Cardiac Output is Not Associated with Plasma NGAL Concentrations Following Cardiopulmonary Bypass for Pediatric Cardiac Surgery

by

Alejandro Abraham Floh

A thesis submitted in conformity with the requirements for the degree of Master of Science (Clinical Epidemiology)
Institute of Health Policy, Management and Evaluation
University of Toronto

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Abstract

Introduction: This study investigated the relationship between postoperative cardiac output (CO) and plasma NGAL concentration, as a potential marker of acute kidney injury, following cardiopulmonary bypass surgery in infants.

Methods: Serial sampling for plasma neutrophil gelatinase-associated lipocalin (NGAL), CO and plasma interleukin-6 (IL-6) occurred at baseline and regular postoperative intervals. NGAL was measured using the Triage NGAL device (Alere, Canada).

Results: Fifty-nine subjects (35 males, median age 134 days, weight 4.98 kg, 54 (90%) with biventricular physiology) completed the study. Lower CO, higher plasma IL-6, inotrope score and omission of preoperative methylprednisolone were significantly associated with plasma NGAL in univariable analyses. Association with CO fell from significance (EST -0.13, p=0.055) while plasma IL-6 correlated strongly (EST 0.15, p<0.0001) with plasma NGAL in a multivariable mixed method model with repeat measures.

Conclusions: No statistically significant association was found between CO and plasma NGAL. Plasma IL-6 was strongly associated with NGAL levels.
I would like to thank Dr. Brian McCrindle and every member of my thesis committee for their help and guidance in completing my master’s project. This study would not have been possible without the support of Dr. Steven Schwartz, my clinical supervisor, and the extraordinary efforts of Drs. Masayuki Nakada, Kandice Mah, and Joshua Blinder, who collected and processed the blood samples at all times of the day and night.
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INTRODUCTION AND RATIONALE

1 Introduction

Acute kidney injury (AKI), defined as an abrupt deterioration in kidney function, is a common complication affecting up to 82% of children undergoing pediatric surgery for congenital heart disease[1-6]. The pathophysiology of AKI is multifactorial and is postulated to result from a cardiopulmonary bypass (CPB) -induced systemic inflammatory response and related ischemia-reperfusion. AKI is associated with a significant health-care burden and correlated with prolonged intensive care and hospital admission, and an increased incidence of infections, organ dysfunction and death[7-10].

Research into CPB-related AKI has generally been limited to observational studies focused on pre-operative and intra-operative contributing factors, with little attention paid to the impact of postoperative management. Postoperative impairment of myocardial performance can lead to a reduction in cardiac output and result in multi-organ dysfunction[11]. Since inadequate kidney perfusion is a known cause of AKI, these alterations to cardiac output may play a defining role in the development of AKI in this vulnerable population (See Figure 1 for conceptual framework). The relative contribution of postoperative cardiac output on the development of AKI in this setting has yet to be thoroughly investigated because of an inability to accurately assess the precise timing of renal injury and limitations on measuring cardiac output, particularly in children. With the use of respiratory mass spectrometry to measure cardiac output and the recent emergence of plasma neutrophil gelatinase-associated lipocalin (NGAL) as a validated biomarker for AKI, this question can now be addressed. This Master’s degree thesis explores the relationship between postoperative cardiac output and plasma concentrations of a biomarker of AKI in a prospective cohort, with the ultimate aim of identifying modifiable factors to target future therapeutic trials.
Figure 1: Conceptual framework for the current study

LITERATURE REVIEW

2 Acute kidney injury after pediatric bypass surgery

The reported incidence of AKI following pediatric CPB surgery has varied widely in the literature, from 33-82%, depending on the AKI definition used[1-6, 12]. Approximately 2-12% of affected children require renal replacement therapy[1, 6, 10, 13]. Although neonates (birth to 28 days old) have been generally excluded from these studies due to observed differences in AKI presentation attributed to changes in kidney development[14], recent studies have employed clinical criteria based on deviation from patient baseline similar to pediatric studies [1, 15, 16]. The incidence of neonatal AKI has been reported at 52-64%, with a dialysis requirement in 19% of patients [6, 17]. AKI evolves rapidly after CPB, with over half of pediatric patients manifesting findings by 24 hours following CPB and nearly 98% of subjects by 48 hours [6].
Perioperative determinants for the development of AKI following CPB surgery have been predominantly explored through observational studies. (Table 1) In a retrospective study in which 481 (31.9%) of 1510 children undergoing CPB surgery developed AKI, younger age, lower weight, preoperative cyanosis, and need for preoperative mechanical ventilation were reported as patient-related risk factors for developing AKI[18]. The association of AKI with younger age was borne out in a separate study in which Li et al. showed that subjects below two years of age were at the highest risk for developing AKI[6]. This finding persisted even in multivariable models accounting for numerous other predictors. Other preoperative factors implicated in postoperative AKI have included use of inotropes, diuretics and nephrotoxic antibiotics (aminoglycosides)[5, 19]. The influence of pre-existing renal dysfunction has been less clear. Adult studies have shown that lower estimated glomerular filtration rates and a higher urine albumin-to-creatinine ratio, representing renal dysfunction, have been correlated with a higher incidence of postoperative injury[20]. The role of preoperative serum creatinine concentrations on postoperative AKI in children has been mixed. Blinder et al. demonstrated that preoperative AKI, assessed by AKIN criteria, increased the risk of postoperative AKI from 26% to 37% (p=0.014) in infants[5]. Other studies, however, have shown no relationship[6] or an inverse relationship between preoperative serum creatinine and the incidence of postoperative AKI[17].

The use of CPB has a significant influence on the development on postoperative AKI. Aydin et al. found that subjects on CPB were at increased odds of developing AKI of 65 when compared to subjects who underwent cardiac surgery off CPB[4]. In subjects who underwent CPB surgery, intra-operative determinants of postoperative AKI have included increased surgical complexity (using the Risk Adjusted Congenital Heart Surgery score – RACHS ), longer CPB time, cross-clamp time, and deep hypothermic arrest time[5, 6, 17]. In the largest prospective cohort study of 311 children undergoing CPB surgery at three institutions, by Li et al., multivariable regression analysis showed age and CPB time to be the factors most strongly associated with developing AKI[6]. A linear association existed between the odds of developing AKI and time on CPB, with a peak adjusted odds ratio of 7.57 for subjects requiring over 180 minutes of CPB compared to those undergoing less than 60 minutes. Other variables including weight, preoperative serum creatinine, and surgical complexity (RACHS) were also associated with
developing AKI but dropped out of significance in multivariable analysis due to high collinearity.
In this group, intraoperative hypotension was found in 87% of those who developed CPB-related AKI, which may reflect the role of impaired renal perfusion on kidney damage. Nonetheless, cardiac output was not quantified and therefore could not be evaluated.

TABLE 1: Risk factors associated with increase in AKI

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Preoperative</th>
<th>Intraoperative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Younger Age</td>
<td>Surgical complexity</td>
</tr>
<tr>
<td></td>
<td>Lower weight</td>
<td>Increased CPB time</td>
</tr>
<tr>
<td></td>
<td>Cyanosis</td>
<td>Increase in cross-clamp time</td>
</tr>
<tr>
<td></td>
<td>? Pre-existing renal dysfunction</td>
<td>DHCA</td>
</tr>
<tr>
<td></td>
<td>Use of diuretics</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Use of nephrotoxic drugs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Use of inotropes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mechanical ventilation</td>
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</tbody>
</table>

AKI has been found to have a significant negative impact postoperatively, with deleterious effects noted for even mild evidence of renal dysfunction[10]. Children with AKI tend to have increased morbidity demonstrated by a longer requirement for ventilation support, intensive care unit (ICU) admission, and hospital admission[4, 17, 21]. AKI has also been associated with increased mortality, approaching an incidence of 80% in subjects requiring renal replacement[19, 22]. Despite the potential influence of developmental differences, neonates have shown a similar risk factor profile[6, 17].

**SUMMARY:** AKI following neonatal and pediatric CPB surgery is common and contributes to postoperative morbidity and mortality. Preoperative risk factors for AKI have included younger age, cyanosis, requirement for hemodynamic support and exposure to nephrotoxic agents. Longer time on CPB, increased surgical complexity, and intra-operative hypotension are also
associated with postoperative AKI. The ability to determine causation has been limited by the observational study designs used and the inability to modify the identified risk factors.

2.1 Pathophysiology of ischemia-induced AKI

The kidney is extremely vulnerable to ischemia and hypoxemia, predominantly at level of the proximal and distal tubules. Despite receiving approximately 20% of systemic cardiac output, the majority of the blood flow is diverted to the renal cortex at the expense of relative hypoxia of the outer renal medulla. This state of oxygen depletion results from a combination of increased oxygen demand in support of the kidney’s concentrating function and a relative limit to blood supply[23]. Ischemic injury to the kidney occurs when this fine balance of supply and demand is disturbed, and results in acute tubular necrosis. Manifestations of ischemic AKI include cellular necrosis and apoptosis that present as tubular dysfunction, decreased effective glomerular filtration rate, and vascular congestion[24]. Following a discrete insult, ischemic AKI progresses through four discrete stages: initiation, extension, maintenance and recovery[25].

Ischemic AKI is the aggregate of complex pathways that lead to cell necrosis and apoptosis (Figure 2). During the initiation phase of AKI, inadequate oxygen delivery leads to rapid depletion of cellular ATP stores, reaching levels of 10-30% within 10 minutes of onset[26, 27]. In response, ATP dependent sodium-potassium channels redistribute from the baso-lateral to apical membrane and become dysfunctional; consequently, oedema ensues as sodium accumulates in the cell and fluid flows across disrupted gap junctions. The degradation of ATP during periods of hypoxia also triggers enzymatic modifications that generate oxygen free radical species upon re-exposure to oxygen during reperfusion[27]. Free radical induced lipid peroxidation and membrane protein oxidation leads to enzyme dysfunction and damage to DNA structure, both strong triggers for cellular necrosis. Free radicals are also released from the migrating neutrophils that accumulate in response to the evolving injury, but the burden of this relative contribution remains unclear. Finally, cell damage is potentiated by the accumulation of intracellular calcium; a high calcium level induces proteases and phospholipases that break down protein, disrupt cellular membranes and interfere with the structure of the cytoskeleton.
Early macroscopic changes of acute tubular necrosis include flattening of cells, blebbing of the apical membrane surface and loss of the brush border[28, 29]. Tubular cells swell and detach from the basement membrane, leading to denuded membranes and gaps through which fluid can leak and exacerbate tissue swelling. The cellular debris that accumulates in tubular lumen coalesces to form casts and obstruct urine flow. The increased pressure is transmitted back to the glomerulus, impeding filtration.

