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Association between Increases in Urinary Neutrophil Gelatinase–associated Lipocalin and Acute Renal Dysfunction after Adult Cardiac Surgery

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Background: **Acute renal dysfunction (ARD) and subsequent acute renal failure after cardiac surgery are associated with high mortality and morbidity. Early therapeutic or preventive intervention is hampered by the lack of an early biomarker for acute renal injury. Recent studies showed that urinary neutrophil gelatinase–associated lipocalin (NGAL or lipocalin 2) is up-regulated early (within 1–3 h) after murine renal injury and in pediatric ARD after cardiac surgery. The authors hypothesized that postoperative urinary NGAL concentrations are increased in adult patients developing ARD after cardiac surgery compared with patients without ARD.**

Methods: **After institutional review board approval, 81 cardiac surgical patients were prospectively studied. Urine samples were collected immediately before incision and at various time intervals after surgery for NGAL analysis by quantitative immunoblotting. ARD was defined as peak postoperative serum creatinine increase by 50% or greater compared with preoperative serum creatinine.**

Results: **Sixteen of 81 patients (20%) developed postoperative ARD, and the mean urinary NGAL concentrations in patients who developed ARD were significantly higher early after surgery (after 1 h: 4,195 ± 6,520 [mean ± SD] *vs.* 1,068 ± 2,129 ng/ml; $P < 0.01$) compared with patients who did not develop ARD. Mean urinary NGAL concentrations continued to increase and remained significantly higher at 3 and 18 h after cardiac surgery in patients with ARD. In contrast, urinary NGAL in patients without ARD decreased rapidly after cardiac surgery.**

Conclusions: **Patients developing postoperative ARD had significantly higher urinary NGAL concentrations early after cardiac surgery. Urinary NGAL may therefore be a useful early biomarker of ARD after cardiac surgery. These findings may facilitate the early detection of acute renal injury and potentially prevent progression to acute renal failure.**

IMPAIRED renal function after cardiovascular surgery is a major contributor to the duration of stay in the intensive care unit (ICU) and hospital, cost, and mortality.[1–3]

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Acute renal dysfunction (ARD) occurs frequently after cardiac surgery and may lead to acute renal failure (ARF) requiring hemodialysis. The 30-day mortality of patients requiring hemodialysis after cardiac surgery is estimated at approximately 64%.[4,5] The risk of renal dysfunction is especially high for patients with impaired preoperative renal function.[2]

Early detection of organ injury with biomarkers contributed significantly to patient care and improved clinical outcome.[6,7] For example, troponin I has revolutionized the diagnosis of cardiac injury.[6,7] Early detection of patients at risk for renal dysfunction with such a biomarker could allow early intervention, prevention of morbidity, and reduction in mortality. However, currently used clinical markers of renal function such as blood urea nitrogen, serum creatinine, estimated creatinine clearance, and urine output may not reflect actual changes in glomerular filtration rate (GFR) and do not accurately predict postoperative ARD.[2] As a consequence of hemodilution during cardiopulmonary bypass (CPB), serum creatinine frequently decreases after cardiac surgery even with significant renal injury. Serum creatinine increases significantly only days after renal tubular damage has occurred.[8] The presence of a sensitive and specific marker for renal injury could allow earlier detection of ARD and potentially facilitate therapeutic intervention at a point where renal dysfunction might still be reversible.

Preclinical studies indicate that neutrophil gelatinase–associated lipocalin (NGAL or lipocalin 2) messenger RNA (mRNA) and protein originating from the kidney are consistently up-regulated and appear in the urine early (within 1–3 h) after murine renal ischemic injury.[9,10] A retrospective study in ICU patients showed that NGAL concentration is highly correlated with septic as well as nonseptic ARF.[11] A study in pediatric cardiac surgical patients also demonstrated that urinary NGAL is significantly increased in patients who subsequently developed ARD.[12] However, a prospective study in adult cardiac surgical patients correlating postoperative urinary NGAL and the postoperative ARD has never been performed. In this study, we hypothesized that urinary NGAL concentration would increase after cardiac surgery and questioned whether we could correlate postcardiac surgery urinary NGAL concentration with the occurrence of postoperative ARD.

