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Original Article

Neutrophil gelatinase-associated lipocalin reflects inflammation and is not a reliable renal biomarker in neonates and infants after cardiopulmonary bypass: a prospective case–control study

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Abstract *Introduction:* Acute kidney injury is a frequent complication after cardiac surgery with cardiopulmonary bypass in infants. Neutrophil gelatinase-associated lipocalin has been suggested to be a promising early biomarker of impending acute kidney injury. On the other hand, neutrophil gelatinase-associated lipocalin has been shown to be elevated in systemic inflammatory diseases without renal impairment. In this secondary analysis of data from our previous study on acute kidney injury after infant cardiac surgery, our hypothesis was that neutrophil gelatinase-associated lipocalin may be associated with surgery-related inflammation. *Methods:* We prospectively enrolled 59 neonates and infants undergoing cardiopulmonary bypass surgery for CHD and measured neutrophil gelatinase-associated lipocalin in plasma and urine and interleukin-6 in the plasma. Values were correlated with postoperative acute kidney injury according to the paediatric Renal-Injury-Failure-Loss-Endstage classification. *Results:* Overall, 48% (28/59) of patients developed acute kidney injury. Of these, 50% (14/28) were classified as injury and 11% (3/28) received renal replacement therapy. Both plasma and urinary neutrophil gelatinase-associated lipocalin values were not correlated with acute kidney injury occurrence. Plasma neutrophil gelatinase-associated lipocalin showed a strong correlation with interleukin-6. Urinary neutrophil gelatinase-associated lipocalin values correlated with cardiopulmonary bypass time. *Conclusion:* Our results suggest that plasma and urinary neutrophil gelatinase-associated lipocalin values are not reliable indicators of impending acute kidney injury in neonates and infants after cardiac surgery with cardiopulmonary bypass. Inflammation may have a major impact on plasma neutrophil gelatinase-associated lipocalin values in infant cardiac surgery. Urinary neutrophil gelatinase-associated lipocalin may add little prognostic value over cardiopulmonary bypass time.

Keywords: Acute kidney injury; neutrophil gelatinase-associated lipocalin; inflammation; interleukin-6; CHD; cardiopulmonary bypass

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ACUTE KIDNEY INJURY FREQUENTLY FOLLOWS cardiac surgery in infants and children with incidence rates of up to 60%.[2] Several studies in the general[3] as well as in the cardiac surgery paediatric intensive care unit population[2,4–10] have demonstrated increased morbidity, length of paediatric intensive care unit stay, and mortality when acute kidney injury occurs.

Creatinine is a late marker of cardiac surgery-associated renal injury. There has been great effort to identify renal biomarkers that indicate prognostically relevant renal injury as early as possible to allow earlier intervention.[1] Among renal biomarkers that have been

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associated with the development of cardiac surgery-associated renal injury, neutrophil gelatinase-associated lipocalin has shown the most consistent results, although not all studies were able to confirm an association with cardiac surgery-associated renal injury.[11] Combining biomarkers[12] or including a wider panel of urinary proteins may be a more sensitive approach.[13]

Efforts to increase biomarker sensitivity and specificity by combining various biomarkers may have inherent statistical problems reducing their validity[14]; moreover, the various reported biomarkers have different cellular sources and reflect different pathogenic mechanisms. In addition, other processes apart from kidney dysfunction may influence levels of biomarkers. In particular, systemic inflammatory diseases may per se increase plasma levels of neutrophil gelatinase-associated lipocalin as shown, for example, for sepsis,[15] Kawasaki disease,[16] inflammatory bowel disease,[17] and acute pyelonephritis.[18] On the other hand, inflammatory markers such as C-reactive protein have been reported to correlate with acute kidney injury incidence and may even play a pathogenic role in the development of kidney failure.[19] Cardiopulmonary bypass per se may elevate cystatin C and C-reactive protein.[20]

We recently reported high sensitivity and specificity of intraoperative and postoperative continuous regional renal near-infrared spectroscopy measurement for the development of cardiac surgery-associated renal injury in a prospective cohort of infants.[21] The aim of this study was to determine the association of neutrophil gelatinase-associated lipocalin with the development of acute kidney injury and inflammation in neonates and infants undergoing cardiac surgery. Therefore, in this report, we performed a secondary analysis of data from our previous study[21] looking at possible correlations between neutrophil gelatinase-associated lipocalin in urine and plasma, serum interleukin-6, and cardiopulmonary bypass time in neonates and infants with and without acute kidney injury. Our hypothesis was that neutrophil gelatinase-associated lipocalin may be associated with surgery-related inflammation.