Ischemic AKI then transitions into the extension phase during which renal endovascular cells are injured. This phase is marked by local and systemic release of pro-inflammatory cytokines, mediated in large part by pro-inflammatory cytokines IL-6 and TNF-α, and results in inflammation of the cortico-medulary junction[25]. Tissue oedema results in capillary congestion, which further exacerbates ischemia, an area susceptible to hypoxic injury. This may account for a 40-50% reduction to renal blood flow even after reperfusion has been
established. A further reduction in GFR may be seen. During the latter two stages, the maintenance phase and recovery phase, tubular cells proliferate or undergo apoptosis as tissue repair is initiated[25].

Induction of ischemia results in a well-established pattern of pathology that makes it an ideal experimental model for AKI[30]. In fact, numerous animal models of AKI have been developed based on renal artery obstruction, including the initial studies that identified NGAL as a potential biomarker of AKI[31].

**SUMMARY:** *Ischemia-reperfusion induces a recognized biochemical response that culminates in cellular oedema and disruption, predominantly of the renal tubules. Tubular urine flow, glomerular filtration and vascular function are disturbed.*

### 3 The effect of cardiopulmonary bypass

The direct contact between blood components and the innate material during the initiation of CPB triggers numerous biochemical pathways that lead to a well-recognized systemic inflammatory response syndrome (SIRS). These complex humoral and cellular cascades activate the coagulation, complement and fibrinolytic systems that have been shown to contribute to systemic organ injury and dysfunction, including AKI. This process is described in this section and summarized in Figure 3.
The earliest manifestation of the contact reaction is the stimulation of the coagulation and fibrinolytic pathways. Blood contact with the CPB circuit promotes conversion of circulating Factor XII into its active form, XIIa. This, in turn, induces the production of kallikrein from prekallikrein, which propagates inflammation by recruiting neutrophils and promoting fibrinolysis. A positive feedback loop between Kallikrein and Factor XII exists, which allows Kallikrein to amplify the inflammatory response by further increasing concentrations of its active metabolite. Factor XIIa actions include cleaving HMWK into bradykinin, a well-recognized vasodilator implicated in increasing vascular permeability and oedema. It can also activate factor XI to initiate the intrinsic limb of the coagulation system that culminates in the production of thrombin, another key inflammatory mediator.

Exposure to CPB also induces the complement system, predominantly through the alternative pathway, to produce the anaphylatoxins C3a and C5a. Complement is also activated by
kallikrein, plasmin, endotoxin and the classical pathway. This arm of the inflammatory response has been implicated in histamine release, increased vascular permeability, neutrophil and monocyte activation, and cytokine release.

Cytokines form a third pathway of the humoral inflammatory response. The mediators are released by primed leukocytes and endothelial cells following exposure to complement, endotoxin and ischemia-reperfusion and can exert a pro- or anti-inflammatory effect. CPB results in a rapid release of a complex array of cytokines including tumor necrosis factor alpha (TNFα), interleukin (IL) 1, IL-6, IL-8, and IL-10. IL-6 levels, which mediate its effects through release of acute phase proteins, have been shown to be strongly correlated with the severity of the inflammatory response and poor outcomes, particularly in children.

Induction of the humoral systems discussed above ultimately results in the activation and chemotaxis of neutrophils, which play a dominant role in tissue inflammation and injury. The first step for migration of neutrophils into affected tissue is the binding onto the endothelial surface. Activated neutrophils and endothelial cells upregulate expression of several adhesion molecules including selectins, integrins, and the immunoglobulin super-gene family whose cross-linking allows neutrophils to strongly bond to the endothelial surface. Neutrophils then undergo conformational changes that allow for transmigration into the subendothelial space. Once in the tissue, neutrophils release cytotoxic granules that contain proteases, oxygen radicals and arachidonic acid derivatives that mediate the inflammatory response.

The magnitude of the inflammatory response following CPB has been associated with increased risk of AKI. Liu et al. reported that children who developed AKI following cardiac surgery had a higher plasma IL-6 and IL-8 concentration at two and twelve hours following initiation of CPB[32]. These cytokines were also associated with the need for prolonged ventilation. Similarly, Miklaszewska and colleagues corroborated the association between plasma IL-6 levels and injury[33]. Endothelial disruptions leading to neutrophil migration and release of cytotoxic agents have also been implicated in the development of AKI, particularly in models of ischemia[34]. In an animal model, Linas et al. reported that primed and activated neutrophils tended to accumulate in injured kidneys. Nevertheless, severity of AKI could be reduced by depleting the blood of circulating leukocytes and by inhibiting neutrophil chemotaxis[35].
SUMMARY: Initiation of CPB incites a complex inflammatory response that activates the coagulation system, increases circulating cytokines and leads to endothelial dysfunction and leukocyte migration. Inflammation has been associated with tissue injury, including AKI, and must be accounted for when studying AKI following cardiac surgery. Plasma IL-6 plays a prominent role in affecting the inflammatory response and its concentration is commonly measured to reflect the height of systemic inflammation.

4 Defining and measuring AKI

4.1 Clinical criteria

In the absence of any direct pathological marker of AKI, surrogate markers of creatinine and urine output have been used to quantify AKI. The AKI literature, however, has been plagued by inconsistent definitions of AKI and disease severity. Creatinine clearance is used to accurately approximate glomerular filtration during periods of steady state, but quantification is time consuming (12-24 hour urine collections) and estimation using conversion formulas has been found unreliable. The use of the Schwartz formula, the reference standard for children, has been shown to be prone to over-estimation[36]; alternatively, isolated measures of plasma creatinine concentrations lack sensitivity and accuracy under various physiologic conditions[16, 37]. These manifestations of AKI usually present 24 to 72 hours after onset of injury. Tubular function is measured by urine production (although urine production can also be impaired due to AKI) and diminished production is nonspecific to the kidney disease and may remain within normal limits despite significant renal disease [16].

The Acute Dialysis Quality Initiative (ADQI) group developed the RIFLE criteria in 2004 to standardize the clinical classification of AKI[16], which assigned renal injury to one of three categories based on severity (R-risk, I-injury, F-failure) and two outcomes (L-loss, E-end-stage kidney disease). Elevation in serum creatinine (≥50% above baseline) in combination with reduced urine production differentiated the degrees of renal impairment (Table 2). This was subsequently modified and validated for pediatric patients and named pRIFLE1 (Table 3). The pRIFLE criteria have been validated as a predictor of morbidity and mortality[1, 16, 38].
### TABLE 2: RIFLE criteria characterizing stages of AKI[16]

<table>
<thead>
<tr>
<th>Stage</th>
<th>GFR Criteria (Serum Creatinine)</th>
<th>Urine Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk</td>
<td>Decrease by &gt; 25% (Increase by 1.5 times)</td>
<td>&lt; 0.5 ml/kg/h for 6 h</td>
</tr>
<tr>
<td>Injury</td>
<td>Decrease by 50% (Increase by 2 times)</td>
<td>&lt; 0.5 ml/kg/h for 12 h</td>
</tr>
<tr>
<td>Failure</td>
<td>Decrease by 75% (Increase by 3 times) or Serum creatinine &gt; 4 mg/dL</td>
<td>&lt; 0.3 ml/kg/h for 24 h or anuric for 12 hours</td>
</tr>
<tr>
<td>Loss</td>
<td>Loss of function for 4 weeks</td>
<td></td>
</tr>
<tr>
<td>End Stage</td>
<td>Loss of function for 4 months</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 3: pRIFLE modification[1]

<table>
<thead>
<tr>
<th>Stage</th>
<th>Estimated Creatinine Clearance</th>
<th>Urine Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk</td>
<td>Decrease by 25%</td>
<td>&lt; 0.5 ml/kg/h for 8 h</td>
</tr>
<tr>
<td>Injury</td>
<td>Decrease by 50%</td>
<td>&lt; 0.5 ml/kg/h for 16 h</td>
</tr>
<tr>
<td>Failure</td>
<td>Decrease by 75% or &lt; 35 ml/min/1.73m2</td>
<td>&lt; 0.3 ml/kg/h for 8 h or anuric for 12 hours</td>
</tr>
<tr>
<td>Loss</td>
<td>Persistent failure for 4 weeks</td>
<td></td>
</tr>
<tr>
<td>End Stage Kidney Disease</td>
<td>Persistent failure for 3 months</td>
<td></td>
</tr>
</tbody>
</table>

In 2007, the RIFLE criteria were further adapted by the Acute Kidney Injury Network (AKIN), a collaborative group of nephrology and intensive care societies, to harmonize the definition and classification of AKI[15]. Under AKIN, severity of AKI was categorized into Stage 1 through 3, reflecting mild, moderate and severe disease based on incremental changes to serum creatinine or decreases in urine output (Table 4). AKIN criteria acknowledged the implication of even a mild increase in serum creatinine by including a rise of greater than 0.3 mg/dL as mild disease. Departing from the RIFLE criteria, the period of change was shortened from 7 days to 48 hours and all individuals requiring renal replacement therapy were classified as severe. When compared in a similar population, AKIN staging was found to be more sensitive than RIFLE, at the potential expense of over-diagnosing AKI but with better predictive power for mortality[39]. Nevertheless, despite the introduction of consensus criteria to standardize measurement of AKI in research, a review of the literature has shown ongoing disparity in its application[40].
### TABLE 4: AKIN classification[15]

<table>
<thead>
<tr>
<th>Stage</th>
<th>Increase in Serum Creatinine</th>
<th>Decrease in Urine Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Increase by 26.4 μmol/L or increase by 100-200% from baseline</td>
<td>&lt; 0.5 mL/kg/hr for over 6 hours</td>
</tr>
<tr>
<td>2</td>
<td>Increase by 200-300% from baseline</td>
<td>&lt; 0.5 mL/kg/hr for over 12 hours</td>
</tr>
<tr>
<td>3</td>
<td>Increase by over 300% from baseline</td>
<td>&lt; 0.3 mL/kg/hr for over 24 hours OR anuria</td>
</tr>
</tbody>
</table>

**SUMMARY:** Clinical AKI is graded on consensus-based clinical criteria, including RIFLE, pRIFLE, and AKIN staging that use ordinal scales for classifying serum creatinine and urine production. There is a time lag of several hours to days from a known insult to the meeting of any of these cut-point criteria before a diagnosis of AKI can be made. Hence, they are not useful for early prediction. Although widely used in the current literature, a clear consensus is lacking.

