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Urinary Neutrophil Gelatinase–Associated Lipocalin (NGAL) Distinguishes Sustained From Transient Acute Kidney Injury After General Surgery

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Introduction: This prospective study tests the hypothesis that after general surgery urinary neutrophil gelatinase–associated lipocalin (NGAL) can distinguish between sustained acute kidney injury (AKI), typical of nephron damage, and transient AKI, commonly seen with hemodynamic variation and prerenal azotemia.

Methods: Urine was collected in 510 patients within 2 to 3 hours after general surgery, and urinary NGAL was determined using enzyme-linked immunosorbent assay. Patients who met AKIN stage 1 criteria of AKI were subclassified into those with sustained AKI (serum creatinine elevation for at least 3 days) and those with transient AKI (serum creatinine elevation for less than 3 days).

Results: Seventeen of 510 patients (3.3%) met the stage 1 AKIN criteria within 48 hours of surgery. Elevations in serum creatinine were sustained in 9 and transient in 8 patients. Urinary NGAL was significantly elevated only in patients with sustained AKI (204.8 $\pm$ 411.9 ng/dl); patients with transient AKI had urinary NGAL that was indistinguishable from that of patients who did not meet AKIN criteria at all (30.8 $\pm$ 36.5 ng/dl vs. 31.9 $\pm$ 113 ng/dl). The area under the curve of the receiver operating characteristic curve of urinary NGAL to predict sustained AKI was 0.85 (95% confidence interval: 0.773–0.929, $P < 0.001$).

Discussion: Urinary NGAL levels measured 2 to 3 hours after surgery were able to distinguish the kinetics of creatinine (sustained AKI vs. transient AKI) over the subsequent week. Transient AKI is an easily reversible state that is likely not associated with substantial tubular injury and therefore NGAL release. Using AKIN criteria, both transient and sustained AKI are classified as AKI even though our data demonstrate that they are possibly different entities.

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Acute kidney injury (AKI) is a diagnosis that is based on the degree of increase in serum creatinine (SCr), the severity and duration of oliguria, or both of these.[1] However, SCr is not a proximate biomarker of kidney tubular injury, but rather an integrator of multiple intrarenal and extrarenal functions, and its final concentration reflects the balance between creatinine production and excretion. Nonetheless, the Acute Kidney Injury Network (AKIN) and Kidney Disease: Improving Global Outcomes (KDIGO) definitions do not differentiate between transient, potentially reversible decreases in glomerular filtration rate that may be due to temporary decreased renal blood flow and sustained increases of SCr caused by intrarenal insults (tubular or glomerular cell death as a result of prolonged ischemia, sepsis, or nephrotoxins). Consequently, the current definitions of AKI may overestimate presumptive tubular cell injury in volume-depleted patients. In addition, patients with decreased muscle mass may not demonstrate a robust increase in SCr even with substantial renal injury. SCr is a delayed and an insensitive marker of renal injury[2] that does not accurately reflect the proportion of injured nephrons.[3] Indeed, recent work shows that the level of SCr does not predict morbidity and mortality as strongly as the duration of its elevation.[4] Hence, it is critical to discover tools in addition to AKIN and KDIGO definitions that distinguish transient from prolonged AKI.

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Using the AKIN or KDIGO SCr criteria, the incidence of postoperative AKI varies from around 1% after general surgery to as high as 25% to 30% after cardiac surgery.[5,6] Preexisting risk factors include advanced age, male gender, obesity (high body mass index), hypertension, diabetes, chronic kidney disease, congestive heart failure, ascites, and a high preoperative American Society of Anesthesiology class. Emergent, i.p., or high-risk surgery poses an increased risk of postoperative AKI, which may be attributed to intraoperative hypotension or shock, and nephrotoxic insults such as i.v. contrast agents, aminoglycoside antibiotics, or immunosuppressive drugs.[5–7]

It is no surprise, therefore, that postoperative AKI is associated with increased intensive care unit and hospital length of stay, cost, and mortality.[8] Postoperative AKI is a major risk factor for the development of nonrenal complications and an independent contributor to mortality in non-cardiac surgery.[9,10] Nonetheless, studies based on the AKIN or KDIGO definitions of AKI may overestimate the effect of tubular damage because SCr does not differentiate between brief and prolonged duration of azotemia, or separate different causes that increase SCr. Prerenal azotemia may progress to intrinsic acute kidney injury if not rapidly treated, but the slow increase in SCr not only delays the diagnosis of AKI, but also prevents timely intervention of protective therapy at an early enough stage of injury to potentially alter outcome. This has sparked an ongoing search for early biomarkers of tubular injury that is typified by prolonged azotemia.