Methods

Patients and study design

The design of the study, the included patient population, as well as the laboratory methodology used has been reported in detail in our previous publication.[21] In short, neonates and infants up to 12 months of age who underwent cardiac surgery on cardiopulmonary bypass were prospectively enrolled. Patients with pre-existing renal disease, infection or dysfunction of other organs were excluded, based on history or baseline laboratory values. Written informed consent of the parents was obtained in every case. Ethical approval was given by the research ethics committee at Deutsches Herzzentrum Muenchen, Technische Universität Muenchen, Munich, Germany.

The following clinical parameters were recorded: time on bypass, aortic cross-clamp time, circulatory arrest time, as well as time on mechanical ventilation and length of stay in the ICU and at the hospital. The Risk Adjustment for Congenital Heart Surgery (RACHS-1) categories were used to compare the severity of the performed cardiac surgery.

The primary outcome was occurrence of acute kidney injury within 3 days post surgery. According to the pRIFLE classification, acute kidney injury was defined as an increase in serum creatinine by 50% or more. This encompasses all pRIFLE stages including Risk, defined as follows: risk, serum creatinine ×1.5; injury, serum creatinine ×2; failure, serum creatinine ×3. Creatinine was measured routinely preoperatively and at least on every morning following surgery during the paediatric intensive care unit stay for a minimum of 5 days. Urine volume criteria were not used because of variable diuretic use. The patients were divided into two groups: the ones who developed postoperative acute kidney injury and the ones who did not.

Laboratory parameters

Interleukin-6 and neutrophil gelatinase-associated lipocalin were measured before cardiopulmonary bypass and at 2–4 hours and 24 hours after termination of cardiopulmonary bypass irrespective of bypass time. Interleukin-6 was measured in plasma using a solid-phase, enzyme-labelled chemiluminescent sequential immunometric assay (Immulite® system; Siemens Healthcare Diagnostics, Henkestraße 127 D-91052 Erlangen). The determination of neutrophil gelatinase-associated lipocalin in ethylenediaminetetraacetic acid anticoagulated plasma was performed with a fluorescence immunoassay using the Triage® Meter (Alere, Köln, Germany). Urinary neutrophil gelatinase-associated lipocalin concentrations were measured using a solid-phase enzyme-linked immunosorbent assay based on the sandwich principle (Hycult Biotech, Plymouth, United Kingdom).

Plasma lactate was measured at 6, 12, and 24 hours postoperatively and $ScvO_2$ and SaO_2 were noted at 2 and 24 hours postoperatively.

Creatinine was determined on the Integra 800 analyser (Roche Diagnostics, Mannheim, Germany) using a kinetic colorimetric assay based on the Jaffé method. Plasma creatinine was measured before cardiopulmonary bypass on the day of the surgical procedure, which, in newborns, was performed between days 5 and 10 after birth. Further measurements of plasma creatinine were performed at the same time points as for neutrophil gelatinase-associated lipocalin, daily, for 5 days postoperatively.

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Statistical analysis

For continuous variables, in case of an asymmetric data distribution, a two-sided Mann–Whitney U test or, for paired analysis, a Wilcoxon test was applied. Data are presented as median and range or as individual values. Correlation analysis was performed with the Spearman ρ coefficient of correlation for asymmetric data distribution. Results were considered statistically significant at $p < 0.05$. The statistical calculations were performed using SPSS 21 software (IBM SPSS Statistics).

Results

From January, 2011 to August, 2011 we enrolled 59 neonates and infants undergoing cardiac surgery on cardiopulmonary bypass. According to the pRIFLE classification,[6] in total, 48% (28/59) of the patients developed acute kidney injury after cardiopulmonary bypass. Of these, 39% (11/28) were classified as risk and 50% (14/28) as injury. Of the patients, three of 28 (11%) were classified as failure. Analysis based on KDIGO criteria, adding an increase of 0.3 mg/dl in plasma creatinine as an acute kidney injury diagnostic criterion, did not change patient numbers per acute kidney injury as a whole and for specific subgroups. All patients in the failure group received renal replacement therapy following cardiopulmonary resuscitation either at the end of surgery or during the following days. The indication for renal replacement therapy was massive haemodynamically intolerable fluid overload in each case. Of these patients, two received circulatory support with extracorporeal membrane oxygenation. Two of 28 (7%) patients with acute kidney injury, one of them with extracorporeal membrane oxygenation, died. All patients without acute kidney injury survived.

