# Urinary Biomarkers in the Early Detection of Acute Kidney Injury after Cardiac Surgery

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**Background and objectives: Serum creatinine (Scr) does not allow for early diagnosis of acute kidney injury (AKI). The diagnostic utility of urinary kidney injury molecule-1 (KIM-1), N-acetyl-β-D-glucosaminidase (NAG), and neutrophil gelatinase associated lipocalin (NGAL) was evaluated for the early detection of postoperative AKI in a prospective study of 90 adults undergoing cardiac surgery.**

**Designs, setting, participants, & measurements: Urinary KIM-1, NAG, and NGAL were measured at 5 time points for the first 24 h after operation and normalized to the urinary creatinine concentration after cardiac surgery. Receiver-operating characteristic curves were generated and the areas under the curve (AUCs) compared for performance of biomarkers in detection of postoperative AKI.**

**Results: Thirty-six patients developed AKI, defined as an increase in Scr of ≥0.3 mg/dl within 72 h after surgery. The AUCs for KIM-1 to predict AKI immediately and 3 h after operation were 0.68 and 0.65; 0.61 and 0.63 for NAG; and 0.59 and 0.65 for NGAL, respectively. Combining the three biomarkers enhanced the sensitivity of early detection of postoperative AKI compared with individual biomarkers: the AUCs for the three biomarkers combined were 0.75 and 0.78. The performance of combining biomarkers was even better among 16 early postoperative AKI patients with AUCs of 0.80 and 0.84, respectively.**

**Conclusions: The results of this study support that a combination of urinary biomarkers may allow for early detection of postoperative AKI after cardiac surgery before a rise in Scr.**

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Acute kidney injury (AKI) is an important cause of morbidity and mortality in hospitalized patients (1). The incidence of hospital-acquired AKI varies from 5% in patients with normal preoperative renal function to 25% in intensive care unit (ICU) patients (2-4). Mortality rates of patients with postoperative AKI range from 40 to 60% among ICU patients who require a renal replacement therapy (5-7). Dialysis remains the only U.S. Food and Drug Administration approved treatment option for established AKI.

Recently, two new definitions of AKI have been developed: RIFLE (risk, injury, failure, loss, and ESRD) and AKIN (acute kidney injury network) criteria (8,9). It is, however, a challenge to detect AKI in a timely fashion with current RIFLE and AKIN criteria because these definitions are entirely based on increases of serum creatinine (Scr) or decreases of urine output. Scr is insensitive for the early detection of AKI because the change in Scr does not discriminate the time and type of renal insult or the site and extent of glomerular or tubular injury (8,10).

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Several proteins and biochemical markers emerged as sensitive and specific biomarkers capable of detecting acute tubular injury early and proved to be promising biomarkers as indicators of AKI in recent human studies (11,12). These include N-acetyl-β-D-glucosaminidase (NAG), neutrophil gelatinase associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), cystatin C, and IL-18 (13-25). However, there are no previously reported data for the temporal expression pattern of urinary KIM-1 and NAG before the development of AKI in adult patients. In addition, there is a large discrepancy in the performance of NGAL as an early AKI marker between adult and pediatric AKI patients (15,17,19,20). Furthermore, there are few data on the influence of prolonged duration of storage at −80°C and the number of freeze-thaw cycles on stability of urinary biomarkers at the present time, which will be critical to ongoing validation studies of potential tandem AKI biomarkers (26).

In this study, we studied the diagnostic utility of urinary KIM-1, NAG, and NGAL as biomarkers separately and in combination for the early detection of postoperative AKI and the temporal expression patterns of urinary biomarkers before the development of postoperative AKI in patients undergoing cardiac surgery. In addition, the stability of urinary KIM-1, NAG, and NGAL were tested with various handling and storing conditions to evaluate the accuracy of the biomarker measurement because our prospective collected urine samples were



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frozen at −80°C for 1 yr and were subjected to at least two freeze-thaw cycles before measurement for KIM-1 and NAG.

