Urinary NGAL in Prediction of Acute Kidney Injury

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Abstract

The incidence of acute kidney injury (AKI, previously referred to as acute renal failure) is reaching epidemic proportions. In this situation, early intervention can significantly improve the prognosis. Despite significant improvements in therapeutics, the mortality and morbidity associated with AKI remain high. A major reason for this is the lack of early markers for AKI, akin to troponins in acute myocardial disease, and hence an unacceptable delay in initiating therapy. Unfortunately, serum creatinine (Scr) is a delayed and unreliable indicator of AKI. The authors have previously shown that urine neutrophil gelatinase-associated lipocalin (NGAL) is an early predictive biomarker of acute kidney injury (AKI) after cardiopulmonary bypass (CPB). In this study, the evidence for the role of NGAL measurements in AKI after CPB is explored. The emerging utility of standardized clinical platforms for reliable measurement of NGAL urine is discussed. In a prospective study with 303 adults undergoing CPB were enrolled and serial urine NGAL measurements were obtained. The primary outcome was AKI, defined as a >50% increase in Scr. AKI developed in 75 patients (25%), but the diagnosis using Scr was delayed by 2 to 3 d after CPB. In contrast, mean urine NGAL levels increased 4-fold within 2 h after CPB and remained significantly elevated for the duration of the study. The 2-h postoperative urine NGAL levels strongly correlated with change in creatinine (r = 0.56, p < 0.001), duration of AKI (r = 0.47, p < 0.001), and length of hospital stay (r = 0.39, p < 0.001). The 12-h urine NGAL levels strongly correlated with death (r = 0.41, p < 0.004). Urine NGAL is an early predictive biomarker of AKI severity and mortality after CPB. It will be important in future studies to validate the sensitivity and specificity of NGAL concentration measurements in clinical samples from large cohorts and from multiple clinical situations. Such studies will be facilitated by the anticipated widespread availability of standardized commercial tools in the near future.

Keywords: Acute kidney injury; Acute renal failure; Biomarker; NGAL; Lipocalin; Serum creatinine; Cardiopulmonary bypass

Introduction

Cardiopulmonary bypass (CPB) is the most frequent major surgical procedure performed in hospitals worldwide, with well over a million operations undertaken each year in adults alone [1]. Acute kidney injury (AKI), previously referred to as acute renal failure, is a frequent and serious complication encountered in 30% to 50% of subjects after CPB [2,3]. A severe AKI increases morbidity and mortality of hospitalized patients [4-6]. Twenty percent to 60% of patients with AKI require dialysis [7], and mortality rates range from 15% in the community setting [8,9] to 50% to 80% in the setting of multiorgan failure [10,11] and more than 80% in the postoperative setting [12,13]. Pathophysiological mechanisms include diminished renal blood flow, loss of pulsatile flow, hypothermia, atheroembolism, and a generalised inflammatory response. Various clinical algorithms have been proposed for prediction of AKI needing dialysis, based on preoperative risk factors [14-17], but no methods are available for the early diagnosis of lesser degrees of renal injury. Outstanding advances in basic research have illuminated the pathogenesis of AKI and have paved the way for successful therapeutic approaches in animal models [18]. However, translational research efforts in humans have yielded disappointing results. Despite significant improvements in therapeutics, the mortality and morbidity associated with AKI remain high. A major reason for this is the lack of early markers for AKI, akin to troponins in acute myocardial disease, and hence an unacceptable delay in initiating therapy [19,20]. Recent evidence suggests that a small reduction in renal function, indicated by serum creatinine (Scr), is an independent predictor of mortality and length of hospital stay [4,21]. In a current clinical practice, the ‘gold standard’ for identification and classification of AKI is dependent on serial Scr measurements [22]. Even minor degrees of postoperative AKI, as manifest by only a 0.2 to 0.3 mg/dL rise in serum creatinine (Scr) from baseline, predict a significant increase in short-term mortality [23,24]. Unfortunately, Scr is an unreliable indicator during acute changes in kidney function [25].

