 (23.7–4120.9)	745.9 (189.4–1577.4)	2072.3 (23.7–4120.9)
Early post-operative	517.9 (320.4–1039.1)	373.5 (125.2–1728.7)	342.6 (193.9–693.1)	1100.2 (431.4–1882.3)
Late post-operative	101.0 (52.8–150.3)	61.0 (5.9–381.5)	61.8 (52.8–101.0)	381.5
UMOD (ng/mL)				
Pre-operative	1520.7 (1212.2–2896.5)	1572.6 (411.7–2271.5)	1466.4 (912.0–2208.6)	1922.1 (1572.6–2271.5)
Early post-operative	1641.3 (1017.1–3361.5)	2240.8 (979.7–2513.9)	2142.0 (893.4–2426.4)	2034.1 (1267.0–3547.4)
Late post-operative	1719.4 (455.3–1988.1)	988.3 (719.3–1300.0)	1300.0 (719.3–1719.4)	988.3
VEGF (pg/mL)				
Pre-operative	410.3 (347.8–534.6)	509.8 (240.3–791.8)	379.0 (294.0–472.4)	650.8 (509.8–791.8)
Early post-operative	725.4 (508.4–1192.4)	1562.2 (532.1–3124.5)	671.0 (472.5–1604.9)	2210.2 (1037.9–3727.4)
Late post-operative	291.6 (234.1–941.6)	270.4 (218.1–452.3)	291.6 (270.4–452.3)	218.1

αGST = α-glutathione S-transferase; Alb = albumin; β2M = β-2 microglobulin; Cal = calbindin; Clust = clusterin; CysC = cystatin C; EGF = epidermal growth factor; KIM1 = kidney injury molecule-1; NGAL = neutrophil gelatinase-associated lipocalin; Osteo = osteoactivin; OPN = osteopontin; TFF3 = trefoil factor-3; UMOD = uromodulin; VEGF = vascular endothelial growth factor.

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CS-AKI+/ FO+. Supplementary Table 2 reports unadjusted biomarkers by CS-AKI phenotype at different perioperative time points. For neonates with the CS-AKI+/ FO+ phenotype, median uCysC and uαGST peaked notably higher in the early post-operative period compared to other phenotypes.

Discussion

In this study, we characterised 14 urine biomarkers at 3 perioperative time points and examined their association with CS-AKI and FO in neonates after cardiac surgery. The aim of this study was to (1) understand the normal time course of these biomarkers in the perioperative period and (2) identify which biomarkers may be most beneficial for further study to evaluate the utility in the early diagnosis of CS-AKI and/or FO. Overall, we found that uαGST, uβ2m, uAlb, uCysC, uNGAL, uOPN, uUMOD, uClust, and uVEGF peaked in the early post-operative period, uKIM1 increased over time, and uTFF3 decreased over time. The remaining biomarkers uCal, uClust, uEGF, and uOsteo did not have clear trends in relation to bypass.

CS-AKI was common in our population, although all CS-AKI was stage 1. The rate of CS-AKI in our cohort is consistent with prior literature, although our cohort may tend towards more mild disease.[2] This is likely multifactorial and may be reflective of earlier age at surgery which may be before maternal SCr is cleared, and the use of STAR perfusion in 31% of neonates. CS-AKI is associated with increased morbidity and mortality in children.[7] In addition to pathophysiologic blood flow secondary to CHD in the pre-operative setting, cardiopulmonary bypass significantly contributes to CS-AKI. Specifically, bypass disrupts the relationship between renal oxygen supply and demand and decreases renal oxygenation after surgery through renal vasoconstriction and hemodilution.[25] Impaired renal perfusion leads to a decrease in glomerular filtration rate via renal inflammation and vasoconstriction and cumulates in further damage to ischaemic tubules.[26] However, the use of SCr levels to define AKI has several limitations, especially in neonates: there is a delay in SCr rise after kidney injury, values vary based on patient characteristics such as sex and muscle mass, and SCr is reflective of maternal renal function in the first days of life.[10,11] The latter limitation was highlighted in our results. Baseline creatinine was higher in neonates without CS-AKI group, suggesting that maternal creatinine levels could be a confounding variable when the diagnosis of CS-AKI relies on a change from baseline.

Because urine biomarkers reflect different mechanisms of renal function, they may serve to elucidate different mechanisms of renal injury and guide potential treatment.[2] For example, NGAL and CysC are synthesised in the proximal tubules, increase before SCr in renal injury, and may reflect injury to proximal renal tubular cells.[9,27] Alb, β2M, and αGST may be specific for proximal renal tubular injury, whereas OPN is secreted in the loop of Henle and the distal tubule, and Cal is found in the distal tubule and collecting duct and may be elevated with damage to these areas of the kidney.[28] While KIM-1 is hardly detected in healthy kidneys, in the setting of ischaemia or nephrotoxic drugs, levels increase in the proximal tubule.[9] CysC may more accurately reflect glomerular filtration rates because it is not affected by age or muscle mass, like creatinine, but is freely filtered by the glomerulus.[29] CysC, Cal, and Alb may also reflect abnormalities in renal protein reabsorption through their expression in the distal tubule.[28] However, when population-specific "normal" values are unknown, the utility of these biomarkers is diminished.