Neutrophil gelatinase–associated lipocalin (NGAL) is a small, 23-kDa protein that is an early biomarker for ischemic, septic, or nephrotoxic kidney injury.[11] It is normally produced at low levels by the epithelial cells of the kidney, but it is quickly upregulated in the thick ascending limb of the loop of Henle and the collecting ducts within 3 hours of tubular epithelial injury.[3,10] Urinary and plasma NGAL increases rapidly and proportionally to the severity and duration of the insult.[3,12–14] These responses were conserved in children and adults, and across many different animal models. Its potential usefulness as a clinical biomarker is enhanced because NGAL is a protease-resistant polypeptide detectable in plasma and urine through automated clinical bioanalyzers, quantitative enzyme-linked immunosorbent assay, or semiquantitative immunoblot techniques.[14]

Urinary NGAL has been evaluated as an early biomarker of renal tubular damage in acute clinical settings such as the operating room, intensive care unit, and emergency department, in high-risk procedures such as cardiac surgery and radiocontrast injection, and after adult and pediatric kidney and liver transplantation.[2,3,10,13,15–18] There is considerable evidence that compared to increases in SCr, NGAL better detects early or subclinical kidney injury, and better predicts dialysis requirement and mortality.[3]

There is also considerable evidence that elevated urinary NGAL can distinguish true renal tubular injury from hemodynamic variations and volume depletion days before serial measurements of SCr make these diagnoses. In an emergency department study on over 600 patients and in a validation study of over 1650 patients, Nickolas *et al*. found that a single urinary NGAL measurement on admission could clearly discriminate between prolonged AKI, defined as an elevation of SCr $\geq$1.5 times baseline sustained for $\geq$3 days, and transient AKI, defined as transient elevated SCr that resolved within 3 days.[3,19] Nearly identical data were found in cirrhotic patients, patients in intensive care units, and in animal models, namely that NGAL levels did not rise outside of the normal range in patients deemed prerenal or transiently azotemic.[20–22] The patients in many of these studies, particularly the emergency department studies, represented a very heterogeneous group of conditions and pathways to renal injury. Here, we designed an observational study to test the hypothesis that urinary NGAL could similarly distinguish sustained AKI from transient AKI in a general surgical population immediately after general surgery.

MATERIALS AND METHODS

We screened all adult patients undergoing any type of general surgical procedure at Columbia University Medical Center who were admitted to the Post Anesthesia Care Unit (PACU) with an indwelling urinary catheter between March and July 2013. Patients were excluded from consideration if they were less than 18 years of age; had preexisting end-stage renal disease (chronic kidney disease stage 5); had undergone nephrectomy; or were either a donor or a recipient of renal transplantation. The Institutional Review Board (IRB) of Columbia University approved the study (IRB #AAAL4657) contingent on written informed consent for the collection of urine prior to testing of the samples in this study.

Within 2 to 3 hours of admission to the PACU, 10 ml urine was collected from the urinary catheter collecting system and centrifuged at 2000*g* for 20 minutes, and the supernatant was frozen at −80 °C and retained for batch analysis approximately 4 months later. We measured urinary NGAL by commercially available enzyme-linked immunosorbent assay kits (Antibody-Shop, Gentofte, Denmark) at the Irving Institute for Clinical and Translational Research of Columbia

University. The limit of NGAL detection with this research assay is 0.5 to 4.0 ng/ml, and intra-assay variation of the urine is 2.1% (range: 1.3%–4.0%).

