Neutrophil Gelatinase-Associated Lipocalin Concentrations Predict Development of Acute Kidney Injury in Neonates and Children after Cardiopulmonary Bypass

Catherine D. Krawczeski, MD, Jessica G. Woo, PhD, Yu Wang, MS, Michael R. Bennett, PhD, Qing Ma, MD, and Prasad Devarajan, MD

Objectives To investigate neutrophil gelatinase-associated lipocalin (NGAL) as an early acute kidney injury (AKI) biomarker after neonatal and pediatric cardiopulmonary bypass (CPB).

Study design Serum and urine samples were obtained before and at intervals after CPB from 374 patients. AKI was defined as a serum creatinine (S_{Cr}) concentration increase from baseline ≥0.3 mg/dL in neonates and ≥50% in children within 48 hours of CPB. Logistic regression was used to assess predictors and clinical outcomes associated with AKI.

Results AKI developed in 30% of patients. Plasma and urine NGAL thresholds significantly increased in patients with AKI at 2 hours after CPB and remained elevated at all points, with 2-hour NGAL the earliest, strongest predictor of AKI. In non-neonates, 2-hour plasma and urine NGAL thresholds strongly correlated with length of hospital stay and severity and duration of AKI.

Conclusion Plasma and urine NGAL thresholds are early predictive biomarkers for AKI and its clinical outcomes after CPB. In neonates, we recommend a 2-hour plasma NGAL threshold of 100 ng/mL and 2-hour urine NGAL threshold of 185 ng/mL for diagnosis of AKI. In non-neonates, recommended AKI thresholds are 50 ng/mL for both 2-hour plasma and urine NGAL. *(J Pediatr 2011;158:1009-15)*.

Acute kidney injury (AKI) after cardiac surgery is associated with a number of adverse outcomes, including prolonged intensive care and hospital stays, diminished quality of life, and increased long-term mortality rate.[1] AKI complicates 30% to 40% of cardiac surgeries.[2,3] Patients who require dialysis are at high risk of mortality,[3] but even minor degrees of postoperative AKI portend a significant increase in mortality[4] and morbidity[5] rates.

The diagnosis of AKI has relied on detection of reduced kidney function with a rise in serum creatinine (S_{Cr}) concentration, which is a delayed and unreliable measure in the acute setting.[6] S_{Cr} concentration may not rise until 50% of kidney function has been lost. In the setting of AKI, S_{Cr} concentration may take days to reach steady state. In addition, S_{Cr} concentration varies by muscle mass, hydration status, age, and sex. In the newborn, the diagnosis of AKI, particularly mild to moderate forms, is even more difficult.[7] S_{Cr} levels in the first few days of life are typically elevated, as a reflection of maternal creatinine, and decline in the first weeks of life as glomerular filtration steadily improves.[8] Thus, mild to moderate decreases in glomerular filtration may not be manifested with a rise in S_{Cr} concentration. Because of this, defining AKI in the neonatal period has been particularly challenging, leading to exclusion of this population in some studies. This is unfortunate, because the neonate is likely even more vulnerable to ischemic or nephrotoxic kidney injury because of incomplete nephrogenesis. In addition, the most complex congenital heart surgeries and longest cardiopulmonary bypass (CPB) times typically occur in the neonatal population, thereby increasing its risk for AKI. Thus, the establishment of non- S_{Cr}–based AKI criteria is even more crucial for this age group.

Experimental studies in animals have identified interventions that may prevent or treat AKI when instituted early in the disease process, well before S_{Cr} concentration rises.[9] Early predictive biomarkers for AKI could allow early intervention and improve outcomes in human AKI. One such biomarker, neutrophil gelatinase-associated lipocalin (NGAL), has been shown to be markedly up-regulated in the kidney tubule cells after ischemic and nephrotoxic injury[2,10-12] and is easily detected in

AKI	Acute kidney injury
AKIN	Acute Kidney Injury Network
AUC	Areas under the curve
CPB	Cardiopulmonary bypass
eCCl	Estimated creatinine clearance
eGFR	Estimated glomerular filtration rate
NGAL	Neutrophil gelatinase-associated lipocalin
RACHS-1	Risk Adjustment for Congenital Heart Surgery 1
ROC	Receiver-operating characteristic
S_{Cr}	Serum creatinine

From the Heart Institute (C.K.) and the Divisions of Nephrology and Hypertension (M.B., Q.M., P.D.), and Biostatistics and Epidemiology (J.W., Y.W.), Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH

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the plasma and urine very early after the insult.[10,13,14] Multiple studies have validated NGAL as a biomarker of ischemic AKI,[2,15] but studies have yielded a wide range of accuracy, with variable optimal cut-off values for the diagnosis of AKI.[16,17]

Our primary objective was to determine the predictive ability and optimal threshold concentrations of plasma and urine NGAL for the diagnosis of AKI in neonates and children after CPB. We hypothesized that NGAL thresholds for these age groups might be different because of functional differences in the neonatal tubule and the inherent difficulty in diagnosing neonatal AKI. Our secondary objective was to determine the relationship between postoperative NGAL concentrations and outcomes, both renal (duration and severity of AKI) and clinical (mortality and length of hospital stay).