There is currently a lack of consensus on the efficacy of urine biomarkers in predicting CS-AKI, with particularly sparse data for neonates. The most reported urine biomarker is uNGAL.[30] A meta-analysis of 37 studies and 10 different biomarkers found that uNGAL had the greatest diagnostic test accuracy in predicting CS-AKI in children and may be a better predictor of CS-AKI in children than in adults.[30] Other studies have reported weaker associations between uNGAL, and CS-AKI and trends may not appear until 48 hours after surgery, which may provide no advantage over current SCr-based diagnosis strategies.[12] Interestingly, in our study, uNGAL was more closely associated with FO than CS-AKI. There are fewer data available for other urine biomarkers. There is some evidence that uKIM-1/creatinine level may help with the detection of early CS-AKI at specific perioperative time points,[12] although most investigation has been done in adult populations and the predictive value of uKIM-1 may be less than that of uNGAL.[30] While a meta-analysis found uCysC to be predictive of AKI in the general paediatric population,[29] analysis of uCysC levels following paediatric cardiac surgery was less predictive.[30] Further study into how specific biomarkers reflective of common mechanisms of CS-AKI may be able to predict CS-AKI earlier than current strategies.

The ability of urine biomarkers to predict FO is a novel area of study with the potential to guide clinical management.[14] FO in neonates undergoing cardiac surgery is common and is associated with poor outcomes independent of CS-AKI.[5] Similar to reported literature rates, FO at 48 hours was common in our cohort.[4,5] Multiple urine biomarkers were higher in the early post-operative period in neonates who developed FO at 48 hours, including uCysC, uβ2m, uVEGF, uNGAL, uKIM1, uClust, uTFF3, and uαGST. Further study into how these biomarkers may predict FO is warranted. The ability to detect which neonates will develop FO will allow for proactive fluid management and may improve outcomes. Evaluating neonates based on CS-AKI phenotypes may further risk stratify the highest-risk patients.[1] A recent study investigating CS-AKI in infants found that early post-operative uNGAL and uCysC levels significantly differed based on CS-AKI phenotype with the highest levels seen in the CS-AKI+/FO+ group.[14] This is consistent with an analysis of the independent effects of FO and AKI on critically ill children, where FO and AKI together predicted the worst outcomes among phenotypes.[6] Studies focused on urine biomarkers and CS-AKI phenotypes may best describe risk factors for poor outcomes but should be population-specific, especially in neonates where renal maturation will affect normal diagnostic measures.[13]

While our study was the first to describe ranges of 14 urine biomarkers in this population and explore their relationship with CS-AKI and FO, it has several important limitations. First, biomarker concentrations were not adjusted for urine output or urine creatinine concentrations. There is no consensus in the literature regarding whether absolute concentrations, concentrations normalised to urine creatinine to account for glomerular filtration rate, or concentrations normalised to urine output to evaluate the excretion rate are most useful in the interpretation of urine biomarker concentrations and their association with CS-AKI.[25] Therefore, we used absolute biomarker concentrations as these are likely to be used in clinical practice. Second, all CS-AKI was stage 1 which limits our ability to comment on the association of these biomarkers with more severe AKI. Third, there is variability in defining the degree of FO and timing of FO that is clinically relevant. Our definitions were based on literature that associates 10% FO with poor outcomes in post-cardiac surgery

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populations.[20] Fourth, we calculated fluid balance by intake and output which does not account for insensible losses but is a recommended calculation by the Pediatric Acute Disease Quality Initiative (ADQI) group.[21] Finally, as with most paediatric studies, our sample size was small and from a single centre. Additionally, our analysis is based on a variable number of samples from each time period, with only four neonates contributing three complete samples over the sampling period.

Our data can be used to inform future studies that interpret urine biomarker concentrations based on age and disease process in the context of CS-AKI and FO, as well as define clinically relevant end points. Establishing population-specific normal urine biomarker values and how deviations from these are associated with CS-AKI and FO in large cohorts may support more widespread implementation to improve outcomes after neonatal cardiac surgery.

Conclusion

In conclusion, in this single-centre prospective cohort study in neonates undergoing cardiac surgery, urine biomarkers varied based on CS-AKI and FO status, and by CS-AKI phenotype. Overall, uαGST, uβ2m, uAlb, uCysC, uNGAL, uOPN, uUMOD, uClust, and uVEGF peaked in the early post-operative period, uKIM1 increased over time, and uTFF3 decreased over time. uβ2M and uαGST were higher in the early post-operative period in neonates who developed CS-AKI. uClust, uCysC, uNGAL, uOPN, and uαGST were higher in the early post-operative period in neonates who developed FO. Future studies should evaluate larger cohorts to establish age- and disease-specific concentration ranges and possible associations between (1) uβ2M, uαGST and CS-AKI and (2) uClust, uCysC, uNGAL, uOPN, uαGST, and FO in a larger cohort of neonates undergoing cardiac surgery.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1047951125000034.

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Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and have been approved by the Duke Institutional Review Board.

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