Weight and RACHS-1 category did not differ significantly between patients with and without acute kidney injury (Table 1). Selected haemodynamic and oxygenation data are presented in Table 2. Patients developing acute kidney injury had a greater need for a vasopressor, significantly longer episodes of arterial hypotension, elevated plasma lactate, and decreased central venous oxygen saturation values compared with patients without acute kidney injury (Table 2). Median cardiopulmonary bypass time was not significantly different in patients with and those without acute kidney injury. Further, no significant difference was found for aortic cross-clamp time and duration of circulatory arrest. In contrast, patients undergoing biventricular repair developed acute kidney injury significantly less often than patients undergoing univentricular palliation (33% versus 65%) or shunt surgery (83%). Of note, all patients undergoing shunt surgery had univentricular physiology.

Neutrophil gelatinase-associated lipocalin in urine and plasma showed a significant increase from the preoperative to the 2–4 hours postoperative values (urine: 2.4 to 25.4 ng/ml, $p < 0.0001$; plasma: 35 to 41 ng/ml, $p = 0.05$) (Table 3). Importantly, neither neutrophil gelatinase-associated lipocalin in urine nor that in plasma at 2–4 and 24 hours postoperatively showed a significant difference between patients with and those without acute kidney injury (Table 4; Figs 1 and 2).

Plasma neutrophil gelatinase-associated lipocalin 24 hours after surgery and interleukin-6 2–4 hours after surgery ($r = 0.45$, $p = 0.001$) and 24 hours post surgery were significantly correlated ($r = 0.47$, $p < 0.0001$). Plasma neutrophil gelatinase-associated lipocalin and urinary neutrophil gelatinase-associated lipocalin as well as interleukin-6 and urinary neutrophil gelatinase-associated lipocalin did not show any significant correlation. In a Spearman's ρ correlation analysis there was no correlation between urinary or plasma neutrophil gelatinase-associated

Table 1. Patient demographics and surgery data.

	AKI	No AKI	p-value
Age (days)	27 (5–274)	105 (8–305)	0.03
Weight (g)	3355 (2445–9100)	4490 (2260–7530)	0.10
Cardiac catherisation, using contrast, before surgery [n (%)]	5 (19)	8 (25)	0.55
Biventricular repair [n (%)]	11 (33)	22 (67)	0.01
Univentricular palliation [n (%)]	17 (65)	9 (35)	0.01
Systemic to pulmonary shunt [n (%)]	15 (83)	3 (17)	<0.01
PCPC [n (%)]	2 (25)	6 (75)	
CPB time (minute)	75 (30–150)	77 (23–160)	0.92
Aortic cross-clamp time (minute)	28 (0–83)	47 (0–110)	0.07
RACHS-1 category			
2 and 3 [n (%)]	15 (56)	22 (69)	0.42
4–6 [n (%)]	12 (44)	10 (31)	0.42

AKI = acute kidney injury; CPB = cardiopulmonary bypass; PCPC = partial cavopulmonary connection
Data are presented as the median and range

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Table 2. Haemodynamic, oxygen data and outcome criteria post surgery.

	AKI	Non-AKI	P
Lactate 6 hours p.s. (μmol/L)	2.2 (0.9–17.0)	1.9 (0.9–7.4)	0.32
Lactate 12 hours p.s. (μmol/L)	3.0 (1.1–19.0)	2.4 (1.0–6.0)	0.08
Lactate 24 hours p.s. (μmol/L)	2.6 (1.4–16.0)	1.7 (0.9–6.5)	0.01
$ScvO_2$ 2 hours p.s. (%)	57.(35–88)	60 (34–87)	0.54
$ScvO_2$ 24 hours p.s. (%)	55 (40–88)	62 (44–89)	0.004
SaO_2 2 hours p.s. (%)	87 (70–100)	92 (55–99)	0.39
SaO_2 24 hours p.s. (%)	83 (69–100)	93 (63–100)	0.76
MAP <50 mmHg for >2 hours within 24 hours p.s. [n (%)]	16 (59)	8 (25)	0.008
Norepinephrine >0.1 μg/kg/minute [n (%)]	5 (19)	2 (6)	0.01
RRT [n (%)]	3 (11)	0 (0)	0.09
Death [n (%)]	2 (7)	0 (0)	0.20

AKI = acute kidney injury; p.s. = post surgery; RRT = renal replacement therapy; SaO_2 = arterial oxygen saturation; $ScvO_2$ = systemic venous oxygen saturation
Data are presented as the median and range or as individual values

Table 3. Preoperative and postoperative plasma and urinary neutrophil gelatinase-associated lipocalin and plasma IL-6 in the whole study population.