First, serum creatinine levels can vary widely with age, gender, lean muscle mass, muscle metabolism, and hydration status. Second, serum creatinine concentrations may not change until about 50% of kidney function has already been lost. Third, at lower rates of glomerular filtration, the amount of tubular secretion of creatinine results in overestimation of renal function. Finally, during acute changes in glomerular filtration, serum creatinine does not accurately depict kidney function until steady-state equilibrium has been reached, which may require several days. However, animal studies have shown that whereas AKI can be prevented and/or treated by several maneuvers, these must be instituted very early after the insult, well before the rise in serum creatinine [19,26]. The quest to improve early diagnosis of AKI is an area of intense research [27,28]. Conventional urinary biomarkers such as casts and fractional excretion of sodium have been insensitive and non-specific for the early recognition of AKI. Other traditional urinary biomarkers such as filtered high molecular weight proteins and tubular proteins or enzymes have also suffered from lack of specificity and a dearth of standardized assays. Fortunately, emerging technologies such as functional genomics and proteomics have uncovered novel candidates that are emerging as biomarkers. Neutrophil gelatinase–associated lipocalin (NGAL) in the plasma was found to be an early predictive biomarker of AKI in a variety of acute clinical settings in pilot studies [27]. NGAL is generally expressed in low concentrations, but it increases greatly in the presence of epithelial injury and inflammation [29-31]. These findings have now been
confirmed in a prospective study of adults who developed AKI after cardiac surgery [22], which found uNGAL to be significantly elevated by one to three hours after the operation. Other human studies [32-34] demonstrated a strong relationship between uNGAL and AKI in renal transplantation, diarrhea-associated hemolytic-uremic syndrome, and lupus nephritis. Preliminary results using the research-based assay also suggest that uNGAL measurements can predict AKI following contrast administration [35], kidney transplantation [33], and in critically ill subjects [34]. The availability of a standardized clinical platform for NGAL measurements could revolutionize diagnostic, especially in intensive care situations [36]. NGAL is a new biomarker for our hospital and therefore we wanted to test the hypothesis that an urinary NGAL immunoassay developed for a standardized clinical platform (ARCHITECT® Analyzer, Abbott Diagnostics) represents an early biomarker of ischaemic renal injury in adults undergoing cardiac surgery in a prospective adult cohort and the extent to which uNGAL concentrations increase before SCR in the setting of an unknown timing of initial kidney injury.

Material and Methods

Patients and study design

All adults undergoing elective CPB for surgical were prospectively enrolled. Exclusion criteria included preexisting renal insufficiency, diabetes mellitus, peripheral vascular disease, and use of nephrotoxic drugs before or during the study period. Preexisting renal insufficiency was defined as a SCR level that was greater than the 90th for the adult’s age and gender. Patients with a history of potential nephrotoxic use (including aminoglycosides, angiotensin inhibitors, and nonsteroidal anti-inflammatory drugs) during the preoperative day or the first two postoperative days were excluded because of potential confounding effects on uNGAL measurements. We therefore studied a homogeneous population of patients with very possibly no major confounding variables, in whom the only obvious renal insult would be the result of ischaemia reperfusion injury after cardiopulmonary bypass.

To obviate postoperative volume depletion and prerenal azotemia, all subjects received at least 80% of their maintenance fluid requirements during the first 24 h after surgery and 100% maintenance subsequently.

Urine and blood specimen collection

We took spot urine samples at baseline and at frequent intervals (2, 12, 24, 48 and 72 h) after initiation of CPB. Urine samples were centrifuged at 2000 x g for 5 min, and the supernatants stored in aliquots at –80°C.

The primary outcome variable was the development of AKI, defined as a 50% or greater increase in serum creatinine from baseline. This corresponds to the risk phase of the RIFLE criteria for diagnosis of AKI [37]. The RIFLE criteria provide a meaningful way to stratify patients at different stages of kidney failure on the basis of severity. Other outcomes included percent change in serum creatinine, days in AKI, dialysis requirement, length of hospital stay, and mortality. Other variables we obtained included age, gender, ethnic origin, CPB time, previous heart surgery, and urine output.

uNGAL and SCR analysis

The ARCHITECT® NGAL (Abbott Diagnostics) assay is a two-step (sandwich) assay using chemiluminescent microparticle immunoassay technology. High affinity antibodies were generated toward distinct epitopes of NGAL and assay standards were prepared using human recombinant NGAL. The assay had a functional sensitivity <2 ng/mL (20% coefficient of variation [CV], 95% confidence) and total CVs <5.0%.

The automated SCR assays were the rate-blanked, compensated Jaffe method and the creatinine plus enzymatic assay performed on the Roche Modular according to the manufacturer’s instructions (Roche Diagnostics Ltd). Between-day imprecision was 3.2% and 2.2% at concentrations of 0.7 and 1.8 mg/dL, respectively.