Urinary sodium (UNa) and urinary creatinine (UCr) were also measured at the Irving Institute. Serum sodium (SNa), SCr, and blood urea nitrogen (BUN) were determined by the clinical laboratory of Columbia University Medical Center as dictated by the clinical care for the patients. The fractional excretion of sodium (FENa) was calculated by the formula (SCr × UNa) / (SNa × UCr). Estimated creatinine clearance was derived using the method of Cockcroft and Gault.[23]

We defined postoperative AKI by the AKIN stage 1 SCr criterion as follows: an absolute increase in SCr ≥ 0.3 mg/dl (≥ 26.4 μmol/l) within 48 hours after surgery. We defined transient AKI as an increase of SCr by ≥0.3 mg/dl (≥26.4 μmol/l) within 48 hours that decreased to less than 0.3 mg/dl (≥26.4 μmol/l) by 72 hours after surgery. Sustained AKI was defined as an increase of SCr by ≥0.3 mg/dl (≥26.4 μmol/l) within 48 hours that remained elevated at 72 hours and more after surgery. These definitions of sustained and transient AKI have previously been used in a cohort of patients admitted to the emergency department.[19]

Comparisons and correlations between groups were made by an unpaired *t*-test or Pearson's test for correlation for values with Gaussian distribution and by Mann–Whitney (Wilcoxon rank) test or Spearman's for correlation for continuous variables without normal distribution. Gaussian distribution was determined using the Kolmogorov–Smirnov test. Categorical data were compared using the χ^2 or Fisher exact test. *P* values were 2-tailed, and $P < 0.05$ was considered significant. Receiver operating characteristic (ROC) curves were plotted, and the point on the ROC curve closest to sensitivity = specificity = 1 was considered the best cutoff value. Analysis of variance (ANOVA) with Tukey's range test was used to compare multiple groups. We used PASW 18.0 (IBM Inc., Armonk, NY) and GraphPad Prism 6.0 (San Diego, CA) software for statistical analysis.

RESULTS

Patient Characteristics

We screened a total of 543 patients between March and July 2013. Of these, 15 patients met exclusion criteria (6 living-donor kidney transplantation; 6 cadaveric kidney transplant recipients; 3 patients undergoing nephrectomy), and 18 patients were excluded because their records were incomplete. We analyzed data from 510 patients, whose mean age was 60.7 ± 15.5 years, and of whom 59.4% were female. The most common procedure groups were major abdominal surgery, including pancreatectomy, exploratory laparotomy, colectomy, or gastric bypass surgery; and major orthopedic surgery, including total knee or total hip replacement and open reduction and internal fixation of hip fractures (Table 1). Patients with AKI had a baseline SCr of 1.46 ± 1.39 mg/dl compared to a baseline SCr of 1.09 ± 3.79 mg/dl in patients without AKI (not significant).

Sustained, Transient, and No AKI by SCr Criteria

Seventeen patients had stage 1 AKI (3.3%) as defined by AKIN criteria (SCr increase ≥0.3 mg/dl [26.5 μmol/l] or increase to 1.5-fold or more from baseline). These patients had higher urinary NGAL levels than patients without AKI: 122.9 ± 305.7 ng/ml versus 31.9 ± 113.0 ng/ml ($P < 0.01$). The area under the curve (AUC) of the ROC curve of urinary NGAL to predict AKI was 0.738 (95% confidence interval [CI]: 0.616–0.860, $P < 0.005$).

Nine of 510 patients (1.76%) met our criteria for sustained AKI as follows: SCr elevated by ≥0.3 mg/dl within 48 hours of surgery that persisted for ≥72 hours. Another 8 patients (1.6%) met our criteria for transient AKI as follows: SCr elevated by ≥0.3 mg/dl within 48 hours of surgery that normalized within 48 to 72 hours. The remaining 493 patients (96.7%) did not meet SCr criteria for either transient or sustained AKI (Figure 1).

We compared urinary NGAL levels in patients who met SCr criteria for sustained AKI, transient AKI, or normal renal function by ANOVA and Tukey's *post hoc*

Table 1. Patient demographics (N = 510)

Patient characteristics	
Age	60.7 (±15.5)
Height (cm)	166.7 (±12.6)
Weight (kg)	82.5 (±24.5)
BMI	30.5 (±20.8)
Female	303 (59.4%)
Surgical procedures	
Major abdominal	113 (22.2%)
Minor abdominal	9 (1.8%)
Gynecological	46 (9.0%)
CNS neurosurgery	24 (4.7%)
Other neurosurgery	24 (4.7%)
Orthopedics	95 (18.6%)
Spine	48 (9.4%)
Thoracic	55 (10.8%)
Vascular major	19 (3.7%)
Vascular minor	5 (1.0%)
Angiogram	7 (1.4%)
Urology major	32 (6.3%)
Urology minor	19 (3.7%)
Other	14 (2.7%)

Data are reported as mean and SD or number and percentage, as appropriate.
BMI, body mass index; CNS, central nervous system.