#### 4.2 Neutrophil Gelatinase-Associated Lipocalin

The significant time delay between an observed insult and the resulting clinical manifestations of AKI prompted investigation into novel biological markers that would allow for earlier detection of injury. Neutrophil gelatinase-associated lipocalin (NGAL) had emerged as the most highly investigated biomarker, particularly in children following CPB surgery. NGAL is a 25 kDa, iron-binding protein that is constitutively expressed in epithelial cells of the kidney, bowel, trachea, lung, breast and ovary[41-44]. As indicated by its name, NGAL was originally identified, isolated and characterized by Kjeldsen from neutrophils in two separate experiments[42, 45]. NGAL was thought to play a fundamental role in the innate immune system by binding siderophores, small iron-binding molecules secreted by bacteria, inhibiting their growth.

NGAL’s potential use as a biomarker for AKI was first recognized in a genome-wide reverse transcription analysis of a Swiss-Webster mice model of acute kidney ischemia[31, 46]. Mishra et al. demonstrated consistent up-regulation of NGAL RNA transcription and translation in the proximal renal tubules by 3 hours of ischemia, with protein detected in plasma and urine. In a subsequent clinical study, Mishra and colleagues demonstrated the predictive power of NGAL in a cohort of 20 children following CPB surgery[47]. Children who developed AKI, defined as a rise in serum creatinine of at least 50% above baseline, were found to have a sharp increase in
urine and plasma NGAL concentrations within two hours of CPB initiation, which peaked by 4 hours. In contrast, NGAL concentrations remained low in those who did not develop AKI. These findings have been confirmed in follow-up studies including larger cohorts from the same institutions[48]. Elevations in urine and plasma NGAL have subsequently been validated to predict clinically relevant renal dysfunction and mortality in adult and pediatric intensive care patients with AKI in numerous clinical situations, including following CPB.[47, 49-52] The predictive power may be increased for children[49].

The biological advantage of NGAL’s iron-binding properties in the setting of kidney injury remains unclear. NGAL can be used as an iron transporter that initiates divergent cellular pathways, from cellular proliferation to apoptosis[53]. The translocation of iron, while bound to NGAL complexes, by renal epithelial cells from renal tubules to extracellular matrix may establish an iron deficient environment that triggers apoptosis. Alternatively, NGAL may modulate cellular proliferation through interaction with hepatocyte growth factor, matrix metallo-proteinase-9, or extracellular protein kinases.

NGAL is also up-regulated by other pathophysiologic processes including sepsis and inflammation. Its utility as a biomarker has been studied in numerous fields including oncology and inflammatory bowel disease[41, 43, 44].

SUMMARY: Plasma NGAL, a protein released by the renal tubular epithelium following AKI, has regularly been used as a biomarker for AKI. It has been shown to predict AKI and is associated with morbidity and mortality, particularly following pediatric CPB, but lacks specificity as it is also released by other epithelial tissues.

4.3 Rationale for NGAL use in cardiac critical care research

The application of clinical criteria as an acute measure of kidney function can also be somewhat questionable in the acute phase of intensive care. Plasma creatinine is considered to best reflect GFR under stable conditions. Critically ill patients are rarely in stable physiologic states, and it is unclear if plasma creatinine accurately represents GFR during these periods of rapid fluctuations. Furthermore, despite advances in the clinical classification of AKI, RIFLE, pRIFLE and AKIN lack responsiveness, requiring 24-48 hours following an insult to demonstrate clinical
changes. This inherent limitation precludes their use as an evaluative index in an intensive care setting where there may be several threats to renal function over a short period of time. Plasma NGAL concentrations, in contrast, respond rapidly to AKI and have been validated in pediatric CPB surgery. As mentioned, children with AKI following CPB surgery experience a sharp rise in plasma NGAL within two hours of initiation of CPB support[47, 48] with the height of plasma concentrations correlating positively with the degree of clinical AKI[48] and postoperative morbidity and mortality[54]. NGAL has therefore been shown to have the responsiveness and predictive power to be well suited to study the effects of cardiac output variability on AKI.

5 Postoperative cardiac output

CPB has been associated with myocardial impairment and dysfunction. It can lead to a well-recognized postoperative decrease in cardiac output that reaches a nadir between 9-12 hours following separation from CPB prior to normalizing by 24 hours[11]. More prolonged depression of cardiac output can occur when there are important residual cardiac lesions or when there has been more comprehensive damage to the myocardium. Approximately 25% of patients manifest symptoms of low cardiac output syndrome with measured flow falling below 2 L/min/m2 indexed to body surface area[11]. Historically, in children undergoing cardiac surgery, a cardiac index below 2 L/min/m2 was strongly associated with death[55].

In general, reduced cardiac output is thought to lead to organ dysfunction due to inadequate tissue perfusion and ischemia. In fact, inducing tissue ischemia by reducing renal blood flow is a common model of AKI in numerous animal studies. However, the role played by the postoperative decline in cardiac output in the development of AKI is unclear. Negative effects of reduced systolic blood pressure, used as a surrogate for cardiac output, on AKI have been demonstrated.[10] However, it is recognized that clinical parameters including heart rate and blood pressure are poor indicators of cardiac output[11].

There are challenges specific to accurately assessing cardiac output in children. Standard techniques used for adult populations, such as thermodilution and echocardiography, are less common due to complication risks and decreased reliability[56, 57]. Respiratory mass spectrometry has been used to very precisely and continuously measure oxygen consumption
(VO2) and then calculate cardiac output using arterial and venous blood gases and the Fick equation[58]. This method of cardiac output monitoring has been used to assess the impact of cardiac output following pediatric heart surgery at the Hospital for Sick Children for many years[6, 59-63].

SUMMARY: CPB can result in a predictable decline in cardiac output that normalizes by the second postoperative day. This decrease in cardiac output may contribute to AKI secondary to renal ischemia. We have the technology and experience to measure cardiac output in postoperative children and to study its effects on kidney injury.

5.1 Evidence to support a link between postoperative cardiac output and AKI

The contribution of postoperative hemodynamics to AKI development has not been studied extensively. Krawczeski et al. demonstrated that subjects with AKI had similar baseline characteristics to those without AKI but had higher inotropic requirements during recovery from CPB surgery[48]. AKI has also been linked to clinical low cardiac output syndrome in several studies: Sethi et al. and Chiravuri et al. found a 5.9 and 20.2 increase in the odds of cardiac failure in subjects with AKI, respectively[19, 64]. Unfortunately neither publication defined “pump” (cardiac) failure, thus limiting the finding’s interpretation and generalizability. In their large retrospective study, Toth et al. reported an ordinal relationship between severity of AKI and inotropic requirement, with higher scores associated with increased severity as determined by pRIFLE score (median inotrope score of 18, 17, 10, and 4 for pRIFLE F, I, R and no AKI, respectively)[18]. A similar step-wise association was found between severity of AKI and incidence of low cardiac output syndrome: low cardiac output rose steadily from 11.2% in those without AKI to 56.7% in those with failure (p<0.05). Low cardiac output syndrome was higher in subjects with AKI, even when matched to unaffected subjects by propensity scoring. The definition of low cardiac output in this study was based on nonspecific clinical markers (e.g., tachycardia, hepatomegaly, or low urine output), increasing subjectivity and potential bias. Furthermore, the temporal relationship among inotropic support, low cardiac output and AKI was not reported, thereby limiting assessment of the role these factors played in the development of AKI. Nevertheless, since higher inotropic scores and clinical low cardiac output
syndrome are frequently found in the setting of poor cardiac performance, these studies all raise a potential association between myocardial impairment and postoperative AKI. This would support the need to further investigate the impact of cardiac output on the subsequent development of AKI, using reliable and quantifiable measurements.
CURRENT STUDY

6 Study aim

To determine the relationship between cardiac output and plasma NGAL concentrations in infants following cardiopulmonary bypass surgery for congenital heart disease.

7 Question

Is cardiac output within the 72 hours following surgery associated with an increase in plasma NGAL concentrations, in term infants undergoing bypass surgery for congenital heart disease?

8 Hypothesis

An inverse relationship exists between postoperative cardiac output within 72 hours following surgery and plasma NGAL concentrations.

9 Methods

9.1 Population

Inclusion and exclusion criteria are summarized in Table 5.

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Term infants following cardiopulmonary bypass</td>
<td>Prematurity</td>
</tr>
<tr>
<td>Endotracheal tube <em>in situ</em></td>
<td>Weight &lt; 2.5 kg</td>
</tr>
<tr>
<td>Arterial line</td>
<td>Congenital nephropathy</td>
</tr>
<tr>
<td>Central venous line</td>
<td>Preoperative renal dysfunction</td>
</tr>
<tr>
<td></td>
<td>Preoperative necrotizing enterocolitis</td>
</tr>
<tr>
<td></td>
<td>Postoperative ECMO</td>
</tr>
</tbody>
</table>
All infants with congenital heart disease undergoing cardiopulmonary bypass surgery were screened for study participation. Potential subjects were identified from hospital inpatient cardiology patients (including cardiac critical care unit and cardiology ward), the preoperative cardiac surgery clinic, and the weekly preoperative surgical conference. Consecutive patients were approached for study enrollment. The study focused on infants as this population was most likely to remain intubated following surgery, which allowed for cardiac output monitoring. Premature subjects born under 37 weeks gestation age who were younger than 28 days of age at time of surgery comprise a group with distinct physiology and renal developmental status and therefore were not included. Subjects with a birth weight below 2.5 kg were also excluded, since the lower body weight does not allow for the same blood sampling schedule. Only patients with appropriately sized endotracheal tubes (with minimal leak) and indwelling lines for arterial and central venous blood sampling were included to ensure accurate measurement of cardiac output; cardiac anaesthesiologists were encouraged to use microcuffed endotracheal tubes to ensure an adequate seal and minimize disqualification of subjects. Subjects requiring postoperative mechanical circulatory support were also excluded, as this support precludes accurate cardiac output measurement and is highly associated with a prolonged systemic inflammatory response and multi-organ dysfunction.