	Before CPB	2–4 hours after CPB	24 hours after CPB	P
NGAL plasma (ng/ml) [median (range)]	35 (16–313)	41 (21–207)	89 (33–498)	0.05*; <0.0001**
NGAL urine (ng/ml) [median (range)]	2.4 (0.3–162.5)	25.4 (0.3–276.7)	7.5 (0.3–138.6)	<0.0001*; <0.0001**
IL-6 (pg/ml) [median (range)]	2.2 (1.0–32.5)	90.1 (8.0–556.0)	136.5 (21.9–1808.0)	<0.00011*; 0.08**

CPB = cardiopulmonary bypass; IL-6 = interleukin-6; NGAL = neutrophil gelatinase-associated lipocalin
*Before CPB versus 2–4 hours after CPB
**2–4 hours after CPB versus 24 hours after CPB

Table 4. Preoperative and postoperative plasma and urinary neutrophil gelatinase-associated lipocalin and plasma IL-6 in neonates and infants with and without acute kidney injury following cardiac surgery.

	Patients with AKI	Patients without AKI	P
NGAL plasma (g/ml) [median (range)]			
Before CPB	29 (20–153)	37 (16–313)	0.05
2–4 hours after CPB	39 (24–169)	45 (21–207)	0.30
24 hours after CPB	90 (33–331)	87 (38–498)	0.60
NGAL urine (ng/ml) [median (range)]			
Before CPB	2.5 (0.3–162.5)	2.4 (0.3–25.3)	0.50
2–4 hours after CPB	31.4 (0.3–276.7)	23.1 (0.6–237.0)	0.90
24 hours after CPB	6.5 (0.3–92.2)	8.3 (0.3–138.6)	0.80
IL-6 (pg/ml) [median (range)]			
Before CPB	2.1 (1.0–30.9)	2.6 (1.0–32.5)	0.50
2–4 hours after CPB	91.2 (26.4–375.0)	89.1 (8.0–556.0)	0.90
24 hours after CPB	146 (21.9–1808.0)	128.5 (32.5–659.0)	0.40

AKI = acute kidney injury; CPB = cardiopulmonary bypass; IL-6 = interleukin-6; NGAL = neutrophil gelatinase-associated lipocalin

lipocalin and urine output at any point of time; data is not shown.

Interleukin-6 increased significantly from a median value of 2.2 pg/ml (1.0–32.5) before cardiopulmonary bypass to 90.1 pg/ml (8.0–556.0) 2–4 hours after cardiopulmonary bypass and to 136.5 pg/ml (21.9–1808.0) 24 hours after cardiopulmonary bypass ($p < 0.0001$) (Fig 3; Table 3). Interleukin-6 showed no significant difference between patients with and those without acute kidney injury at any time point (Table 4).

There was a moderate correlation between cardiopulmonary bypass time and urinary neutrophil gelatinase-associated lipocalin 2–4 hours after cardiopulmonary bypass ($r = 0.50$, $p < 0.0001$) and 24 hours after cardiopulmonary bypass ($r = 0.49$, $p < 0.0001$). We divided the patient population dichotomously with the median cardiopulmonary bypass time as a cut-off point to compare patients with lower versus those with higher cardiopulmonary bypass times. This was possible because of the

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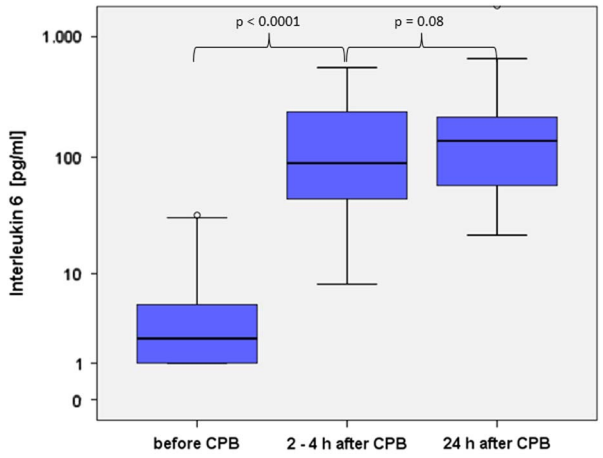

Figure 1.
Interleukin 6 before and after cardiopulmonary bypass in neonates and infants.