Statistical analysis

SAS version 8.2 was used for analyses. To compare continuous variables, we used a two-sample t test or Mann-Whitney rank sum test, and to compare categorical variables we used the x² or Fisher’s exact test, as indicated. The associations between variables were assessed by Spearman rank order correlation analysis. Univariate and multivariate stepwise regression analyses were undertaken to assess predictors of AKI after CPB. Potential independent predictor variables included age, sex, CPB time, urine output and history of prior cardiac surgery.

Results

In this prospective study, serial urine samples from 303 adults who met the inclusion and exclusion criteria undergoing CPB were assayed for uNGAL to assess its ability to predict AKI and other adverse outcomes. Approximately one half of the patients were male. Mean age was 52.1 years (SD, 0.6). Seventy-five patients (25%) met the criteria for AKI within a 3-day period. Of these, SCr rose 24–48 h after CPB in twenty-two (29%), but in the other 12 (70%) the increase happened 48–72 h after the procedure. Thus, the diagnosis of acute renal injury using currently accepted practices could be made only days after the inciting event. However, the increase in SCr by 50% or greater from baseline was continued by 2 to 3 days after CPB. No differences were noted with respect to age, or sex (Table 1). All patients received a similar postoperative fluid regime, and there were no differences in the volume status or urine output between the two groups.

In patients that developed AKI, the clinical outcomes were significantly worse. Patients who developed acute renal injury were older and had longer CPB times compared with those who did not develop AKI. The SCR rose by a greater percentage in the AKI group, and both length of hospitalization and mortality rate were significantly higher (Table 1). Among patients with AKI, twenty-three (30.6%) required dialysis, primarily for fluid overload. There were a total of two deaths, all in the AKI group. The causes of death were multiorgan failure in those patients.

Urinary NGAL was low in the urine of all patients before surgery. uNGAL measurements at baseline were not comparable in the AKI and non-AKI groups (Table 1). In the 228 adults who never developed AKI, a small increase was noted in uNGAL at 2 h after CPB (p=0.005 vs baseline) and 12 h after surgery (p=0.005 vs baseline), which normalized back to baseline levels at the 24-hour time point (Figure 1). In marked contrast, in patients who subsequently developed AKI there was a robust fourfold increase in uNGAL at 2 hours after CPB, which persisted at the 12-hour and 24-hour time points. The pattern of uNGAL excretion was characterised by a peak very early after the precipitating event followed by a lesser but sustained increase over the entire duration of the study.

To test the hypothesis that urinary NGAL levels measured soon after CPB could be used to predict eventual clinical outcomes, a
Spearman rank order correlation analysis was performed. uNGAL at 2 h levels strongly correlated with percentage change in SCr (r= 0.56, p<0.001), duration of AKI (r=0.47, p<0.001), and length of hospital stay (r=0.39, p<0.001). The 12-hour NGAL levels strongly correlated with mortality (r=0.41, p=0.004).