In order to avoid the potential confounding effects of preoperative kidney dysfunction, all subjects with plasma creatinine concentrations one-and-a-half times the upper limit of normal for age or meeting the requirement for renal replacement therapy were excluded. Although not all structural abnormalities are associated with functional impairment, a reliable test to identify at-risk subjects was not available and, therefore, subjects with congenital nephropathy on abdominal ultrasound were not included. Evidence of preoperative necrotizing enterocolitis also warranted exclusion, as it may also affect NGAL concentrations and act as a confounder.

9.2 Study design

This was a prospective, observational cohort study.

9.3 Setting

The study was conducted in the Cardiac Critical Care Unit at the Hospital for Sick Children.
9.4 Measurements

9.4.1 Exposure

Absolute cardiac output value was used as the primary exposure/predictor variable of this study. A respiratory mass spectrometer (AMIS 2000, Innovision A/S, Odense, Denmark) was connected in-line with the ventilator to continuously measure oxygen consumption (VO2) according to a previously described protocol[59]. Contemporaneous arterial and venous blood gases were drawn and used to calculate cardiac output according to the Fick equation[58]. Cardiac output was recorded upon admission to the cardiac critical care unit and at six-hourly intervals for the first 72 hours or until time of extubation, whichever occurred first (Figure 4).

Figure 4: Timeline of study-related measurements
9.4.2 Outcomes

9.4.2.1 Primary outcome

Plasma NGAL concentration was used as the primary outcome for the study. Whole-blood samples (0.5 mL) were drawn prior to CPB, following separation from CPB, at CCU admission and at six-hourly intervals, corresponding to timing of the cardiac output measurement. The Triage® NGAL point-of-care immunoassay was used in conjunction with the Triage® Meter (Biosite, Inc) to rapidly quantify plasma NGAL concentrations, within the range of 60 to 1300 ng/ml. The EDTA-anticoagulated whole blood was injected into a single use cassette in which the plasma was separated from the blood cells across a filter and is reconstituted with the fluorescent-labeled antibody that is used to fill the measurement strip. Dissolved plasma NGAL prevents binding of the fluorescent-labeled antibody to the fixed NGAL particle on the assay such that the degree of fluorescence is inversely proportional to the plasma concentration of NGAL. In a study conducted by Dent and colleagues, plasma NGAL concentrations measured by this bedside test correlated positively (Pearson r=0.94, p<0.001) with ELISA quantification[54]. Measured NGAL concentrations have been shown to predict clinical AKI and are correlated with increased morbidity and mortality[48].

9.4.3 Outcome modifiers

9.4.3.1 Nephrotoxic agents – aminoglycosides

Subjects were followed for use of aminoglycosides and categorized by use (yes/no) at every individual time point. Aminoglycosides may be used as prophylaxis (when chest is open) or treatment in postoperative subjects, particularly in subjects at higher risk for low cardiac output. These antibiotics have known toxic side effects on the kidney and therefore must be considered in the analysis to minimize bias.

9.4.4 Potential confounders

9.4.4.1 Inflammatory cytokines

Blood (1mL whole blood) was drawn for the inflammatory cytokine IL-6 during sampling for plasma NGAL measurements. Plasma IL-6 was measured in order to quantify the systemic inflammatory response that results from CPB and that has been linked to AKI. The magnitude
and effect of inflammation must be accounted for when examining the relationship between cardiac output and plasma NGAL concentrations, and can be explored and adjusted for in multivariable modeling.

9.4.4.2 Inotrope-vasopressor score

Total inotropic and vaso-active support was quantified at each blood sampling using the modified inotrope-vasopressor score (IS= dopamine dose (mcg/kg/min) + dobutamine dose (mcg/kg/min) + 100 x epinephrine dose (mcg/kg/min) + 10 x milrinone dose (mcg/kg/min) + 10,000 x vasopressin dose (U/kg/min) + 100 x norepinephrine dose (mcg/kg/min))[65]. Lower cardiac output may be associated with a higher score as higher doses are used when output is deemed clinically insufficient. These agents can also contribute to AKI. As such, the use of inotropes and vasopressors may act as confounders and must be incorporated into the analysis.

9.5 Statistical analysis

All demographic data were described by means with standard deviations, medians with interquartile ranges, and frequencies as appropriate. For the purpose of analysis, NGAL data were paired with previous cardiac output measurements that were offset by six hours, to account for the biological relationship between cardiac output and AKI. (Figure 5) The effect of systemic inflammation was assessed as a potential confounder on plasma NGAL concentrations. Due to a similar biological temporal relationship, plasma NGAL concentrations were modeled with the preceding plasma IL-6 concentrations. In light of the large variability in individual plasma IL-6 concentrations, systemic inflammation was also quantified by peak IL-6 plasma concentrations and by calculating the area under the curve for the first twenty-four postoperative hours using the trapezoidal rule. Appropriate transformations were made to achieve normal distributions in non-normally distributed data. Univariable mixed regression models were used to determine the direction and strength of the associations among potential predictor variables and serum NGAL concentrations, while accounting for repeated measures with time as a fixed effect and patient as a random effect. Mixed models allow for variation in individual subject intercepts, slopes, and randomly missed samples. All predictor variables with p<0.4 were considered for the final multivariable mixed regression model. Predictor variables were assessed for multicollinearity and removed if analysis for tolerance was below 0.25 or the
Variance inflation factor was above 4. The final model incorporated cardiac output and time from surgery based on clinical considerations and additional predictors based on change in estimates strategy; all variables that resulted in a greater than 10% increase in beta value of Ln cardiac output were included.

Figure 5: Modeling of predictor variables: Statistical pairing of plasma NGAL concentrations with the prior measurement of cardiac output and plasma IL-6 concentrations.

9.5.1 Sample size

Sample size for this study was estimated using the assumption that the correlation between NGAL and cardiac output would be $r > 0.40$ (adjusted for repeated measures). In order to detect this degree of correlation, at a power of 90% using a p-value of 0.05, a total of 60 patients were required. (Table 6) Because of the repeated measures analysis, subjects with early study termination contributed to the study and did not adversely affect the study’s power.

<table>
<thead>
<tr>
<th>Power/Correlation</th>
<th>r=0.50</th>
<th>r=0.40</th>
<th>r=0.30</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>29</td>
<td>46</td>
<td>84</td>
</tr>
<tr>
<td>85%</td>
<td>32</td>
<td>53</td>
<td>96</td>
</tr>
<tr>
<td>90%</td>
<td>37</td>
<td>61</td>
<td>112</td>
</tr>
</tbody>
</table>
10 Results

10.1 Study population

Sixty subjects were enrolled into the study. Fifty-three subjects completed the study, providing 316 paired observations of cardiac output and plasma NGAL. Demographic, surgical and outcome data are presented in Table 7. Subjects were predominantly male (58%) infants undergoing biventricular repair (90%). Median time to extubation and ICU discharge was 1.5 and 4 days, respectively. Only one subject experienced a cardiac arrest and none died during the study.

Table 7: Patient demographics and surgical characteristics

<table>
<thead>
<tr>
<th>Demographics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male)</td>
<td>35 (58%)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>4.98 (3.68, 6.36)</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>0.3 (0.24, 0.36)</td>
</tr>
<tr>
<td>Baseline serum creatinine (μmol/L)</td>
<td>29 (24, 42)</td>
</tr>
<tr>
<td>Age at surgery (days)</td>
<td>134 (16,187)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Surgical Complexity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Biventricular physiology</td>
<td>54 (90%)</td>
</tr>
<tr>
<td>Cardiopulmonary bypass time (min)</td>
<td>118 (93, 156)</td>
</tr>
<tr>
<td>Cross clamp time (min)</td>
<td>80 (64, 110)</td>
</tr>
<tr>
<td>Deep hypothermic circulatory arrest</td>
<td>10 (17%)</td>
</tr>
<tr>
<td>Deep hypothermic circulatory arrest time (min)</td>
<td>22 (20, 26)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical Outcomes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to extubation (Days)</td>
<td>1.5 (1, 5)</td>
</tr>
<tr>
<td>ICU length of stay (Days)</td>
<td>4 (2,9)</td>
</tr>
<tr>
<td>Hospital length of stay (Days)</td>
<td>9 (6, 16)</td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Death</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Continuous nonparametric data presented as median (interquartile range). Count data presented as frequencies.
10.2 Postoperative cardiac output:

Cardiac output data were available for fifty-six subjects; data were not captured on four subjects due to lack of availability of a respiratory mass spectrometer. Given the variable timing of extubation, the number of subjects undergoing cardiac output sampling declined over time, with only sixteen subjects having data recorded at 72 hours following surgery. A total of 382 cardiac output observations were recorded. Median cardiac output increased steadily from 2 L/min/m² immediately following CCU admission to 2.9 L/min/m² at 72 hours. (Figure 6) Approximately 66% (37 of 56 patients) had at least one cardiac output measurement that fell below 2 L/min/m². Overall, only 39% (148 of 382) of individual cardiac output measurements fell below that threshold.

Figure 6: Box-plot of cardiac output over time. Solid lines represent the median value, open boxes represent the inter-quartile range and lines represent maximum and minimum values. N=56 subjects; Asterisk (*) denotes maximum values truncated.
10.3 Plasma NGAL concentrations

Plasma NGAL concentrations were not collected on three subjects due to lack of available NGAL assays. A total of 341 NGAL samples were measured with only fifteen patients having NGAL concentrations recorded for the entire duration of study. Preoperative NGAL concentrations tended to be below 60 nanogram/mL, the lower limit of detection for the Triage® NGAL point-of-care immunoassay. Plasma NGAL concentrations rose sharply after cardiac surgery, reaching a median (IQR) peak value of 96 (89, 314) at 18 hours (12, 30) following separation from bypass. Median NGAL concentrations remained relatively stable across the sampling period with a range from 113 to 190 nanograms/mL. (Figure 7)

Figure 7: Box-plot of plasma NGAL concentration over time. Solid lines represent the median value, open boxes represent the inter-quartile range and lines represent maximum and minimum values. N=57 subjects. Asterisk (*) denotes maximum values truncated.