Materials and Methods

Patients

All adult patients undergoing cardiac surgery at Columbia University Medical Center (New York, New York) were eligible for enrollment. A total of 81 patients were prospectively studied from July 26 to November 2, 2004. The Institutional Review Board of Columbia University Medical Center approved the study, and informed consent was obtained. The exclusion criterion for the study was preexisting renal failure requiring hemodialysis. Postoperative ARD was defined as an increase in serum creatinine in the postoperative period by more than 50% compared with preoperative serum creatinine. This is the definition of renal risk suggested by the Second International Consensus Conference of the Acute Dialysis Quality Initiative Group as part of the Risk–Injury–Failure–Loss–End-stage renal disease classification criteria.[13] We calculated Parsonnet scores for all patients.[14] The Parsonnet score (range, 0–38) has been developed for risk stratification of cardiac surgical patients, and the patients received points according to their age, sex, cardiovascular status, preoperative comorbidities, and extent of surgery.[14]

The preoperative creatinine clearance was estimated using the method described by Cockcroft and Gault.[15] ICU- and hospital-free days within 30 days were defined as the number of days a patient was outside the ICU or hospital within the first 30 days postoperative and were zero if the patient died in the ICU or in the hospital, respectively.

Sample Collection

Five milliliters of urine was collected from 81 patients at the following time points: (1) preoperative; (2) immediately after CPB or, in the case of off-pump coronary artery bypass grafting, after reperfusion of the last bypass graft; (3) at 1, 3, 18, and 24 h after arrival at the ICU or at the time of discharge from the ICU, whichever occurred first. The urine was immediately centrifuged at 1,000*g* for 15 min. and the supernatant was frozen at −20°C for NGAL immunoblotting and urinary creatinine analysis.

Quantitative Immunoblotting for Urinary NGAL

Human recombinant NGAL protein standard was expressed as a glutathione S-transferase-fusion protein in BL21 strain of *Escherichia coli*, and glutathione S-transferase was removed by thrombin cleavage as previously described.[11,12] Ten microliters of urine was mixed with 10 μl nonreducing Laemmli buffer (Bio-Rad, Hercules, CA) and electrophoresed along with 10 μl recombinant human NGAL standards (0.1–1 ng/μl) at room temperature through 4–20% gradient polyacrylamide gel (Bio-Rad) for 1.5 h at 80 V. The primary monoclonal antibody for NGAL (Antibody Shop, Gentofte, Denmark) was diluted 1:1,000. Immunoblotting continued as described previously.[16–18] The densities of three known standard concentrations (1, 5, and 10 ng/ml) were used to create a standard curve. The standard curve was used to calculate the concentration of each sample by plotting the density of the sample band on the standard curve. The detectable limit of NGAL with our immunoblotting was 0.5 ng/ml. To confirm the precision of the immunoblot, we analyzed how precisely the 5-ng/ml sample is positioned on the line between 1 and 10 ng/ml and found a mean deviation of 4.4% in eight samples.

The NGAL band intensities were quantified using LabWorks 4.5 software (UVP Inc., Upland, CA), and the urinary NGAL concentrations were calculated from the NGAL standard curve generated from each blot.

Urinary Creatinine Assay

Urinary creatinine levels were determined by a colorimetric method based on the Jaffe reaction.[19] Briefly, 10 μl urine was added to 1 ml mixture (2:2:1) of 0.0 5M sodium phosphate containing 0.5 M sodium borate (pH 12.7–12.8), 4% sodium dodecyl sulfate, and saturated picric acid. After 45 min, absorbance was measured at 510 nM. Twenty-five microliters of 60% acetic acid was then added. After vigorous mixing, the solution was allowed to stand for 6 min before absorbance was again measured at 510 nM. Comparison with a 15-mg/dl creatinine standard allowed for calculation of urinary creatinine values.

Statistical Analysis

Before enrolling subjects, we performed a power analysis. We assumed a difference of urinary NGAL of 500 ng/ml between the groups with ARD and without ARD with a common SD of 500 ng/ml and an incidence of ARD of 25%. We concluded that we would require 63 subjects to achieve a power of $(1 - \beta) = 0.8$. We enrolled 18 patients more to compensate for potential problems with sample procurement and processing.