## Materials and Methods

### *Patient Selection*

All adult patients undergoing cardiac surgery at Columbia University Medical Center (New York, NY) were eligible for enrollment. A total of 90 patients were prospectively studied from June 25, 2005 to January 11, 2006. Exclusion criteria for the study were patients who depended on chronic dialysis support and patients who died within the first 24 h after surgery. Postoperative AKI was defined by using modified Stage 1 and Stage 2 categories of AKIN criteria, which is an absolute increase in Scr of ≥0.3 mg/dl from baseline or an absolute increase in Scr of 2- to 3-fold from baseline within the first 72 h after cardiac surgery, respectively (9). In a subgroup analysis, early postoperative AKI was defined as an absolute elevation in Scr of ≥0.3 mg/dl from baseline within the first 24 h after operation. Late postoperative AKI was defined as absolute elevation in Scr of ≥0.3 mg/dl from baseline between 24 and 72 h after operation.

To evaluate the effect of handing of urine sample at the time of collection, protease inhibitor, prolonged freezing, and repeated freeze-thaw cycles before biomarker measurement, additional urine samples were collected from 15 established AKI patients seen for renal consultation at Brigham and Women's Hospital and Thomas Jefferson University Hospital (TJUH).

The preoperative GFR was estimated using the method described by Cockcroft and Gault (27). A total 20 patients (12 non-AKI and 8 AKI patients) had underlying chronic kidney disease with estimated GFR ≤60 ml/min, according to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative guidelines (28). ICU- and hospital-free days within 30 d were defined as the number of days a patient was outside of the ICU or hospital within the first 30 postoperative days and were zero if the patient died in the ICU or in the hospital, respectively. The institutional review boards of TJUH, Brigham and Women's Hospital, and Columbia University approved the study.

### *Urine and Serum Sample Collections and Storage*

Ten milliliters of urine were collected at the following time points: (1) preoperative; (2) immediately after cardiopulmonary bypass (CPB), or, in case of off-pump coronary artery bypass grafting, after reperfusion of the last bypass graft; and (3) 3, 18, and 24 h later. A total of 429 urine samples were collected from 36 patients with AKI and 54 patients without AKI. The urine samples were centrifuged at 1000 *g* for 5 min and the supernatants were stored at −20°C for up to 20 mo, then stored at −80°C. Scr was measured at baseline, immediately postoperative, and then at least daily during the hospital stay.

Urinary NGAL was measured within the first 3 mo without repeated freeze-thaw cycles. The time between initial urine collection and assays for NAG and KIM-1 at TJUH was about 1.5 yr. Urine samples were subjected to two freeze-thaw cycles before assays for KIM-1 and NAG at TJUH.

### *Stability of Urinary Biomarkers*

Thirty milliliters of freshly voided urine samples from established AKI patients were collected and centrifuged at 1000 *g* for 5 min. The supernatant was aliquoted into ten eppendorf tubes and processed in varying manners for comparisons. The reference eppendorf tubes were frozen at −80°C within the first 20 min after collection. Other urine samples were allowed to sit at room temperature or refrigerated at 4°C for 2, 4, 6, and 24 h before freezing at −80°C. Biomarkers were measured within 2 wk after initial urine collection and then remeasured after prolonged freezing at −80°C for 1 and 2 yr with and without repeat freeze-thaw cycles. To determine the need for protease inhibitor, reference eppendorf tubes with and without addition of a protease inhibitor cocktail tablet (complete mini, Roche, Mannheim, Germany) were frozen at −80°C soon after collection.

In addition, we performed comparative stability studies to evaluate the effect of prolonged storage at −20 *versus* −80°C using duplicate urine samples that were stored at −80 and −20°C for 2 yr, respectively.

### *Measurement of Urinary KIM-1, NAG, and NGAL*

Urinary soluble KIM-1 protein was quantified by a modified ELISA system using polyclonal KIM-1 antibodies as described previously (14). Fifty microliters of urine sample were used for detection of urinary KIM-1 protein. Urinary NAG was measured by colorimetric assay by using a commercially available kit (Roche Applied Science, Indianapolis, IN) (14) according to the manufacturer's protocol. Urinary NGAL concentration was measured using a commercially available ELISA kit (Antibody Shop, Gentofte, Denmark). Urine samples were diluted to 1:50 and 1:5000 dilutions before performing an ELISA assay to fit the concentrations of respective NGAL protein in the linear range of the standard curve. The measured values were normalized to the urinary creatinine concentration. The inter- and intraassay coefficients of variation for KIM-1, NAG, and NGAL were <10%. The measurements were made in duplicate and in a blinded fashion.

### *Serum and Urinary Creatinine Determination*

Scr and urine creatinine concentrations were measured by the Jaffé assay using Roche/Hitachi 917 systems (Roche Diagnostics, Indianapolis, IN) and Creatinine Companion (Exocell, Philadelphia, PA) (29), respectively.