Although smaller patients tended to have lower peak NGAL concentrations (weight: parameter estimate (EST)=0.13, p=0.02, r=0.3; body surface area: EST=2.8, p=0.05, r=0.26), no association was found with age, CPB time, aortic cross-clamp time and deep hypothermic circulatory arrest
time. No specific association was observed in the relationship between these predictors and plasma NGAL concentration at each individual time point. 

(Table 8)

Table 8: Univariate linear regression modeling independent predictor variables and plasma NGAL concentrations. All statistically significant values (p < 0.05) are in bold. BSA — body surface area; CPB – cardiopulmonary bypass; DHCA – deep hypothermic circulatory arrest.

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>Age EST</th>
<th>p-value</th>
<th>Weight EST</th>
<th>p-value</th>
<th>BSA EST</th>
<th>p-value</th>
<th>CPB time EST</th>
<th>p-value</th>
<th>Cross-clamp time EST</th>
<th>p-value</th>
<th>DHCA time EST</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.002</td>
<td>0.02</td>
<td>0.13</td>
<td>0.01</td>
<td>2.8</td>
<td>0.02</td>
<td>0.002</td>
<td>0.16</td>
<td>0.004</td>
<td>0.05</td>
<td>-0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>0.002</td>
<td>0.02</td>
<td>0.11</td>
<td>0.02</td>
<td>2.2</td>
<td>0.05</td>
<td>0.001</td>
<td>0.64</td>
<td>0.002</td>
<td>0.39</td>
<td>-0.02</td>
<td>0.10</td>
</tr>
<tr>
<td>12</td>
<td>0.001</td>
<td>0.27</td>
<td>0.08</td>
<td>0.22</td>
<td>1.4</td>
<td>0.32</td>
<td>0.002</td>
<td>0.18</td>
<td>0.01</td>
<td>0.07</td>
<td>-0.02</td>
<td>0.27</td>
</tr>
<tr>
<td>18</td>
<td>0.001</td>
<td>0.28</td>
<td>0.05</td>
<td>0.39</td>
<td>0.7</td>
<td>0.58</td>
<td>0.002</td>
<td>0.17</td>
<td>0.005</td>
<td>0.05</td>
<td>-0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>24</td>
<td>-0.0002</td>
<td>0.85</td>
<td>0.02</td>
<td>0.80</td>
<td>-0.2</td>
<td>0.92</td>
<td>0.002</td>
<td>0.34</td>
<td>0.005</td>
<td>0.08</td>
<td>-0.02</td>
<td>0.30</td>
</tr>
<tr>
<td>30</td>
<td>0.001</td>
<td>0.36</td>
<td>0.11</td>
<td>0.11</td>
<td>2.7</td>
<td>0.13</td>
<td>0.001</td>
<td>0.52</td>
<td>0.003</td>
<td>0.29</td>
<td>-0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>36</td>
<td>0.003</td>
<td>0.02</td>
<td>0.19</td>
<td>0.004</td>
<td>4.8</td>
<td>0.003</td>
<td>0.001</td>
<td>0.79</td>
<td>0.002</td>
<td>0.47</td>
<td>-0.01</td>
<td>0.38</td>
</tr>
<tr>
<td>42</td>
<td>0.002</td>
<td>0.15</td>
<td>0.16</td>
<td>0.03</td>
<td>3.9</td>
<td>0.04</td>
<td>-0.0001</td>
<td>0.98</td>
<td>0.001</td>
<td>0.76</td>
<td>-0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>48</td>
<td>0.002</td>
<td>0.17</td>
<td>0.13</td>
<td>0.07</td>
<td>3.2</td>
<td>0.07</td>
<td>-0.001</td>
<td>0.76</td>
<td>0.001</td>
<td>0.73</td>
<td>-0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>54</td>
<td>0.001</td>
<td>0.68</td>
<td>0.07</td>
<td>0.47</td>
<td>1.5</td>
<td>0.45</td>
<td>-0.001</td>
<td>0.54</td>
<td>-0.0001</td>
<td>0.97</td>
<td>-0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>60</td>
<td>0.0001</td>
<td>0.94</td>
<td>0.07</td>
<td>0.45</td>
<td>1.4</td>
<td>0.45</td>
<td>-0.001</td>
<td>0.60</td>
<td>0.0003</td>
<td>0.91</td>
<td>-0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>66</td>
<td>0.002</td>
<td>0.08</td>
<td>0.14</td>
<td>0.09</td>
<td>3.0</td>
<td>0.09</td>
<td>-0.001</td>
<td>0.56</td>
<td>-0.001</td>
<td>0.87</td>
<td>-0.01</td>
<td>0.23</td>
</tr>
<tr>
<td>72</td>
<td>0.001</td>
<td>0.41</td>
<td>0.05</td>
<td>0.51</td>
<td>1.2</td>
<td>0.51</td>
<td>-0.001</td>
<td>0.58</td>
<td>-0.00001</td>
<td>1.0</td>
<td>-0.02</td>
<td>0.04</td>
</tr>
</tbody>
</table>
10.4 Systemic inflammatory profile

The median plasma IL-6 concentrations rose sharply following cardiac surgery, reaching a peak (IQR) of 187 (108, 276) picogram/mL at time of CCU admission. Concentrations subsequently declined and stabilized at a low level of inflammation by 48 hours following cardiac surgery. (Figure 8)

Figure 8: Box-plot of plasma IL-6 concentrations over time. Solid lines represent the median value, open boxes represent the inter-quartile range and lines represent maximum and minimum values. Asterisk (*) denotes maximum values truncated.

10.5 Censoring of subjects and bias

Only 16 subjects of the entire cohort provided CO measurements at 72 hours of observation. Given the number of subjects censored throughout the study because of earlier extubation and ICU discharge, we compared the demographic characteristics, preceeding cardiac output and
NGAL values of censored subjects with individuals not censored at all individual time points. This analysis was undertaken to assess potential selection bias. There were no observed differences in the demographic, operative and cardiac output profiles of censored individuals compared to those remaining in the study at any timepoint.

### 10.6 Predictor variables and plasma NGAL concentrations

The relationship between cardiac output and plasma NGAL concentrations was examined using a mixed regression model to account for repeated measures, random intercepts and slopes, and values missed at random. Cardiac output, plasma IL-6 concentrations and plasma NGAL concentrations were log transformed to approximate normal distribution for use in statistical analyses. A statistically significant inverse association, with lower cardiac output linked to higher plasma NGAL concentrations, was noted (EST -0.23, p=0.0012). This analysis was repeated for other predictor variables and potential confounders. (Table 9)

Table 9: Univariable models for factors associated with higher NGAL concentrations (log transformed). (N=450)

<table>
<thead>
<tr>
<th>Predictor Variable</th>
<th>EST</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Older Age (days)</td>
<td>0.001</td>
<td>0.108</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.079</td>
<td>0.090</td>
</tr>
<tr>
<td>Body Surface Area (m²)</td>
<td>1.55</td>
<td>0.130</td>
</tr>
<tr>
<td>Use of Preoperative Methylprednisolone</td>
<td>-0.46</td>
<td>0.0001</td>
</tr>
<tr>
<td>Longer CPB Time (min)</td>
<td>0.001</td>
<td>0.270</td>
</tr>
<tr>
<td>Longer Cross-Clamp Time (min)</td>
<td>0.003</td>
<td>0.017</td>
</tr>
<tr>
<td>Longer Deep Hypothermic Circulatory Arrest Time (min)</td>
<td>-0.021</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Higher Inotrope Score</td>
<td>-0.0001</td>
<td>0.023</td>
</tr>
<tr>
<td>Shorter Scheduled Time (Hrs)</td>
<td>-0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Postoperative Aminoglycoside Use</td>
<td>0.105</td>
<td>0.340</td>
</tr>
<tr>
<td>Lower Previous CO (L/min/m²) Log Transformed</td>
<td>-0.23</td>
<td>0.0012</td>
</tr>
<tr>
<td>Higher Previous IL-6 (nanogram/mL) Log Transformed</td>
<td>0.117</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Higher IL-6 AUC (nanogram/mL*hours) / 1000</td>
<td>0.04</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
All predictors had a p-value $\leq 0.4$ and were therefore considered for the final multivariable model. Body surface area, x-clamp time, and deep hypothermic circulatory arrest were highly correlated and determined to be collinear with other predictor variables (variance inflation factor greater than 4 and tolerance below 0.25) and therefore excluded in a stepwise fashion from the final multivariable model.

The relationship between cardiac output and plasma NGAL concentration was examined in a final multivariable mixed regression model that included cardiac output, time after surgery and predictor variables identified through the change in estimate process. The final model, with age included as the only additional predictor variable, found a trend toward higher plasma NGAL concentration with lower cardiac output (EST -0.13, p=0.055). Plasma IL-6 concentrations were strongly associated with increases in NGAL concentrations (EST 0.155, p<0.0001).