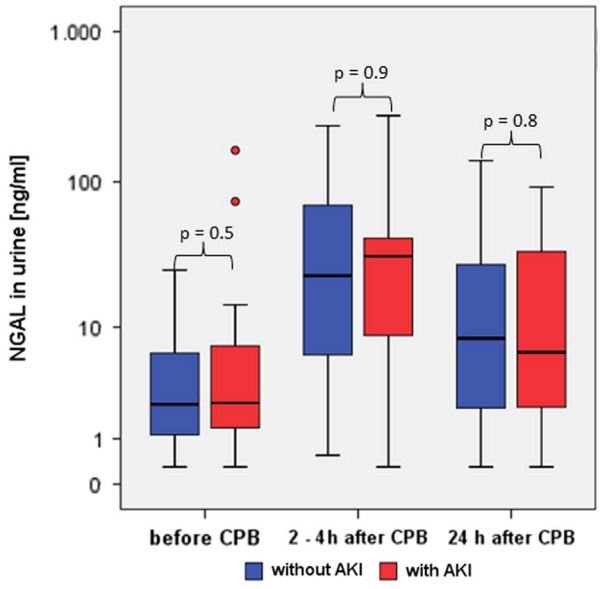

Figure 2.
Neutrophil-gelatinase associated lipocalin in urine before and after cardiopulmonary bypass in neonates and infants. NGAL = neutrophil-gelatinase associated lipocalin.

near identity of median cardiopulmonary bypass times in the acute kidney injury and in the non-acute kidney injury group. A total of 27 patients had bypass times below 75 minutes and 32 infants had times above 75 minutes. Patients with a bypass time ⩾75 minutes showed significantly higher urinary neutrophil gelatinase-associated lipocalin values 2–4 and 24 hours after cardiopulmonary bypass compared with patients with bypass times below 75 minutes: at 2–4 hours after cardiopulmonary bypass, in patients with less than 75 minutes bypass time, values were 8.7 pg/ml (0.3–215.2 pg/ml) versus 41.3 pg/ml

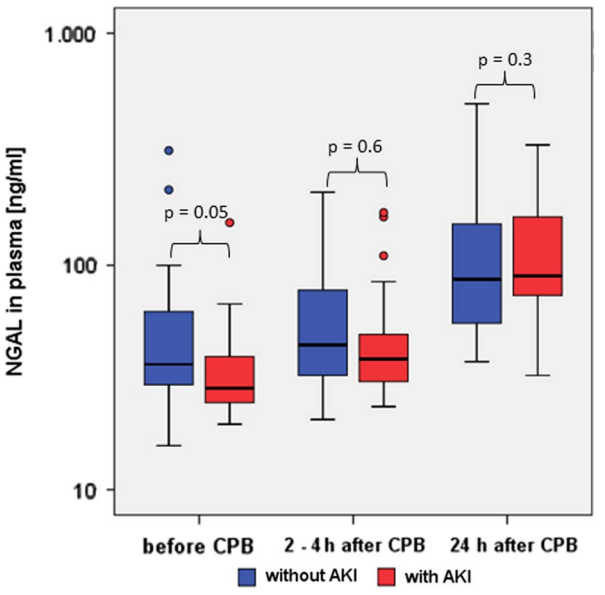

Figure 3.
Neutrophil-gelatinase associated lipocalin in plasma before and after cardiopulmonary bypass in neonates and infants with and without acute kidney injury. AKI = acute kidney injury; NGAL = neutrophil-gelatinase associated lipocalin.

(2.1–276.7 pg/ml) with bypass time greater or equal to 75 minutes ($p = 0.001$); and at 24 hours after cardiopulmonary bypass, in patients with <75 minutes bypass time, values were 2.2 pg/ml (0.3–126.5 pg/ml) versus 16.8 pg/ml (2.0–138.6 pg/ml) with bypass time ⩾75 minutes ($p = 0.001$) (Table 5; Fig 4). In contrast, plasma neutrophil gelatinase-associated lipocalin and interleukin-6 were not significantly correlated with cardiopulmonary bypass time.