To assess independent predictors for the development of AKI in the entire cohort, multivariate logistic regression was performed. All variables that were found by univariate analysis to display a p<0.1 were entered into the model. Urinary NGAL measurement at 2 hours after CPB was the most powerful independent predictor of AKI (r=0.82, p=0.0001). Also, a significant correlation was found between AKI (50% or greater in serum creatinine) and the following: included history of previous cardiac surgery (r=0.52, p=0.001), urinary output (r=0.42, p=0.0001). Also, a significant correlation was found between AKI (50% or greater in serum creatinine) and the following: included history of previous cardiac surgery (r=0.52, p=0.001), urinary output (r=0.42, p=0.0001). Also, a significant correlation was found between AKI (50% or greater in serum creatinine) and the following: included history of previous cardiac surgery (r=0.52, p=0.001), urinary output (r=0.42, p=0.0001) and CPB time (r=0.43, p=0.003). Univariate analysis of our data showed that the following outcomes were not predictive of AKI: age and sex.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No AKI (n=228)</th>
<th>AKI (n=75)</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (SD), y</td>
<td>48 (8.5)</td>
<td>53 (4.5)</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Male, %</td>
<td>54</td>
<td>58</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Prior surgery, %</td>
<td>20</td>
<td>45</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Bypass time (SD), min</td>
<td>42 (2.3)</td>
<td>142 (4.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Urine output, mL/kg per h</td>
<td>1.7 (0.10)</td>
<td>1.2 (0.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change in SCr (%)</td>
<td>8.6 (2.1)</td>
<td>87.3 (7.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SCr baseline, mg/dL</td>
<td>0.72 (0.11)</td>
<td>0.71 (0.04)</td>
<td>NS</td>
</tr>
<tr>
<td>SCr 2 h, mg/dL</td>
<td>0.71 (0.11)</td>
<td>0.71 (0.04)</td>
<td>NS</td>
</tr>
<tr>
<td>SCr 12 h, mg/dL</td>
<td>0.70 (0.11)</td>
<td>0.72 (0.04)</td>
<td>NS</td>
</tr>
<tr>
<td>SCr 24 h, mg/dL</td>
<td>0.71 (0.10)</td>
<td>0.73 (0.09)</td>
<td>NS</td>
</tr>
<tr>
<td>SCr 48 h, mg/dL</td>
<td>0.72 (0.12)</td>
<td>1.0 (1.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SCr 72 h, mg/dL</td>
<td>0.80 (0.08)</td>
<td>1.2 (0.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>uNGAL baseline, ng/mL</td>
<td>76.2 (12.1)</td>
<td>82 (9.1)</td>
<td>&lt;0.7</td>
</tr>
<tr>
<td>uNGAL 2 h, ng/mL</td>
<td>89 (11.4)</td>
<td>371 (24.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>uNGAL 12 h, ng/mL</td>
<td>122 (10.3)</td>
<td>522 (21.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>uNGAL 24 h, ng/mL</td>
<td>78 (8.5)</td>
<td>592 (32.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>uNGAL 48 h, ng/mL</td>
<td>76 (4.2)</td>
<td>838 (22.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>uNGAL 72 h, ng/mL</td>
<td>75 (8.1)</td>
<td>823 (24.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Duration of AKI (SD), day</td>
<td>0</td>
<td>3.2 (0.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hospital stay (SD), day</td>
<td>0</td>
<td>13 (2.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dialysis, no.</td>
<td>1</td>
<td>23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Death, no.</td>
<td>0</td>
<td>2</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*P < 0.0001 comparing AKI versus no AKI groups

Table 1: Patient characteristics and clinical outcomes.

**Discussion**

The issue of the early diagnosis of acute kidney injury (AKI) has been debated for years. Partially this has been due to the lack of a suitable and consistent definition. Other limitations are the paucity of available experimental models of AKI and the inadequate capability of selected marker molecules to detect the impairment of kidney function in real time. Desirable characteristics of clinically applicable AKI biomarkers include: (a) they should be noninvasive and easy to perform at the bedside or in a standard clinical laboratory using easily accessible samples such as blood or urine, (b) they should be rapidly and reliably measurable using a standardized assay platform, (c) they should be highly sensitive to facilitate early detection and with a wide dynamic range and cutoff values that allow for risk stratification, (d) they should be highly specific for AKI and enable the identification of AKI subtypes and etiologies, and (e) they should exhibit strong biomarker properties on receiver-operating characteristic (ROC) curves.

In 2002, during the second Acute Dialysis Quality Initiative (ADQI) Consensus Conference held in Vicenza, a new classification of AKI called RIFLE (Risk, Injury, Failure, Loss, End-stage renal disease) was proposed based on the level of serum creatinine rise or urine output reduction [37]. The RIFLE classification has been validated now in many papers and it is the most current and accurate tool to define the level of AKI and the level of associated risk of mortality [29].

The initial measurement of SCr cannot reflect the extent of injury because its accumulation always lags behind the insult [39]. Even a large decline in glomerular filtration rate (GFR) may manifest as a small change in serum creatinine level, particularly in the initial 48 hours after acute kidney injury before steady-state equilibrium is reached [39,40]. As a result, the diagnosis of acute kidney injury currently requires measuring serum creatinine repeatedly and delaying maneuvers to prevent ongoing kidney damage, such as stopping use of nonsteroidal anti-inflammatory drugs, adjusting medication dosages, or correcting hemodynamic status.