Values are presented as mean ± SD. Comparisons between groups were made by unpaired *t* test for values with gaussian distribution and by Mann–Whitney (Wilcoxon rank) test or Spearman test for correlation for continuous variables without normal distribution. Gaussian distribution was determined using the Kolmogorov–Smirnov test. Chi-square test, Fisher exact test, or one-way analysis of variance was used as appropriate. *P* values were two tailed, and $P < 0.05$ was considered significant. Receiver operating characteristic curves were generated for peak urinary NGAL and immediately after surgery and 1, 3, 18, and 24 h later. The area under the curve for each receiver operating characteristic curve was used to quantify the ability of urinary NGAL to predict postoperative ARD. We calculated the "best threshold" for urinary NGAL 18 h after CPB defined as the urinary NGAL value that is closest to the point on the

Table 1. Patient Characteristics

	No ARD (n = 65)	ARD (n = 16)	*P* Value*
Preoperative			
Female	23 (35%)	5 (31%)	NS
Age, yr	67 (49–76)	73 (66–76)	NS
BMI, kg/m^2	26 (23–29)	27 (24–30)	NS
Serum creatinine, mg/dl	0.9 (0.8–1.1)	1.2 (0.8–1.5)	NS
Estimated creatinine clearance, ml/min	73 (52–100)	57 (37–92)	0.024
Parsonnet score	11 (5–18)	13.5 (7–22)	NS
Operation			
CABG–CPB, n	6 (9%)	0	NS
OPCAB, n	8 (12%)	3 (19%)	NS
Single valve, n	29 (45%)	1 (6%)	0.0038
Multiple valve, n	4 (6%)	3 (19%)	NS
CABG + valve, n	12 (19%)	5 (31%)	NS
Reoperation, n	15 (32%)	3 (19%)	NS
Other, n	6 (9%)	4 (25%)	NS
Intraoperative			
CPB time, mean ± SD, min	120 ± 43	162 ± 60	0.005
AXC time, mean ± SD, min	82 ± 36	137 ± 31	< 0.001
Postoperative			
ICU-free days within 30 days	29 (28–29)	26 (8.5–29)	0.003
Hospital-free days within 30 days	24 (22–26)	19.5 (0–22)	< 0.001
Peak serum creatinine, mg/dl	1.0 (0.8–1.3)	2.1 (1.5–2.75)	< 0.001
Δ Serum creatinine, %	+8.4 ± 16.5	+89 ± 57	< 0.001
Requiring CVVHDF, n	0	5 (31%)	< 0.001
Mortality, n	1 (1.5%)	5 (31%)	< 0.001

Data are expressed as mean ± SD or median (25–75% interquartile range) for nonparametric variables, or number (percentage).

* $P < 0.05$.

ARD = acute renal dysfunction (see text for definition); AXC = aortic cross clamp; BMI = body mass index; CABG–CPB = coronary artery bypass grafting with cardiopulmonary bypass; CPB = cardiopulmonary bypass; CVVHDF = continuous veno-venous hemodialysis and filtration; ICU = intensive care unit; NS = not significant; OPCAB = off-pump coronary artery bypass grafting.

receiver operating characteristic curve where sensitivity = 1 − specificity = 1. We used this value as a cutoff value for urinary NGAL to calculate sensitivity, specificity, and negative and positive predictive values.

SPSS 11.0.4 (SPSS Inc., Chicago, IL) and Graphpad Prism 4.0 (San Diego, CA) were used for the statistical analysis.

Results

Patient Characteristics

Patient characteristics are listed in table 1. Eighty-one patients were enrolled, and urinary NGAL was successfully analyzed in all patients.

Of the 81 patients studied, 16 (20%) developed postoperative ARD as defined by a peak postoperative increase in serum creatinine by more than 50% compared with preoperative values (fig. 1A). Patients who developed ARD had longer CPB times (162 ± 60 *vs.* 120 ± 43 min; $P = 0.005$) and longer aortic cross clamp times (137 ± 131 *vs.* 82 ± 36 min; $P < 0.001$) than the non-ARD cohort. Five of the 16 patients with postoperative ARD developed ARF and required continuous veno-venous hemodialysis. Also, 5 of the 16 patients with ARD (including 3 of the 5 patients requiring continuous veno-venous hemodialysis) developed multiple organ failure and died in the hospital compared with 1 of 63 patients without ARD (31% *vs.* 1.5%, $P < 0.001$) (table 1).