Table 10: Final multivariable model for independent factors associated with higher NGAL concentrations (log transformed). (N=316)

<table>
<thead>
<tr>
<th>Predictor Variable</th>
<th>EST</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>4.37</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lower Previous CO (L/min/m$^2$) Log Transformed</td>
<td>-0.13</td>
<td>0.055</td>
</tr>
<tr>
<td>Shorter Scheduled Time (Hrs)</td>
<td>-0.001</td>
<td>0.55</td>
</tr>
<tr>
<td>Higher Previous IL-6 (nanogram/mL) Log Transformed</td>
<td>0.15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Older Age (days)</td>
<td>0.001</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Given the high variability in individual IL-6 measurements, total inflammation was approximated by calculating the area under the curve (AUC) of IL-6 during the first 24 postoperative hours using the trapezoidal rule. The AUC of IL-6 was strongly associated with plasma NGAL concentrations. (Table 10) However, when the AUC of IL-6 plasma concentrations was incorporated into the final multivariable model, the effects of cardiac output on plasma NGAL concentrations fell further from significance. (Table 11)
Table 11: Multivariable model for independent factors associated with higher NGAL concentrations (log transformed). In this model, inflammation is quantified by a 24 hour AUC of IL-6. (N=316)

<table>
<thead>
<tr>
<th>Predictor Variable</th>
<th>EST</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>5.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lower previous CO (L/min/m$^2$) log transformed</td>
<td>-0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>Shorter Scheduled Time (Hrs)</td>
<td>-0.008</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Higher IL-6 AUC (nanogram/mL*hours) / 1000</td>
<td>0.04</td>
<td>0.0006</td>
</tr>
<tr>
<td>Older Age (days)</td>
<td>0.0003</td>
<td>0.6</td>
</tr>
</tbody>
</table>

10.7 Predictor variables and plasma IL-6 concentrations

To better understand the role of inflammation, we analyzed the relationship between numerous predictor variables and plasma IL-6 concentrations. Once again, mixed method models accounting for repeated measures were employed. (Table 12) Plasma IL-6 concentrations were not influenced by patient age or size, or by duration of CPB or aortic cross clamp. An inverse relationship was observed between cardiac output and plasma IL-6. Similar results were found with univariate linear regression models of these predictor variables, the height of inflammation represented by peak plasma IL-6 concentrations, the area under the curve for the first twenty-four hours after CPB.

Table 12: Univariate models for independent factors associated with higher log transformation of plasma IL-6 concentrations and predictor variables (N=571)

<table>
<thead>
<tr>
<th>Predictor Variable</th>
<th>EST</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Older Age (days)</td>
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<td>0.40</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.10</td>
<td>0.15</td>
</tr>
<tr>
<td>Body Surface Area (m$^2$)</td>
<td>2.47</td>
<td>0.14</td>
</tr>
<tr>
<td>Use of Preoperative Methylprednisolone</td>
<td>-0.28</td>
<td>0.20</td>
</tr>
<tr>
<td>Longer CPB Time (min)</td>
<td>-0.0026</td>
<td>0.22</td>
</tr>
<tr>
<td>Longer X-clamp Time (min)</td>
<td>-0.004</td>
<td>0.15</td>
</tr>
<tr>
<td>Longer Deep Hypothermic Circulatory Arrest Time (min)</td>
<td>-0.05</td>
<td>0.002</td>
</tr>
<tr>
<td>Shorter Scheduled Time (Hrs)</td>
<td>-0.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Higher Inotrope Score</td>
<td>0.0012</td>
<td>0.06</td>
</tr>
<tr>
<td>Lower CO (L/min/m$^2$) Log Transformed</td>
<td>-0.58</td>
<td>0.0003</td>
</tr>
</tbody>
</table>
10.8 Clinical AKI

Preliminary analysis of the role of clinical AKI within this cohort is presented in Appendix A.

11 Discussion

This study found that postoperative cardiac output was not statistically associated with plasma NGAL concentrations measured by the Triage® NGAL point-of-care system. It was the height of the systemic inflammatory response that appeared to play a central role in determining postoperative NGAL levels. Although the data supports these assertions on face value, an association may still exist, given that the p-value for the association between cardiac output and plasma NGAL approached statistical significance (p=0.055). This proximity to significance, therefore, merits further evaluation for potential biases and systematic errors, and tempers the strength of the final conclusion.

11.1 Systemic inflammatory response

IL-6, a pro-inflammatory cytokine, was measured in this study to help account for potential confounding effects of inflammation on AKI. Although systemic inflammation has been shown to affect kidney injury, it has not been accounted for in the previous studies that measured NGAL following CPB[47, 48, 54].

In this study, elevated IL-6 concentration was shown to be the strongest independent predictor of plasma NGAL in both the univariable and the final mixed-model regressions. Low cardiac output was associated with higher NGAL concentrations in the univariable model, but this relationship was lost when incorporating IL-6 in the model; in that setting there was, at best, a trend for low cardiac output to increase NGAL concentrations. This finding raises the question of potential inaccuracies in the conceptual framework from which this study was initially developed. (Figure 1) It was originally postulated that inflammation acted as a confounder, leading to low cardiac output and AKI through two independent pathways. Alternatively, in light of these results, systemic inflammation and cardiac output may be individual elements in a single causative pathway with potential positive feedback mechanisms. (Figure 9)
An increase in plasma IL6 levels is still associated with low cardiac output; however, in this proposed model, the effects of cardiac output on plasma NGAL would be directly linked to plasma IL-6 concentrations.

The uncertainty in the conceptual framework illustrates that a complex biological relationship exists between ischemia, inflammation and AKI. As previously described, ischemia disrupts a cell’s biochemical and structural integrity and leads to cellular injury and death (via apoptosis or necrosis). Cell breakdown results in release of chemokines, endothelial membrane receptors tasked with recruitment and activation of leukocytes, which initiate an inflammatory response that can further augment tissue injury, particularly in the kidney. This is due, in part, from a positive feedback loop between vasoconstriction and circulating pro-inflammatory cytokines (including TNF-α, IL-1β, IL-6, IL-12, and IL-18), that worsen blood flow to the susceptible outer medulla causing increased ischemic injury[66]. Pro-inflammatory cytokines, TNF-α and IL-1, are also intimately involved in disruption of cellular adhesion of the tubular epithelium to the basement membrane, which results in shedding into tubules and formation of tubule casts and obstruction to urine flow[67].

Tubule cells, the site of ischemic AKI, are not only the targets of the inflammatory response but also active participants in perpetuating it. When injured, tubular epithelium generate many of
the pro-inflammatory cytokines and chemokines, including TNF-α, IL-6, IL-1β, and IL-8[67]. The up-regulated chemo-attractants promote leukocyte infiltration and activation. Recently, ischemia of tubular epithelium has been shown to increase expression of toll-like receptors (TLR) implicated in the propagation of the inflammatory response[68, 69]. TLRs are membrane-spanning proteins that, when stimulated, activate the innate immunological response[70]. In AKI, TLRs are activated by endogenous byproducts released during cellular injury and necrosis. Systemic inflammation is amplified through further release of cytokines and chemokines[29].

An interruption to this pathway has been associated with decreased inflammation and reduced injury[71]. Lastly, injured tubular epithelium activates T-cells acting as MCHII antigen presenting cells; this further facilitates cytokine production.

The use of NGAL as a measure of AKI in setting of inflammation has become increasingly problematic, as other studies have also raised concern that NGAL levels may reflect the magnitude of systemic inflammation, independent of AKI. NGAL had previously been shown to discriminate between septic neonates with clinical AKI from those without renal dysfunction[72]. However, in a separate study of neonates presenting with sepsis, urine and plasma NGAL were associated with markers of inflammation (C-reactive protein and procalcitonin) and not with clinical AKI[73]. A critique of the study is that a universally accepted definition for clinical AKI was not used; however, it highlights concern over the specificity of NGAL to detect AKI. A casual relationship between inflammation and NGAL has been supported by in vitro studies demonstrating an up-regulation of NGAL by several pro-inflammatory cytokines including IL-1β and TNF-α[74, 75].

In light of the biological underpinning described, the strength of the relationships between inflammation, ischemia (represented by cardiac output) and AKI in a clinical, observational study is limited to being described merely in terms of associations. From an epidemiological perspective, causation cannot be ascertained through this research design. An interventional study controlling for one of the elements would be required to study the hypothesized causative pathway.
11.2 Most appropriate biomarker

11.2.1 Urine versus Plasma NGAL

Following ischemic injury, NGAL protein can be found both in plasma and urine [31, 47]. Although both have been used to identify and quantify AKI, they may originate from different sources and ultimately reflect different processes. Some data even suggest that urinary NGAL better reflects renal injury than its plasma counterpart, but this is not supported by other studies.

In her original study, Mishra et al showed that NGAL transcription was dramatically up-regulated in the kidney. While the protein was localized primarily to in the proximal tubules, macroscopic evidence of cell damage, i.e. apoptosis, was greatest in the distal tubules[31]. The combination of these findings led many to believe that the distal tubule cell produced NGAL and released it simultaneously into the urine and blood. Plasma NGAL was then filtered at the glomerulus and reabsorbed by the proximal tubule cells. Corroborating evidence came from a separate mouse model in which NGAL was found across sections of the nephron, including the proximal tubules, ascending loop of Henle, distal tubules, and collecting ducts[76]. However, in this model, the distal tubules and collecting apparatus were found to produce urinary NGAL, while plasma NGAL originated from injury to the proximal tubules. A complex picture emerged in which urinary NGAL was specific to distal tubule injury, while plasma NGAL may represent proximal tubule injury. This model starts to break down when considering NGAL in the context of clinical situations and not isolated animal models and may be particularly important when using plasma NGAL as an AKI biomarker in the setting of systemic pathology such as the generalized inflammation following CPB. NGAL expression is not limited to the kidney and found in several organs including liver, lung, pancreas, and ovaries[77]. It is, therefore, possible that the NGAL found in the proximal tubule may reflect reabsorption of filtered plasma NGAL from a distant source, whereas urinary NGAL reflects renal pathology. Alternatively, urinary NGAL may also reflect plasma NGAL from other sources in the setting of impaired or over-saturated endocytosis by injured proximal renal tubules [78]. Finally, the contribution of infiltrating neutrophils to the NGAL stores has not been delineated.
When urine and plasma NGAL assays were assessed in clinical models, both showed similar performance to detect clinical AKI, specifically after pediatric cardiac surgery. The area under the receiver operator curve (ROC) for urine NGAL was 0.998 at 2 hours following CPB and 1.00 at 4 hours after CPB. For plasma NGAL, the area under the ROC curve was approximately 0.91 at 2 hours following CPB in that study; this improved to 0.96 (95% CI 0.94-0.99) in a follow-up study using the Triage® NGAL assay[54]. A recent meta-analysis by Haase et al confirmed this finding using aggregate data from 18 individual studies[49].

At this junction, it remains unclear if one source of NGAL measurements offers a significant advantage in detecting AKI. Review of recent literature indicates that both plasma and urine NGAL are being used.