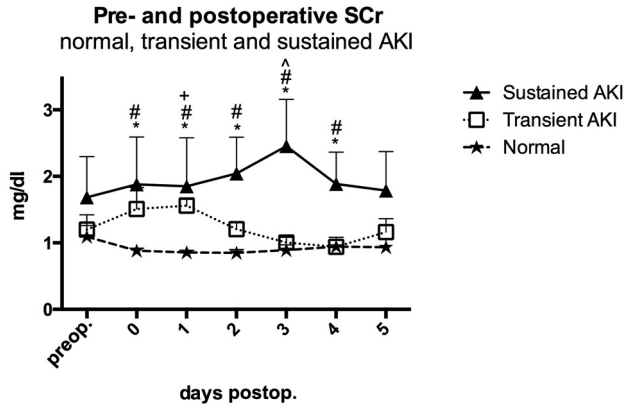

Figure 1. Perioperative changes in SCr in patients with sustained (≥3 days) or transient (<3 days) AKI or no AKI. Mean ± SEM, $P < 0.05$: *ANOVA comparison of groups, Tukey's *post hoc* test; #sustained versus no AKI; ^transient versus sustained AKI; +transient versus no AKI. AKI, acute kidney injury.

range test. Mean immediate postoperative urinary NGAL levels in patients who developed sustained AKI were 204.8 ± 411.9 ng/dl, and were significantly higher by ANOVA ($P < 0.001$) than in those who developed transient AKI (30.8 ± 36.5 ng/dl) or who had normal renal function (31.9 ± 113 ng/dl) (Figure 2). Tukey's *post hoc* test confirmed that urinary NGAL levels in patients with sustained AKI were significantly higher than those in patients with transient AKI or those with normal renal function, and that there was no significant difference in urinary NGAL between the latter 2 groups. The AUC of the ROC curve of urinary NGAL to detect sustained AKI was 0.851 (95% CI: 0.773–0.929, $P < 0.001$). The best cutoff was 12.52 ng/ml, with a sensitivity of 70.1% (95% CI: 65.8%–74.0%), a specificity of 88.9% (95% CI: 51.8%–99.7%), and a likelihood ratio of 6.305. In comparison, the AUC of UNa to predict sustained AKI was 0.786 (95% CI: 0.673–0.900, $P < 0.005$) (Table 2).

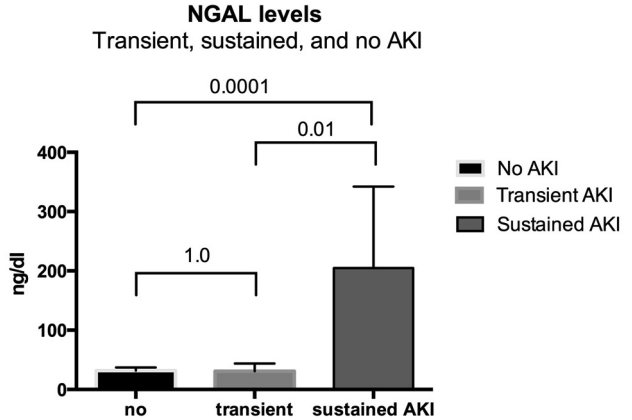

Figure 2. Urinary NGAL levels in patients with no AKI, transient AKI (<3 days), or sustained AKI (≥3 days) by AKIN criteria. *P* values derived from ANOVA with Tukey's *post hoc* tests. AKI, acute kidney injury; NGAL, neutrophil gelatinase–associated lipocalin.