Urinary NGAL

Patients who developed ARD had significantly higher peak postoperative urinary NGAL concentrations (5,994 ± 7,616 ng/ml, n = 16) compared with patients who did not develop postoperative ARD (1,760 ± 3,527 ng/ml, n = 65; $P = 0.0014$; 95% confidence interval of the difference of the mean, 1,679–6,789 ng/ml). Representative immunoblots for urinary NGAL are shown in fig. 2.

When evaluating all patients (ARD and no ARD), peak urinary NGAL did not correlate with peak Δ creatinine (Spearman $R = 0.20$, $P = 0.07$).

Postoperative Urinary NGAL Kinetics

Urinary NGAL levels were equivalent in patients with or without ARD immediately after the termination of CPB (or reperfusion of last bypass graft in off-pump coronary artery bypass grafting patients; fig. 1B). In both groups, urinary NGAL levels (ARD: 2,516 ± 2,405 ng/ml, n = 16; non-ARD: 1,590 ± 3,490 ng/ml, n = 65) immediately after surgery were significantly increased compared with preoperative values (ARD: 112 ± 238 ng/ml; non-ARD: 44 ± 129 ng/ml). However, within an hour after surgery, urinary NGAL concentration had de-

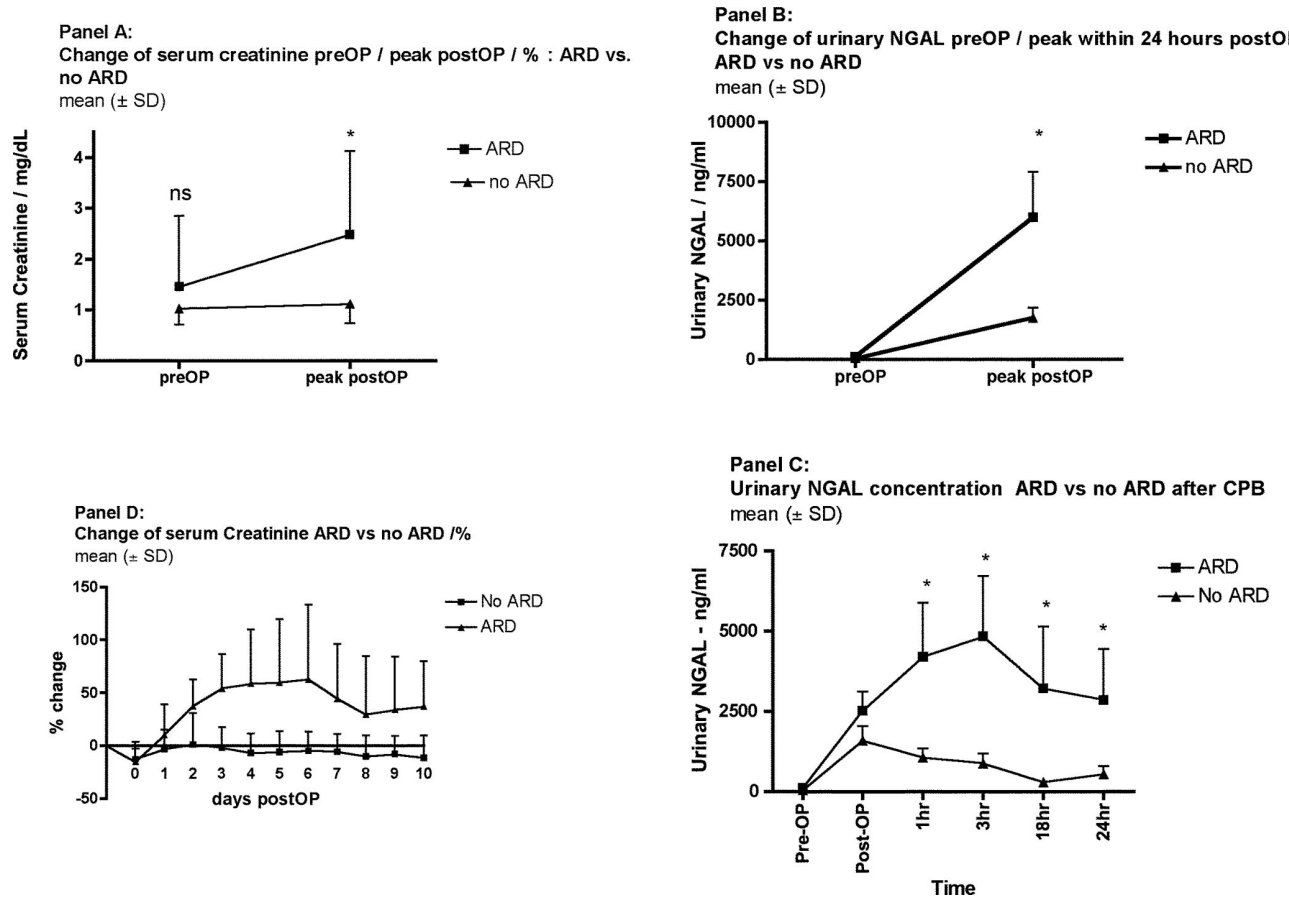

Fig. 1. Change of serum creatinine and urinary neutrophil gelatinase–associated lipocalin (NGAL): postoperative acute renal dysfunction (ARD) *versus* no ARD. (*A*) Mean (± SD) preoperative and peak postoperative serum creatinine in patients with ARD and without ARD. (*B*) Mean (± SD) preoperative and peak postoperative urinary NGAL in patients with ARD and without ARD. (*C*) Mean (± SD) urinary NGAL concentration in patients with ARD and without ARD. (*D*) Mean (± SD) change of serum creatinine as percent of preoperative serum creatinine in patients with ARD and without ARD. * $P < 0.05$ for ARD *versus* no ARD. CPB = cardiopulmonary bypass; ns = not significant; postOP = postoperative; preOP = preoperative.

creased in non-ARD patients but continued to increase in patients who manifested ARD (4,195 ± 6520 *vs.* 1,068 ± 2,129 ng/ml; $P = 0.002$; 95% confidence interval of the difference of the mean, 1,181–5,073 ng/ml; fig. 1C). Urinary NGAL subsequently continued to increase in the ARD group, peaking at 3 h and remaining increased for 24 h postoperatively. Urinary NGAL levels at 1, 3, and 18 h after cardiac surgery were significantly higher in

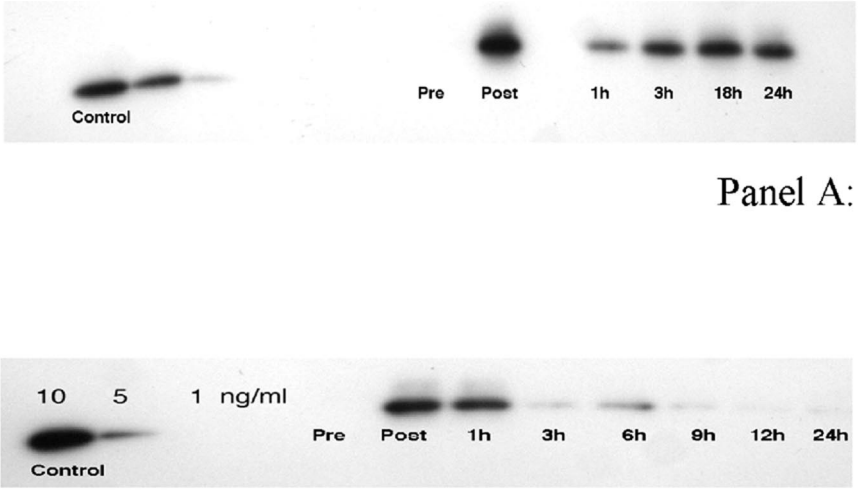

Fig. 2. (*A*) Representative immunoblot in a patient who developed acute renal dysfunction: Urinary neutrophil gelatinase–associated lipocalin remains increased 18 and 24 h after cardiopulmonary bypass. (*B*) Representative immunoblot in a patient without acute renal dysfunction: Urinary neutrophil gelatinase–associated lipocalin increases immediately after cardiac surgery but then decreases rapidly.

patients with ARD compared with non-ARD patients ($P < 0.01$ at 1, 3, and 18 h).