Using genomic and protein microarray technology, a series of molecules have been identified as potential markers for AKI; among them NGAL which is a 25 kDa protein, generally expressed in low concentrations, and is greatly increased in the case of epithelial damage [29,40,41]. In several papers NGAL has been demonstrated to rise significantly in patients with AKI but not in the corresponding controls [22,27,42]. Mishra and coworkers observed a significant rise in urine NGAL (uNGAL) 2 days before the rise in SCr in children with AKI following CPB [42]. Furthermore, this rise in NGAL occurs in various studies at 24 to 48 hours before the rise in creatinine is observed. NGAL both in urine and plasma is an excellent early marker of AKI with an area under the receiver operator characteristic curve (AUC) in the range of 0.9. The molecule still requires a complete evaluation with an area under the receiver operating characteristic (ROC) curves.

The NGAL both in urine and plasma is an excellent early marker of AKI with an area under the receiver operator characteristic curve (AUC) in the range of 0.9. The molecule still requires a complete evaluation with an area under the receiver operating characteristic (ROC) curves.
In a cross-sectional study, subjects in the intensive care unit with established acute renal failure displayed a greater than 10-fold increase in plasma NGAL concentration and more than a 100-fold increase in urine NGAL concentration by Western blotting when compared to normal controls [44]. Both plasma and urine NGAL concentrations correlated highly with serum creatinine concentrations. Kidney biopsies in these patients showed intense accumulation of immunoreactive NGAL in 50% of the cortical tubules. These results identified NGAL as a widespread and sensitive response to established AKI in humans.

A urine NGAL immunoassay has been developed for a standardized clinical platform (ARCHITECT® analyzer, Abbott Diagnostics). Urine NGAL concentration measured by ARCHITECT assay was found to be an early predictive biomarker of AKI severity after CPB [45]. In a pilot study with 136 urine samples and 6 calibration standards, NGAL concentrations by research ELISA and by the ARCHITECT assay were highly correlated (r=0.99). In a subsequent study, 196 children undergoing CPB were prospectively enrolled and serial urine NGAL concentrations obtained by ARCHITECT assay [45]. AKI developed in 99 patients, but the diagnosis using serum creatinine concentration was delayed by 2-3 days after CPB. In contrast, mean urine NGAL concentrations increased 15-fold within 2 h, and by 25-fold at 4 and 6 h after CPB. In this study of adults undergoing cardiopulmonary bypass, AKI (defined as a 50% increase in serum creatinine) occurred in 25% of subjects, but the diagnosis using serum creatinine was only possible 1–3 days after surgery. In marked contrast, NGAL measurements by enzyme-linked immunosorbent assay (ELISA) revealed a robust four-fold or greater increase in the urine and plasma within 2–6 h of surgery in patients who subsequently developed AKI. In a prospective study of adults who develop AKI after cardiac surgery, urinary NGAL was significantly elevated by 1–3 h after the operation [22]. AKI, defined as a 50% increase in serum creatinine, did not occur until the third postoperative day. However, patients who did not encounter AKI also displayed a significant increase in urine NGAL in the early postoperative period although to a much lesser degree than in those who subsequently developed AKI. The AUC reported in the adult study was 0.74 for the 3-h NGAL and 0.80 for the 18-h NGAL, which is perhaps reflective of the confounding variables that typically accumulate with age. NGAL has also been evaluated as a biomarker of AKI in kidney transplantation. Biopsies of kidneys obtained 1 h after vascular anastomosis revealed a significant correlation between NGAL staining intensity and the subsequent development of delayed graft function [46].

Urinary NGAL measurement at 2 hours after CPB was the most powerful independent predictor of AKI (r=0.82, p=0.0001). Also, a significant correlation was found between AKI (50% or greater in serum creatinine) and the following: included history of previous cardiac surgery (r=0.52, p=0.001), urinary output (r=0.42, p=0.001) and CPB time (r=0.43, p=0.003). The 2-h urine NGAL levels correlated with severity and duration of AKI, length of stay, dialysis requirement and death. This assay is easy to perform with no manual pretreatment steps, a first result available within 35 min and it requires only 150 microlitres of urine.

**Conclusion**

In summary, NGAL is emerging as a center-stage player in the AKI field as a novel predictive biomarker. However, it is acknowledged that the studies published thus far are small, in which NGAL appears to be most sensitive and specific in relatively uncomplicated patient populations with AKI. NGAL measurements may be influenced by a number of coexisting variables, such as preexisting renal disease [47] and systemic or urinary tract infections [48,49]. Large multicenter studies to further define the predictive role of plasma and urine NGAL as a member of the putative AKI panel have been initiated, robust assays for commercialization are nearly complete, and the results are awaited with optimism.

**References**