Table 2. Urinary sodium, urinary creatinine, fractional excretion of sodium, and urinary NGAL in patients with sustained and transient acute kidney injury and normal postoperative serum creatinine

	Sustained AKI $N = 9$	Transient AKI $N = 8$	No AKI $N = 493$	*P* value
Urinary sodium (mmol/l)	60.9 ± 34.8	75.5 ± 41.7	114.6 ± 54.7	$P < 0.05$
P values *post hoc* test (Tukey)	↓ 0.84 ↑	↓ 0.11 ↑		
	↓ 0.01	↑		
Urinary creatinine (mg/dl)	93.6 ± 64.6	108.2 ± 50.7	68.6 ± 55.9	NS
FENa	2.07 ± 3.74	1.45 ± 1.57	2.66 ± 12.27	NS
NGAL (ng/ml)	204.8 ± 412.0	30.8 ± 25.9	31.9 ± 113.0	$P < 0.0001$
P values *post hoc* test (Tukey)	↓ 0.01 ↑	↓ 1.0 ↑		
	↓ 0.0001	↑		

One-way analysis of variance and Tukey's *post hoc* multiple comparison test to compare mean values across the groups.
AKI, acute kidney injury; FENa, fractional excretion of sodium; NGAL, neutrophil gelatinase–associated lipocalin; NS, not significant.

Impact of AKI and Urinary NGAL on Post-operative Outcome

Patients who developed sustained AKI had significantly longer hospital length of stay after surgery (22.4 ± 18.5) than patients who had transient AKI (13.4 ± 10.5 days) or normal renal function (4.3 ± 5.0) ($P < 0.001$, ANOVA with Tukey's *post hoc* test).

DISCUSSION

Our study demonstrates that in a large cohort of patients undergoing general surgery, immediate postoperative urinary NGAL levels were significantly more elevated in patients who went on to have sustained (≥3 days) elevations of SCr (sustained AKI) compared to those with transient AKI or no elevations in SCr (normal). Urinary NGAL was able to predict sustained AKI with an AUC of the ROC curve of 0.851, indicating that urinary NGAL levels correlated with severe renal injury days before SCr increases and demonstrates a sustained course. Urinary NGAL had greater discriminatory power to detect sustained AKI immediately after surgery than either UNa, FENa, or the BUN-to-SCr ratio, supporting its potential usefulness in providing prognostic information and guiding the nature and timing of potential protective interventions. UNa is seldom used to determine prerenal state, as it depends on diuretics or the type of i.v. fluids administered perioperatively.

Patients in our study who developed transient SCr elevations had urinary NGAL levels indistinguishable from those of patients whose SCr remained normal. Transient elevations of SCr most likely reflect a decrease in glomerular filtration rate caused by hypovolemia rather than tubular or glomerular cell death. This is frequently referred to as a prerenal syndrome or

prerenal azotemia when certain criteria are met (BUN/SCr >20, FENa <1%), in humans[3] and in mice.[22] Our observations, which confirm those of Nickolas *et al.*[3,19] and other investigators,[20,21,24] provide strong clinical evidence that transient azotemia does not necessarily cause tubular injury and the genetic expression of urinary NGAL. Instead, transiently elevated SCr is a consequence of renal hypoperfusion that is reversed with volume restoration or hemodynamic stabilization without obvious long-term SCr elevation. However, using AKIN criteria these patients would be assigned the same category as patients who have sustained, intrinsic AKI. Therefore a substantial number of patients who meet the RIFLE, AKIN, or KDIGO criteria of "AKI" may not have tubular injury resulting in the release of NGAL into the urine; that is, they may have SCr elevation without true acute kidney tubular injury. Moreover, it has recently been observed that false-positive diagnosis of AKI is relatively common with small changes in SCr because of its inherent variability when baseline values are elevated.[25] Conversely, the ability of NGAL to predict AKI in some studies may have been impaired by including patients whose transient elevations of SCr may have met AKIN criteria without actual tubular injury and NGAL expression.

As a consequence of the "overdiagnosis" of AKI by RIFLE, AKIN, or KDIGO criteria, the incidence and prevalence of mild to moderate kidney injury may be overestimated in many current studies, and the adverse impact of "true" AKI may be underestimated by dilution with the more benign syndrome of transient azotemia that can be due to hemodynamic variation and volume depletion ("prerenal azotemia"). This is further emphasized by our observation that patients who had substantial early elevation in urinary NGAL (AKI) went on to have sustained (≥3 days) elevation in SCr that was associated with significantly prolonged hospital length of stay.