11.2.2 Other biomarkers

Research into the use of a biomarker as an early detection system for AKI has exploded over the last decade. The features sought for an ideal AKI biomarker included high sensitivity to detect renal injury while discriminating from distant organ dysfunction often associated with systemic pathology causing AKI (i.e. high specificity), responsiveness (reacting quickly to physiologic changes), and reliability to predict severity of illness[79, 80]. In addition to NGAL, several other candidate molecules have been found to be increased in the setting of AKI, including Cystatin C, IL-18, kidney injury molecule (KIM) 1, and NGAL. At the time of our protocol development, NGAL was chosen since the majority of studies were measuring NGAL levels, which had been shown to reliably predict AKI following CPB, particularly in children. In light of this current study and updated research presented above, reconsideration of other biomarkers would be appropriate.

*Cystatin C (CyC)* – CyC is a 13 kDa cysteine protease inhibitor produced by all nucleated cells in the body. It is readily filtered by the glomerulus and reabsorbed at the proximal tubules. Plasma concentrations have been used in pediatrics for over a decade to detect AKI from various underlying aetiologies[81]. Conflicting data have emerged over its utility as a biomarker for AKI. Plasma CyC levels have demonstrated poor specificity in detecting clinical AKI in many studies[81]. This was recently verified in a study by Zheng et al in which plasma CyC showed poor discrimination between subjects with and without AKI in the first 48 hours after cardiac
surgery[82]. In contrast, Krawczeski et al demonstrated in a cohort of 370 children undergoing CPB that plasma CyC reliably predicted AKI, particularly at 12 and 24 hours from surgery. CyC concentrations correlated well with AKI severity and longer intervals of care.

**IL-18** – IL-18 is a pro-inflammatory cytokine elevated in setting of AKI. Its pathogenic role in AKI was first suggested in animal models in which inhibition of IL-18 production or activity conferred from protection from injury[83]. Early clinical studies corroborated the use of IL-18 as a biomarker for AKI, particularly in the setting of critical illness[84]. Its role in pediatric cardiac surgery was validated by Parikh et al. in a study of 311 subjects following CPB[21]. IL-18 concentrations rose in all subjects by 6 hours following surgery, but were significantly increased in those with clinical AKI. Higher IL-18 levels were associated with increased odds of severe AKI, even after accounting for other predictors, and correlated directly with increased intervals of care. The reliability of IL-18 to predict clinical AKI in pediatric bypass patients was supported by two other studies[85, 86]. The performance of this biomarker, however, was found to be superior for pediatric patients compared to adult cohorts[87].

**Kidney injury molecule -1 (KIM-1)** – KIM-1 is a trans-membrane glycoprotein up-regulated by proximal renal tubules and shed into urine as a response to ischemic injury[88, 89]. Initial clinical studies have shown an increase in urinary KIM-1 in subjects with AKI, which has been associated with increased morbidity and mortality in adult patients[90, 91]. It can also reliably differentiate ischemia-induced injury from prerenal azotemia[80]. In children, elevation to KIM-1 levels has been shown to predict AKI in the emergency setting, but its role following pediatric surgery has been questioned in a recent study revealing a poor association with clinical AKI after accounting for other predictors[92].

**Liver fatty acid-binding protein (L-FABP)** – L-FABP is a 14 kDa protein belonging to the lipocalin superfamily, constitutively expressed by proximal tubules[93]. Under normal physiologic conditions, L-FABP physiology parallels that of NGAL, being freely filtered at the glomerulus and reabsorbed by active endocytosis at the level of proximal tubules. Its ability to identify AKI was shown in an animal study of cisplatin-induced nephrotoxicity in which L-FABP was produced by proximal tubules and released in urine[94]. Results were duplicated in a clinical study of 40 children after CPB surgery, where a significantly larger increase in urine L-FABP levels was
noted in subjects who developed clinical AKI[95]; this difference was detected by 4 hours after CPB, whereas changes in serum creatinine presented only after 24-48 hours. Plasma concentrations of L-FABP also discriminated for AKI, but only at 12 hours after surgery. In a subsequent study, urine L-FABP was found to be the most reliable biomarker to detect severe AKI following pediatric CPB surgery[96]. Despite L-FABP being produced by hepatocytes as well, hepatic production is thought to cause an increase in plasma levels while urine concentrations reflect AKI[97]. Association between L-FABP and AKI has since been established under numerous clinical conditions, including hypoxia. Concentrations correlate strongly with severity of injury and poor outcomes in some studies[98, 99], but not all[96].

Recent studies have questioned the utility of using a single biomarker for prediction of AKI in this setting[100]. In a study of 380 adults following cardiac surgery, Koyner and colleagues showed that although plasma NGAL concentration (measured using a Triage® Meter) was the best individual biomarker to predict progression of AKI following CPB surgery, performance was raised by the use of multiple assays[101]. Similar conclusions were drawn from a separate study of 220 children following CPB surgery. Urine NGAL was found to best predict clinical AKI soon after surgery in the 27% of subjects who developed clinical AKI. Prediction improved over time with the incorporation of urine IL-18, KIM-1, and liver fatty-acid binding protein[85]. A similar stepwise improvement in prediction of clinical AKI was demonstrated by Parikh et al when using urinary IL-18, urinary NGAL, and plasma NGAL[21].

### 11.3 Triage® NGAL assay

The Triage® NGAL point-of-care system was developed by Biosite Inc. and initially validated by Dent and colleagues[54]. In that study the assay demonstrated a precision of 14% and a within-day coefficient of variance (CV) of 11%, compared with a %CV between 12.5-14.7 reported by the parent company. It was highly correlated (Pearson correlation 0.94, p<0.0001) with the ELISA quantification assay against which it was validated. The assay strongly predicted AKI and levels correlated well with changes in creatinine, length of hospitalization and mortality. These promising results formed the basis for choosing the Triage® NGAL system for use in our study. However, since starting the study, new information has emerged that questions the applicability of this assay. In a multi-centre study of 311 children undergoing CPB, plasma NGAL
concentrations measured with the Triage® NGAL point-of care system did not accurately detect severe AKI and were only weakly correlated with the mildest AKI (RIFLE R)[21]. During this time research revealed that the human NGAL protein was found in several isoforms: monomer, disulfide-linked homodimer and heterodimer. Although the kidney and neutrophils both produce NGAL in the monomer form, renal tubule cells also produce the heterodimer form and NGAL is released from neutrophils as a homodimer[102]. Biosite Inc. subsequently released an updated, reportedly more renal-specific, version of the assay and has since terminated production of the original assay which accounted for these differences in isoforms. These findings raise a concern that the Triage® NGAL point-of care system may have lacked the expected specificity to detect AKI and is measuring the inflammatory response, resulting in a Type II error.

11.4 Power
This study was powered to detect an association between plasma NGAL concentrations and cardiac output in a setting of repeated measurements over the entire study period. It was assumed that the majority of enrolled subjects would have at least twelve samples over seventy hours, such that early termination of a minority of subjects would not impact the required sample size. However, during the time of this study, the ICU underwent a paradigm shift in patient management with a concerted effort to extubate children early, occasionally even in the operating room. Earlier extubation resulted in a higher incidence of censoring than expected, with over half the subjects removed after the first postoperative day (Figure 6). The reduction in the number of overall samples may have contributed to a Type II error in which there was insufficient power to detect a true difference. This could have been further exacerbated by the large inflammatory effect on NGAL concentrations. Such a dominant impact could have masked the more subtle effect of cardiac output on NGAL levels.

11.5 Selection bias
Several factors in our study raise the possibility of a selection bias that must be considered. The height of plasma NGAL concentration was lower in our study compared to similar trials using the same assay. The median plasma NGAL concentrations increased in our study from 60 nanogram/mL prior to CPB to 185 nanogram/mL at 6 hours, and then slowly decreasing to 126
nanogram/mL by 72 hours. Although similar in pattern and trajectory, other studies showed a higher peak in plasma NGAL concentrations, which generally fell between 200-250 nanogram/mL [21, 48, 54]. This observed difference may be attributed to an unanticipated recruitment bias of subjects that were healthier than the representative population, or may merely reflect a true difference in AKI due to variations in perioperative practices.

In this study, age, weight, body surface area and cardiopulmonary bypass time did not influence plasma NGAL levels (all p > 0.05). This differs from the current literature in which younger age at surgery and longer CPB are associated with an increase in NGAL concentrations and higher incidence of clinical AKI[48], a relationship that was seen both in neonatal and non-neonatal groups. Even though the subjects in our study, infants with median age of 4.5 months, were similar to the cohort included by Krawczeski and colleagues at Cincinnati Children’s Hospital, a closer examination reveals influential clinical differences. Our study cohort tended to have lower serum creatinine concentrations, shorter time on bypass, and spent less time in hospital. Lower preoperative creatinine has been associated with a reduction in AKI and may explain the lower median NGAL observed. A potential inference is that the subjects in other studies were sicker than those included in this study and therefore more susceptible to injury from CPB. Relatively healthier subjects may better tolerate CPB and not be greatly affected by the duration on CPB. The lack of association in this study may be attributed to the analysis that was undertaken. Krawczeski employed linear regression models to examine the effects of predictors on plasma NGAL concentrations at every time point. In our analysis, this evaluation was undertaken using linear regression adjusted for repeated measures, an analysis known to minimize effects of static factors such as age and CPB time.

Lastly, the median cardiac output in our subject population increased progressively from 2.01 L/min/m² to 2.82 L/min/m². Unlike previous studies, the expected nadir in blood flow was not observed. It is unclear if this represents a healthier patient cohort with more robust hemodynamics or a difference in clinical practice that ameliorates this potential decline. Nevertheless, over 50% of subjects and nearly 40% of all paired measurements had cardiac output measurement below 2, and therefore was likely still representative of the population at large.
12 Synthesis and future directions

The underlying motivation for this Master’s thesis was to identify modifiable factors that contribute to the burden of AKI in children recovering from surgery with CPB. Although the vast majority of those affected recover, AKI can impact short-term morbidity and mortality and long-term impairment, and increase medical costs. Age, preoperative kidney injury, surgical complexity and CPB time have been consistently associated with AKI; however, these patient-specific elements cannot usually be influenced. Therefore, we focused on postoperative cardiac output, a patient level factor that could be accurately measured and possibly manipulated.