In contrast to urinary NGAL, serum creatinine in patients who developed ARD reached its peak only on postoperative day 4 (±2.2; fig. 1D). Serum creatinine at 24 h for patients who developed ARD remained equivalent to the preoperative values.

NGAL:Urinary Creatinine Ratio

Urinary creatinine immediately after the termination of CPB (or reperfusion of last bypass graft in off-pump coronary artery bypass grafting patients) was significantly lower in patients in the ARD group (5.0 ± 0.7 mg/dl, n = 10) compared with the non-ARD group (10.7 ± 1.0 mg/dl, n = 35; $P < 0.01$). Although there was no significant difference in urinary NGAL concentrations between the two groups at this time point, the ratio of urinary NGAL to urinary creatinine was significantly higher (71.6 ± 18.0, n = 10 *vs.* 36.4 ± 9.5, n = 35; $P < 0.05$). This suggests that the urinary NGAL:urinary creatinine ratio could be used to predict postoperative ARD at an early stage. Three hours after the termination of CPB (or reperfusion of last bypass graft), urinary creatinine was similar in both groups (ARD: 33.5 ± 6.3 mg/dl, n = 7; non-ARD: 27.3 ± 3.9 mg/dl, n = 28), but the urinary NGAL:urinary creatinine ratio at 3 h after arrival in the ICU was significantly higher for the ARD *versus* the non-ARD group (16.8 ± 5.6, n = 7 *vs.* 7.7 ± 1.9, n = 28; $P < 0.05$).

Sensitivity and Specificity of Urinary NGAL after Cardiac Surgery

We generated receiver operating characteristic curves for urinary NGAL 18 h after surgery (fig. 3) and for peak urinary NGAL and immediately postoperatively and 1, 3, and 24 h later (not shown). The areas under the curve are listed in table 2.

Using urinary NGAL as a test for acute renal dysfunction, the test performed fairly (area under the curve, 0.7–0.8) at 18 h (area under the curve, 0.80 [0.573–1.027]; $P = 0.017$). The best threshold at 18 h was 213 ng/ml. We calculated sensitivity, specificity, and positive and negative predictive values for this best threshold at 18 h at 213 ng/ml (table 3).

Discussion

In this study, we showed that renal dysfunction after cardiac surgery is associated with increased urinary NGAL levels after CPB. Urinary NGAL was increased significantly immediately after CPB in all patients compared with preoperatively, but patients who later developed acute renal dysfunction demonstrated a sustained increase of urinary NGAL over the next 24 h with a peak at 3 h. This suggests that early and persistent increase of urinary NGAL concentration after cardiac surgery may correlate with the development of ARD after cardiac surgery.

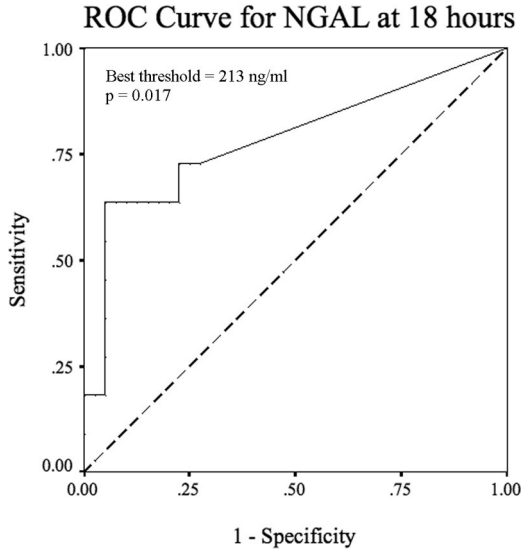

Fig. 3. Receiver operating characteristics (ROC) of urinary neutrophil gelatinase–associated lipocalin (NGAL) to detect acute renal dysfunction 18 h after cardiac surgery. The area under the curve is 0.80 (95% confidence interval, 0.573–1.027). The *dotted line* is the no-discrimination curve.