Although, as mentioned above, our observations support those of Nickolas *et al.*,[3,19] there are some very important differences between the 2 studies. In the emergency department study the Risk of the RIFLE criteria was used, a much more stringent criterion for AKI (increase of SCr by 1.5 times baseline) than stage 1 of the AKIN criteria (increase in SCr by 0.3 mg/dl from baseline). The incidence of sustained AKI and transient azotemia in our study was much lower (1.6% vs. 4.7% and 1.76% vs. 13.8%, respectively). This is consistent with a previous large population-based study that found that the incidence of AKI was only 0.8% in patients with previously normal renal function undergoing non-cardiac surgery.[7] Despite the low incidence of sustained AKI and transient azotemia in general surgical patients, urinary NGAL was clearly able to distinguish between the 2 entities immediately after surgery. This could be particularly useful if rapid methods of measuring NGAL are used that provide results within hours. Due to its longer processing time, enzyme-linked immunosorbent assay to detect NGAL is not feasible for clinical use.

We did not measure plasma NGAL, which has the advantage of allowing its determination in the setting of rapidly changing urine output. However, plasma NGAL derives not only from the kidney but also from other organs exposed to the same injury. Nonetheless, meta-analysis suggests that plasma and urinary NGAL measurements perform similarly well in the diagnosis of AKI and both offer an advantage over SCr.[13]

NGAL has been studied in a range of clinical settings in children and adults, but most studies have focused on patients undergoing cardiac surgery or liver transplantation or who are critically ill. Initial studies of NGAL in pediatric patients demonstrated much higher AUCs of the ROC curve of >0.9;[3,16,26–28] but this has not been sustained in some recent studies in adult patients—for example, undergoing cardiac surgery; critically ill in the ICU; and after contrast exposure in coronary angiography.[12,17,29–32] NGAL's lower discriminatory power in adults (0.85 for sustained AKI in this study) is likely attributable most critically to variables such as the prevalence of transient prerenal azotemia and preexisting kidney disease and other comorbidities resulting in a more heterogeneous study group[10,33] and potentially contributions from different NGAL molecular species, which older assays failed to differentiate.

A limitation of our study is that the timing of urinary sampling for NGAL in the PACU was rather loosely defined as within a few hours after admission. In contrast, most studies on renal biomarkers sample at specific time points after surgery or potential renal insults so as to capture the optimal timing to utilize NGAL.[2,14,16,32] NGAL levels do change with time after surgery,[15] but most diagnostic tests of organ injury—troponin being the most obvious example—are performed at varying time points after the insult. In our study we tried to replicate the potential clinical use of measuring NGAL in the PACU, and a specific time point for sampling is not practical in normal clinical practice. Our study was designed to most realistically simulate diagnostic testing in a typical clinical setting while sampling fell within the window of peak performance of urinary NGAL (3–12 hours after renal injury).

A further limitation of our study is the low rate of events. This incidence of AKI that we observed is similar to previously reported frequency of AKI in patients undergoing general, non-cardiac surgery.[5] Even with this low event rate our results are robust and comparable to those of the study of NGAL in patients

admitted to the emergency department.[19] Studying higher-risk patients will likely increase the incidence of AKI and therefore the statistical power, but results of such studies may be less generalizable.

In conclusion, we have demonstrated that in patients undergoing general surgery, the incidence of SCr elevation is low, less than 4%. Nonetheless, postoperative urinary NGAL concentrations predicted the subsequent development of sustained AKI in a subset of those patients. Our study confirms the observations of Nickolas *et al.*[3,19] and other investigators[20,21,24] that transient elevations in SCr seen with prerenal azotemia are not associated with NGAL expression outside of the normal range, and therefore likely do not represent tubular injury. Urinary NGAL measurements may therefore facilitate the rapid diagnosis of true intrinsic AKI and distinguish it from transient SCr elevation due to hemodynamic variations and volume depletion previously called prerenal azotemia.

DISCLOSURE

All the authors declared no competing interests.

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