Discussion

In this report, we present further data from our previously published prospective study in infants after cardiac surgery on early markers of acute kidney injury[21] and show evidence that plasma and urinary neutrophil gelatinase-associated lipocalin do not correlate with acute kidney injury. We propose that plasma neutrophil gelatinase-associated lipocalin may reflect surgery-associated inflammation.

Extracorporeal circulation comprises many mechanisms that lead to a systemic inflammatory state in addition to haemodynamic alterations including non-pulsatile circulation. Plasma interleukin-6 has been shown to be elevated after cardiopulmonary bypass in children.[22] In our study, neither urinary and plasma neutrophil gelatinase-associated lipocalin nor interleukin-6 correlated with acute kidney injury occurrence. This is in accordance with another study, which showed that inflammatory mediators were not

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Table 5. Preoperative and postoperative plasma and urinary neutrophil gelatinase-associated lipocalin (NGAL) and plasma interleukin-6 (IL-6) in neonates and infants after cardiac surgery with cardiopulmonary bypass (CPB) time less and more than 75 minutes.

	CPB time		
	<75 minutes (n = 27)	⩾75 minutes (n = 32)	P
NGAL urine (ng/ml) [median (range)]			
2–4 hours after CPB	8.7 (0.3–215.2)	41.3 (2.1–276.7)	0.001
24 hours after CPB	2.2 (0.3–126.5)	16.8 (2–138.6)	0.001
NGAL plasma (ng/ml) [median (range)]			
2–4 hours after CPB	40 (21–207)	41 (24–162)	0.90
24 hours after CPB	131 (33–498)	87 (38–331)	0.20
IL-6 (pg/ml) [median (range)]			
2–4 hours after CPB	73.0 (8.0–556.0)	110.0 (13.9–447.0)	0.50
24 hours after CPB	159.0 (32.3–659.0)	115.0 (21.9–1808.0)	0.40

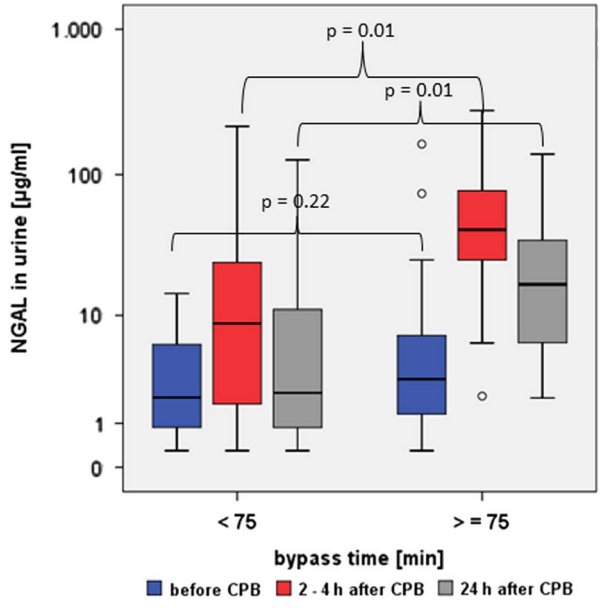

Figure 4.
Urinary neutrophil-gelatinase associated lipocalin in neonates and infants after cardiac surgery with CPB time less and more than 75 minutes. NGAL = neutrophil-gelatinase associated lipocalin.

associated with acute kidney injury development.[23] On the other hand, we noted a clear correlation of plasma neutrophil gelatinase-associated lipocalin levels with interleukin-6, an important but non-specific marker of inflammation. This is in accordance with reports on systemic inflammatory diseases.[16,17] Therefore, plasma neutrophil gelatinase-associated lipocalin may predominantly be associated with inflammation and not specifically be mirroring renal injury.

In our study population, 48% of infants developed acute kidney injury after cardiopulmonary bypass surgery, which is comparable to recently published series.[2,4,6–10] Known risk factors for the development of acute kidney injury are being of a younger age,[2,9,10] bypass time,[2,3,9] RACHS-1 category classification,[2,9] and vasopressor use.[3] In our study, patients in the acute kidney injury group were significantly younger than patients in the non-acute kidney injury group, confirming these findings, whereas there was no difference in RACHS-1 category classification. Further, patients with acute kidney injury had higher vasopressor use as well as higher lactate and lower $ScvO_2$ values. Infants with univentricular physiology including univentricular palliation and shunt surgery developed acute kidney injury more often than did infants undergoing biventricular repair. As shown in our previous study[21] regional renal oxygenation measured using near-infrared spectrometry correlates with acute kidney injury development, although within the cyanotic group and also within the acyanotic group arterial oxygen saturation did not differ significantly between those developing acute kidney injury and those who did not.