The results of this study were not definitive, with a trend to increased AKI indicated through elevated plasma NGAL concentrations at lower cardiac output. Inflammation, quantified with plasma IL-6 concentration, had a more significant association with NGAL levels. Despite the numerous limitations (as discussed above), this study raises several avenues for future investigation that merit closer inspection and can form the basis of future research program.

12.1 The “right” modifiable risk factor

As previously mentioned, an association between postoperative cardiac output and AKI may not exist or may be effectively muted in the presence of inflammation, which plays a more dominant role. Although this study does not delve into the exact mechanism by which inflammation contributes to postoperative AKI, its strong association should be further explored. Systemic inflammation has long been identified as a contributor to post-CPB organ dysfunction, with an emphasis on improving outcomes by reducing the inflammatory burden. An extensive body of research exists for anti-inflammatory strategies using corticosteroids, post-CPB ultrafiltration and administration of aprotinin. Many studies have shown overall benefit, but little data exist on their impact on postoperative AKI.

Use of preoperative and intraoperative corticosteroids has been associated with attenuation of the inflammatory response. In a randomized study of 29 children, Bronicki et al. demonstrated a significant reduction in postoperative complement and plasma IL-6 concentrations when 1 mg/kg dexamethasone was administered immediately before surgery[103]. Similarly, use of methylprednisolone has demonstrated a consistent decrease in pro-inflammatory cytokines.
including IL-6 and IL-8\cite{104-106}. Use of corticosteroids in many studies has been associated with improved clinical outcomes, including a decrease in ventilation time, ICU admission, and mortality; the relationship between the anti-inflammatory effects of steroids on development of AKI was directly assessed. In neonates undergoing CPB surgery, Graham et al. found that subjects randomized to receive combined preoperative and intraoperative methylprednisolone had higher serum creatinine concentrations than those who received only intraoperative methylprednisolone (54 ± 16 mmol/L versus 47 ± 11 mmol/L)\cite{104}; however, this finding has not been duplicated in other studies\cite{107, 108}. In our study, administration of preoperative steroids was associated with a reduction in postoperative NGAL. This relationship lost significance in multivariable modeling and was not used in the final model. This preliminary data reinforces the potential relationship raised above. Theoretically, corticosteroids could have exerted an anti-inflammatory effect that decreased plasma IL-6 concentrations, resulting in a reduction in plasma NGAL concentrations. As such, it would lose significance when IL-6 concentrations were included in the same model. It is important to note, however, that preoperative use of methylprednisolone was not associated with a reduction in plasma IL-6 on univariable modeling in our study. This may reflect that the anti-inflammatory effects of methylprednisolone are mediated through an alternative pathway, such that IL-6 levels would not be impacted; however, this would be in direct contradiction to the data presented above. Alternatively, the use of preoperative methylprednisolone was limited to neonatal subjects and those who underwent deep hypothermic circulatory arrest, as per our institution’s practice. This observed association could, therefore, reflect other relationships and not its potential anti-inflammatory effects.

A deeper understanding of the role of steroids in AKI would be best assessed with a prospective interventional trial. A properly designed prospective study confined to subjects in our institution would be difficult to complete. As per usual practice, steroid use is restricted to a limited population that would not have a suitable matched comparison group to help delineate the desired relationship. Expanding the comparison group to include patients from other institutions or other age and surgical groups would introduce numerous confounders that would limit interpretation of the study results. There is sufficient preliminary data, and likely clinical equipoise, to inform an interventional trial in higher risk patients currently not receiving
preoperative corticosteroids. The study intervention would consist of methylprednisolone dosing according to current protocols; the primary outcome would be incidence of AKI. Secondary outcomes would include biochemical markers of inflammation (IL-6) and other clinical markers of morbidity.

Use of corticosteroids prior to CPB is not the only anti-inflammatory strategy currently employed. Ultrafiltration following CPB has been used to remove circulating inflammatory mediators and to attenuate a SIRS response since the 1990s. This strategy has gained significant traction, being used in as many as 80% of institutions[109]. Ultrafiltration has been shown to decrease inflammatory markers, possibly more effectively than the preoperative corticosteroid strategy[110-112]. The role of ultrafiltration in AKI, however, is unclear. In a case-controlled study of 308 subjects, Chiravuri showed that the quantity of volume removed by ultrafiltration correlated directly with the odds of developing acute kidney failure[19]. Hassinger et al. found no significant relationship between ultrafiltrate volume and AKI in a separate study of 100 subjects[113]. In these studies, AKI was classified as an increase of 50% from baseline creatinine concentrations (RIFLE R or AKIN 1). Modulation of ultrafiltration may, therefore, still provide a practical and effective means by which the inflammatory response can be attenuated. We would be unable to address the question if the use of ultrafiltration improves outcome compared to placebo or control, as all subjects within this cohort underwent modified ultrafiltration according to routine operative care. Withholding this therapy would not be acceptable, but the analysis for an association between volume of ultrafiltrate removed and plasma IL-6 (area under the curve or peak) is possible; it could inform a larger retrospective study to determine whether this general association is seen in all children after surgery with CPB.

12.2 The “right” AKI biomarker

Research into novel biomarkers has exploded in an effort to discover accurate, early indicators of disease. For AKI, NGAL has become the dominant assay used in clinical studies, having shown its ability to predict clinically apparent kidney injury in numerous disease entities, including pediatric CPB. For that reason, NGAL was chosen for this study. Other potential candidate molecules include IL-6, interleukin-18, Cystatin C, kidney-injury-molecule 1 (KIM-1),
and liver-type fatty acid-binding protein. Unfortunately, all currently available biomarkers lack organ specificity, as described in a recently published position paper from the ADQI consensus conference: “in most biomarker studies, proof of the specificity of biomarker changes to diagnose actual changes in renal pathology (the ‘gold standard’) has been lacking”[100]. This lack of organ specificity may significantly limit the use of these biomarkers in subjects with systemic involvement such as that experienced following CPB. As such, plasma NGAL concentrations may parallel kidney injury and perform well as a prognosticating tool, but also measure other injury and, therefore, lack accuracy in quantitating AKI. Further study into the ability of other biomarkers to quantify kidney injury is required prior to conducting a similar study. The difficulty remains that there is no “gold-standard” against which a biomarker can be compared to validate its quantifying ability. This may be overcome by a study design that compares a biomarker against newer MRI or nuclear medical viability modalities.

13 Conclusion

Increased systemic inflammation, and not lower cardiac output, appears to be significantly associated with higher plasma NGAL concentrations measured by the Triage® NGAL point-of-care system in infants following cardiac surgery with CPB. Further study is required to ascertain whether this phenomenon is measurement-related or physiologically-based. This study can form the foundation of a research program focused on minimizing AKI following CPB by reducing systemic inflammation and/or identifying an organ-specific biomarker able to quantitate AKI in an ICU setting.


Appendix A – Clinical AKI

Sixty-five percent of subjects (39/60) met criteria for clinical AKI according to the RIFLE criteria. Only half the subjects satisfied criteria based on changes in creatinine, and 42% (25/60) met criteria for AKI based on changes to urine output (Table 13). Changes to both creatinine and urine output were seen in 15 (25%) of subjects. A single subject required renal replacement therapy.

Table 13: Incidence of clinical AKI according to RIFLE classification

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Total</th>
<th>GFR Criteria</th>
<th>Urine Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>21 (35%)</td>
<td>30 (50%)</td>
<td>35 (58%)</td>
</tr>
<tr>
<td>Risk</td>
<td>26 (43%)</td>
<td>21 (35%)</td>
<td>20 (33%)</td>
</tr>
<tr>
<td>Injury</td>
<td>12 (20%)</td>
<td>8 (13%)</td>
<td>5 (8%)</td>
</tr>
<tr>
<td>Failure</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Subjects with clinical AKI tended to be younger and smaller than those without AKI. Baseline serum creatinine was lower in setting of AKI. There was no difference in cardiac physiology, surgical time intervals or duration of care (Table 14).

Plasma NGAL concentrations increased sharply following CPB in all subjects. There was no statistically significant difference in creatinine concentration between subject who developed AKI and those who did not (Figure 10).
Table 14: Patient demographics and surgical characteristics according to AKI status

| Demographics                                    | No AKI (N=21) | AKI (N=39) |
|------------------------------------------------||--------------|------------|
| Sex (male)                                      | 13 (62%)     | 22 (56%)   |
| Weight (kg)                                     | 4.09 (3.48, 5.4) | 5.7 (4.09, 7.1) |
| Body surface area (m²)                          | 0.26 (0.23, 0.32) | 0.34 (0.26, 0.4) |
| Baseline serum creatinine (μmol/L)              | 34 (25, 52)  | 28 (24, 32) |
| Age at surgery (days)                           | 45 (11,153)  | 151 (24,209) |

| Surgical Complexity                             |               |            |
|------------------------------------------------||--------------|------------|
| Biventricular physiology                        | 20 (95%)     | 34 (87%)   |
| Cardiopulmonary bypass time (min)               | 119 (102, 152) | 117 (91, 161) |
| Cross clamp time (min)                          | 81 (58, 119) | 80 (64, 107) |
| Deep hypothermic circulatory arrest             | 5 (24%)      | 5 (13%)    |
| Deep hypothermic circulatory arrest time (min)  | 22 (21, 26)  | 20 (17, 25) |

| Clinical Outcomes                               |               |            |
|------------------------------------------------||--------------|------------|
| Time to extubation (Days)                       | 2 (1, 5)     | 1 (1, 5)   |
| ICU length of stay (Days)                       | 4 (2, 8)     | 3 (2,9)    |
| Hospital length of stay (Days)                  | 11 (7, 23)   | 7 (6, 15)  |
| Morbidity Composite Score                       | 5 (24%)      | 11 (28%)   |
| Cardiac arrest                                  | 0 (0%)       | 1 (5%)     |
| Death                                           | 0 (0%)       | 0 (0%)     |
Figure 10: NGAL concentration over time according to clinical AKI status. The solid line represents the median value of subjects with AKI; dashed line represents median value for those without AKI. Interquartile range presented by error bars. Total number of subjects included at each time point indicated in bottom line.