Renal injury is relatively common after cardiac and aortovascular surgical procedures because of renal hypoperfusion, reperfusion injury, and inflammatory responses. ARD, even if it does not progress to ARF, is associated with significantly increased morbidity and mortality after cardiac surgery[2,3] and increases long-term mortality.[20] Unfortunately, no definitive, early diagnostic marker of ARD exists. ARD is usually diagnosed by monitoring urine output, measuring serum creatinine, measuring blood urea nitrogen, and estimation of creatinine clearance. However, urine output correlates poorly with GFR, and blood urea nitrogen, serum creatinine, and creatinine clearance may be affected by the dilutional effect of increased total body water in the early postoperative period after cardiac surgery. Moreover, it may take several days before the serum creatinine increases to a peak that represents the nadir of GFR.[8] This hampers our ability to intervene at a stage of ARD early

Table 2. Areas under the Curve for the Receiver Operating Characteristics of Urinary NGAL

	AUC (95% CI)	*P* Value
Peak NGAL	0.686 (0.451–0.920)	0.138
NGAL immediately after surgery	0.674 (0.44–0.907)	0.164
NGAL 1 h after surgery	0.68 (0.449–0.911)	0.151
NGAL 3 h after surgery	0.737 (0.504–0.97)	0.059
NGAL 18 h after surgery	0.8 (0.573–1.027)	0.017
NGAL 24 h after surgery	0.68 (0.458–0.902)	0.151

AUC = area under the curve; CI = confidence interval; NGAL = urinary neutrophil gelatinase–associated lipocalin.

Table 3. Sensitivity, Specificity, Positive and Negative Predictive Values, and Accuracy (95% Confidence Intervals) of the Ability of Urinary NGAL to Predict Postoperative Acute Renal Dysfunction*

NGAL > 213 ng/ml	Sensitivity	Specificity	PPV	NPV	Accuracy
Immediately after surgery	0.81 (0.54–0.96)	0.48 (0.36–0.61)	0.28 (0.17–0.43)	0.91 (0.76–0.98)	0.58 (0.49–0.62)
1 h after surgery	0.80 (0.52–0.96)	0.59 (0.46–0.71)	0.32 (0.18–0.49)	0.93 (0.80–0.99)	0.63 (0.54–0.68)
3 h after surgery	0.69 (0.39–0.91)	0.65 (0.52–0.77)	0.29 (0.15–0.49)	0.91 (0.79–0.98)	0.65 (0.57–0.71)
18 h after surgery	0.73 (0.39–0.94)	0.78 (0.62–0.89)	0.47 (0.23–0.72)	0.91 (0.23–0.72)	0.77 (0.65–0.84)
24 h after surgery	0.80 (0.44–0.97)	0.73 (0.54–0.87)	0.47 (0.23–0.72)	0.92 (0.75–0.99)	0.74 (0.62–0.81)
Peak NGAL	0.81 (0.54–0.96)	0.48 (0.25–0.60)	0.28 (0.16–0.43)	0.91 (0.76–0.98)	0.53 (0.46–0.59)

* The cutoff value for urinary neutrophil gelatinase–associated lipocalin (NGAL) is the "best threshold" at 18 h postoperative = 213 ng/ml.
NPV = negative predictive value; PPV = positive predictive value.

enough to potentially prevent its progression to established ARF, with its even higher morbidity and mortality.

Human NGAL was originally identified as a 25-kd protein covalently bound gelatinase from human neutrophils.[21] NGAL is expressed in very low levels in several human tissues, including the kidney, trachea, lung, and colon. However, NGAL concentration in renal proximal tubules increases rapidly after renal ischemia–reperfusion injury.[10,12] Initially, gene chip array studies identified renal NGAL mRNA to be up-regulated more than 10,000-fold at 24 h after renal ischemia.[12] A consistent 3- to 4-fold up-regulation of NGAL mRNA has been found in proximal tubules as early as 2–3 h after injury.[12] NGAL protein was detected in the urine even when the ischemic injury was very mild (5 min of renal artery occlusion). Recently, Mori *et al.* [11] demonstrated that NGAL levels correlate with septic as well as nonseptic ARF in humans. The reason for NGAL up-regulation is not clear, but it may represent a defense mechanism to protect against tubular cell death and preserve renal function, because exogenous NGAL protects against murine ischemia–reperfusion injury.[11] A recent study in pediatric cardiac surgery patients showed that urinary NGAL is highly predictive of postoperative ARD in this patient population.[12]