Acute kidney injury pathogenesis is complex and not thoroughly elucidated and includes, among others, haemodynamic factors as well as inflammatory and cell-death pathways. In the search for biomarkers indicating developing renal injury early after cardiac surgery, several molecules have been identified such as kidney injury molecule-1, interleukin-18, liver fatty acid-binding protein, and neutrophil gelatinase-associated lipocalin neutrophil gelatinase-associated lipocalin[11,24]; however, none of them has shown consistent sensitivity and specificity in follow-up studies. Recently, the product of insulin-like growth factor-binding protein 7 and tissue inhibitor of metalloproteinases-2 has been shown in a large population of critically ill adult patients to predict acute kidney injury with an "area under the curve"value of 0.70.[25] In a paediatric study involving patients with cardiac surgery-associated acute kidney injury the predictive value of this parameter has also been supported; however, the number of patients with more severe acute kidney injury stages was very small and further studies are needed to validate these results.[26] A recently reported proteomic approach includes a

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wider array of urinary proteins, and in an adult cardiac surgery population an area under the curve value for predicting acute kidney injury of 0.81 has been found. In this study neutrophil gelatinase-associated lipocalin performed poorly comparably to our results with an area under the curve value of 0.63.[13]

Important cellular sources of neutrophil gelatinase-associated lipocalin are proximal tubules, the ascending loop of Henle, and collecting ducts, although a further major source is constituted by leucocytes. The physiological function of neutrophil gelatinase-associated lipocalin is not entirely clear but may include chelation of free iron, which leads to a reduction in the production of reactive oxygen species. Neutrophil gelatinase-associated lipocalin is highly up-regulated in sepsis with or without acute kidney injury[15] and elevated plasma and urine levels have been found in inflammatory bowel disease[17] and Kawasaki disease[16] among other systemic inflammatory diseases. Of note, acute kidney injury was not associated with increased neutrophil gelatinase-associated lipocalin levels in these reports.

We observed a moderate correlation of urinary but not of plasma neutrophil gelatinase-associated lipocalin with cardiopulmonary bypass time. This is consistent with findings from earlier studies in which a correlation analysis of urinary neutrophil gelatinase-associated lipocalin and cardiopulmonary bypass time was performed and yielded a positive result[27,28] but in contrast to a report showing a significant correlation of plasma neutrophil gelatinase-associated lipocalin with cardiopulmonary bypass time in children.[27] On the other hand, plasma neutrophil gelatinase-associated lipocalin has less consistent predictive value for acute kidney injury than urinary neutrophil gelatinase-associated lipocalin.[27,29] Some[22,27] but not all studies adapted the sampling point of time according to the duration of cardiopulmonary bypass: for example, with cardiopulmonary bypass times above 2 hours plasma samples for neutrophil gelatinase-associated lipocalin were collected immediately after surgery, whereas with cardiopulmonary bypass times below 2 hours samples were collected 2 hours after surgery. This may have captured earlier plasma peaks of neutrophil gelatinase-associated lipocalin in more prolonged surgical procedures although no study has investigated this appropriately. Urinary neutrophil gelatinase-associated lipocalin values may cover a period of neutrophil gelatinase-associated lipocalin increase over several hours and, therefore, be more sensitive than plasma neutrophil gelatinase-associated lipocalin levels. In an effort to increase the ability to predict cardiac surgery-associated acute kidney injury other authors have used clinical models incorporating cardiopulmonary bypass time.[24,29] Adding urinary neutrophil gelatinase-associated lipocalin to these models increased the area under the curve value by a small extent.[25,30] For example, in one study the area under the curve of 0.85 for prediction of acute kidney injury by the clinical model was increased to 0.91 by adding urinary neutrophil gelatinase-associated lipocalin.[30]