Methods

This study was approved by the institutional review board of Cincinnati Children's Hospital Medical Center. All patients <18 years of age undergoing cardiac surgery with CPB at our center between January 2004 and May 2007 were approached for study inclusion. Written informed consent was obtained from the legal guardian of each patient, with assent from the patient when appropriate, before enrollment. Patients with severe pre-existing renal insufficiency (S_{Cr} concentration >2 times reference range, on the basis of age-adjusted reference ranges) were excluded. Past nephrotoxin (such as contrast or aminoglycoside) use was not an exclusion factor as long as the S_{Cr} concentration was within reference range at the time of surgery. Complexity of surgery was categorized according to the Risk Adjustment for Congenital Heart Surgery 1 (RACHS-1) consensus-based scoring system.[18]

To obviate postoperative volume depletion and prerenal azotemia, subjects received at least 80% of maintenance fluid requirements (on the basis of body weight) during the first postoperative 24 hours and 100% maintenance subsequently. The serum albumin level was routinely maintained >2.5 mg/dL, with supplementation as needed. Serum and urine samples were obtained before CPB and at specific intervals after CPB and stored in aliquots at –80°C. S_{Cr} concentration was measured at baseline and was routinely monitored at least daily in the postoperative period.

The primary outcome variable was the development of AKI. For the neonatal group (age ≤30 days), AKI was defined as a ≥0.3 mg/dL absolute increase in S_{Cr} concentration from baseline within 48 hours of surgery, equivalent to stage 1 in the Acute Kidney Injury Network (AKIN) definition of AKI.[19] For the non-neonatal group (age >30 days), AKI was defined as a ≥50% increase in S_{Cr} concentration from preoperative baseline within 48 hours of surgery.[20] This definition has been derived by expert consensus and has been validated in other pediatric populations.[21] Secondary outcomes included severity of AKI on the basis of the pediatric RIFLE criteria,[22] duration of AKI, hospital length- of-stay, and hospital mortality rate. The pediatric RIFLE classification stratifies kidney injury according to severity: Risk of renal dysfunction, Injury to the kidney, Failure of kidney function, Loss of kidney function, and End-stage renal disease, by using creatinine clearance or urine output criteria. The first 3 categories correspond to AKI, and the latter two categories correspond to chronic kidney disease. Because urine output may be affected by multiple intraoperative and postoperative variables such as the use of ultra-filtration or the administration of diuretics, we calculated pediatric RIFLE scores by using creatinine clearance criteria estimated with the modified Schwarz formula,[23] with "Risk" corresponding to an estimated creatinine clearance (eCCl) decrease of 25% from preoperative baseline, "Injury" corresponding to an eCCl decrease of 50%, and "Failure" corresponding to an eCCl decrease of 75% or absolute value <35 mL/min/1.73m^2. Duration of AKI was defined as the number of days S_{Cr} concentration was elevated ≥0.3 mg/dL (for neonates) or ≥50% (for non-neonates) higher than baseline. To provide a quantified index of the subject's postoperative hemodynamic status, we used dosages of inotropic infusions to calculate an inotrope score 24 hours after CPB, as previously described for similar co-horts.[24]

Biomarker Measurements

The laboratory investigators were blinded to the sample sources and clinical outcomes. The plasma NGAL enzyme-linked ammunoassay was performed by using a validated assay, as previously described.[2,25] The inter- and intra-assay co-efficient variations were <5% for batched samples analyzed on the same day and <10% for the same sample measured 6 months apart. The urine NGAL enzyme-linked ammunoassay was performed with a commercially available assay (NGAL ELISA Kit 036; AntibodyShop, Grusbakken, Denmark) that specifically detects human NGAL.[26] The intra-assay co-efficients of variation were 2.1% (range, 1.3% to 4.0%), and inter-assay variation was 9.1% (range, 6.8% to 18.1%).

Statistical Methods

Statistical analysis was performed with SAS software version 9.2 (SAS Institute, Cary, North Carolina). Descriptive analyses are reported as medians, with interquartile ranges for continuous variables and frequency and proportion for categorical variables. The Wilcoxon rank-sum test was used to test for group differences in continuous variables, and the χ^2 or Fisher exact test was used for categorical variables, as indicated.

Patients were divided in neonatal (≤30 days of age) and non-neonatal (>30 days of age) groups. For each group, least square means and their standard errors were calculated for NGAL concentrations by using generalized estimating equation models to account for the repeated biomarker measurements at multiple points. Spearman correlation co-efficients were calculated between NGAL concentrations at each point and these clinical variables: age, CPB duration, RACHS-1 score, postoperative inotrope score, percent change in S_{Cr} concentration, hospital length-of-stay, and duration of AKI.

Univariable and multivariable stepwise logistic regression analyses were undertaken to assess predictors of AKI. Potential