Our study extends these preclinical and clinical findings to describe an association of urinary NGAL release after adult cardiac surgery with the incidence of ARD. We were able to show that patients who developed postoperative ARD had significantly higher urinary NGAL and could be differentiated from patients who did not develop ARD within 1 h of admission to the ICU. Urinary NGAL peaked at 3 h after surgery and continued to be significantly increased at 18 h after cardiac surgery in patients with postoperative ARD. In contrast, in patients who did not develop ARD, NGAL levels peaked immediately after CPB and declined rapidly thereafter. Although net urinary NGAL levels were equivalent immediately after CPB, the urinary NGAL:urinary creatinine ratio was significantly higher in ARD patients, suggesting that it might be detected even at this very early stage when using NGAL:urinary creatinine ratio as a marker.

Neutrophil gelatinase–associated lipocalin is a polypeptide that is not degraded by proteases and can be stored without significant degradation until detection. Our study suggests that measurement of urinary NGAL concentration may be a clinically practical early biomarker of ischemic renal injury. Recently, an enzyme-linked immunosorbent assay for human NGAL has become commercially available (Antibodyshop, Gentofte, Denmark) that would further increase the sensitivity as well as reduce the assay time for detection for urinary NGAL.

We defined postoperative ARD as an increase in peak serum creatinine (Δ Cr_{max}) by more than 50% compared with preoperative values. In large outcome studies, Δ Cr_{max} correlates with postoperative renal dysfunction, dialysis-dependent acute renal failure, ICU and hospital duration of stay, and in-hospital mortality.[3,22] The Second International Consensus Conference of the Acute Dialysis Quality Initiative Group recommended this definition as the criteria of "risk of renal failure" as part of the Risk–Injury–Failure–Loss–End-stage renal disease classification.[13]

Our definition of ARD (an increase in serum creatinine greater than 50%) represents a significant impairment of GFR in the ARD group. The NGAL concentrations were significantly different 1 h after surgery, whereas serum creatinine peaked only at postoperative day 4 (fig. 1D). This is clinically important, because increases in postoperative serum creatinine of as little as 0.3 mg/dl within the first 48 h (or decreases in creatinine clearance by as little as 10%) are associated with significantly increased ICU and hospital duration of stay and morbidity and mortality after cardiac surgery[3,22] and may increase the probability of developing renal failure at a later point. Therefore, early recognition and therapy of ARD could potentially have a positive impact on outcome.

There are a number of limitations in this study. It was insufficiently powered to establish a direct correlation of urinary NGAL with the probability of postoperative renal dysfunction or other clinical outcomes. We were not able to precisely determine a cutoff value for urinary NGAL that is predictive for ARD. We did not directly measure creatinine clearance, which reflects GFR. Although we may infer GFR from serum creatinine, this does not accurately reflect GFR. Nevertheless, we showed conclusively that the 16 of 81 patients who developed ARD (defined as creatinine increase by more

than 50%) after cardiac surgery had on average higher NGAL levels than patients without postoperative renal dysfunction. We did not attempt to distinguish increases in serum creatinine related to prerenal states *versus* intrarenal injury. This concept is intriguing because patients with congestive heart failure may be prerenal as a consequence of preoperative diuretic therapy and fluid restriction, as well as inadequate blood flow to the kidney. Perioperative volume loading may reverse or obscure a prerenal state; however, patients who have inadequate cardiac output after surgery may remain prerenal—indeed, postoperative cardiac function is a major determinant of renal outcome. Unfortunately, the most convenient indices of a prerenal state (urinary osmolality, sodium, fractional secretion of sodium) are rendered uninterpretable by the ubiquitous use of saluretic agents in the perioperative period. Moreover, prerenal states may precede or develop into acute renal injury, so NGAL may predict those patients at risk whether or not the initial state is "prerenal." The ultimate determinant is outcome, which we are currently testing in a follow-up study involving a larger number of patients at our institution.

In conclusion, our study demonstrated that increased urinary NGAL concentrations are associated with an increased occurrence of postoperative acute renal dysfunction in adult cardiac surgical patients. It is possible that urinary NGAL could provide a screening tool for renal injury immediately after cardiac surgery, and conceivably in other high-risk procedures such as renal transplantation or aortovascular surgery. This in turn might allow appropriate intervention in the narrow therapeutic window between ARD and ARF.

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