Further, in our study, the kinetics of urinary and plasma neutrophil gelatinase-associated lipocalin values differed with plasma neutrophil gelatinase-associated lipocalin values showing a later peak. Therefore, urinary and plasma neutrophil gelatinase-associated lipocalin may reflect different pathological processes. Neutrophil gelatinase-associated lipocalin is markedly upregulated in kidney tubular cells very early after ischaemia and is rapidly excreted in the urine, as shown in animal and human studies.[31,32] Our data suggest that urinary neutrophil gelatinase-associated lipocalin may reflect processes during surgery or cardiopulmonary bypass that are injurious to tubular cells but transient and, therefore, not severe enough to cause acute kidney injury. Most of the paediatric cardiac surgery-associated acute kidney injury reports note much longer cardiopulmonary bypass times and higher average neutrophil gelatinase-associated lipocalin values in patients developing acute kidney injury compared with ours.[27–30,33,34] Prolonged surgery with cardiopulmonary bypass times comparatively longer than those in our study may accentuate these processes leading to acute kidney injury. Our study was unique in that patients in the acute kidney injury and non-acute kidney injury group showed equal median cardiopulmonary bypass times. Therefore, one important risk factor for acute kidney injury cancelled out, which simplified and strengthened the statistical analysis regarding neutrophil gelatinase-associated lipocalin in our study; moreover, most previous studies included exclusively children[27–29] in contrast to our study population of neonates and infants. Differing results may, therefore, reflect different age-specific sensitivity of neutrophil gelatinase-associated lipocalin. One study[34] reports high sensitivity of neutrophil gelatinase-associated lipocalin for developing acute kidney injury in a population comparable to ours. Few newborns were included, whereas in infants cardiopulmonary bypass times differed significantly in those with acute kidney injury and those without acute kidney injury.

Our results suggest that plasma and urinary neutrophil gelatinase-associated lipocalin values are not reliable indicators of an impending acute kidney injury in neonates and infants after cardiac surgery with cardiopulmonary bypass. Inflammatory status may have a major impact on plasma neutrophil gelatinase-associated lipocalin values in the setting of cardiac surgery, whereas an increase in urinary neutrophil

gelatinase-associated lipocalin may add little prognostic value over cardiopulmonary bypass time.

Our study has several limitations. First, the small sample size limits the power of our conclusions. We concentrated on only one, although prototypical, inflammatory parameter, namely interleukin-6. In further studies, a broader array of inflammatory parameters in plasma as well as urine may better characterise the nature of the inflammatory response. Some error may have been introduced by varying sampling time between 2 and 4 hours after termination of cardiopulmonary bypass although hourly dynamics of plasma neutrophil gelatinase-associated lipocalin have not been rigorously investigated. Previous studies[22,27] have reported sampling times of 2 hours after termination of cardiopulmonary bypass with earlier sampling when cardiopulmonary bypass times exceeded 2 hours. In our cohort cardiopulmonary bypass times were <2 hours in most cases leading to comparable sampling times. In contrast, other important studies[28,34] showing a correlation of neutrophil gelatinase-associated lipocalin with acute kidney injury development started sampling 2 hours after initiation of cardiopulmonary bypass. They demonstrated peak neutrophil gelatinase-associated lipocalin values at this early sampling time, which we may have missed with our measurements of plasma neutrophil gelatinase-associated lipocalin at 2–4 hours after termination of cardiopulmonary bypass.

We defined an arbitrary, though convenient and plausible, cut-off point of median cardiopulmonary bypass time to compare lower with higher cardiopulmonary bypass times. This was made possible by the near identity of cardiopulmonary bypass times in patients with and those without acute kidney injury, which may have been due to chance although this should not have influenced the results. We have included newborn patients in our study at an earliest age of 5 days at surgery when maternal plasma creatinine values may still confound the diagnosis of renal failure. This may lead to underdiagnosis of acute kidney injury in patients in the first week of life. In our study most patients were older so this may not be a significant confounder. We did not include urinary output and fluid overload in our analysis although these may be regarded as important parameters in the diagnosis of acute kidney injury in other settings. Depending on the haemodynamic characteristics of the individual patient and the prevailing goal of haemodynamic stabilisation, fluid – and diuretic – therapy often differs greatly between patients after cardiac surgery lessening the value of fluid parameters regarding acute kidney injury development and impeding comparisons.

In conclusion, in our study in neonates and infants undergoing cardiac surgery with cardiopulmonary bypass, plasma neutrophil gelatinase-associated lipocalin correlates with inflammatory status but not with the occurrence of acute kidney injury. In this specific population, neutrophil gelatinase-associated lipocalin may not be a reliable marker of developing acute kidney injury.

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Conflicts of Interest

None.

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