### *Statistical Analyses*

Continuous variables were compared using the *t* test or the nonparametric Mann–Whitney U test, as appropriate. For analysis of single biomarkers, receiver-operating characteristic (ROC) curves were generated and the areas under the curve (AUCs) calculated and compared using the method of Hanley and McNeil for comparison for a single time point (30). Two-tailed *P* values <0.05 were considered statistically significant. For joint analysis of multiple biomarkers, a fitted multiple logistic regression model was used to give maximum sensitivity and specificity (Table 1). Statistical analyses were performed using SAS Version 9.1 (SAS Institute, Cary, NC) and MedCalc Version 9.3.1 (MedCalc, Inc., Mariakerke, Belgium).

## Results

### *Stability of Urinary KIM-1, NAG, and NGAL Proteins*

Urinary KIM-1, NAG, and NGAL proteins did not degrade significantly up to 24 h at 4°C and at room temperature, and up to 1 yr in prolonged storage at −80°C with repeat freeze and thaw cycles (Table 2A). An addition of protease inhibitor was not necessary to prevent degradation of urinary KIM-1, NAG, and NGAL proteins. In addition, urinary NAG remained stable even after 2 yr of prolonged storage at −80°C with repeat freeze-thaw cycles. There was no significant degradation of urinary NAG and KIM-1 proteins after prolonged storage at −20°C for 2 yr (Table 2B), but urinary NGAL protein degraded significantly.

*Table 1.* Formula for fitted multiple logistic regression model for combining biomarkers[a]

| | |
|---|---|
| For AKI ($n = 36$) *versus* all non-AKI ($n = 54$) combined | |
| pre-OP | $[-0.9596 + 0.01386 \times NAG + 0.3102 \times KIM1 + 0.0006661 \times NGAL]$ |
| immediately post-OP | $[-2.0269 + 0.001718 \times NAG + 0.7716 \times KIM1 + 0.0001331 \times NGAL]$ |
| 3 h post-OP | $[-2.0673 + 0.01182 \times NAG + 1.0965 \times KIM1 + 0.00009982 \times NGAL]$ |
| 18 h post OP | $[-1.6097 + 0.03977 \times NAG + 0.2894 \times KIM1 + 0.0005103 \times NGAL]$ |
| 24 h post-OP | $[-0.5933 + 0.007838 \times NAG + 0.06978 \times KIM1 + 0.0008006 \times NGAL]$ |
| For early AKI ($n = 16$) *versus* all non-AKI ($n = 54$) combined | |
| pre-OP | $[-2.0360 - 0.0005436 \times NAG + 0.5281 \times KIM1 + 0.0008857 \times NGAL]$ |
| immediately post-OP | $[-3.3182 + 0.00212 \times NAG + 1.088 \times KIM1 + 0.00009552 \times NGAL]$ |
| 3 h post-OP | $[-3.4557 + 0.01294 \times NAG + 1.4386 \times KIM1 + 0.0001215 \times NGAL]$ |
| 18 h post-OP | $[-3.8619 + 0.06381 \times NAG + 0.5328 \times KIM1 + 0.0008294 \times NGAL]$ |
| 24 h post-OP | $[-2.2619 - 0.01722 \times NAG + 0.2097 \times KIM1 + 0.0007784 \times NGAL]$ |
| For late AKI ($n = 20$) *versus* all non-AKI ($n = 54$) combined | |
| pre-OP | $[-1.3241 + 0.01832 \times NAG + 0.1329 \times KIM1 + 0.0003802 \times NGAL]$ |
| immediately post-OP | $[-2.4918 + 0.002208 \times NAG + 0.6224 \times KIM1 + 0.0001534 \times NGAL]$ |
| 3 h post-OP | $[-2.4066 + 0.01427 \times NAG + 0.977 \times KIM1 + 0.00006504 \times NGAL]$ |
| 18 h post-OP | $[-1.3878 + 0.03679 \times NAG + 0.08044 \times KIM1 + 0.00009971 \times NGAL]$ |
| 24 h post-OP | $[-0.5948 - 0.0148 \times NAG - 0.2848 \times KIM1 + 0.0008516 \times NGAL]$ |

[a]AKI, acute kidney injury; OP, open-heart operation; KIM-1, kidney injury molecule-1; NAG, N-acetyl-$\beta$-D-glucosaminidase; NGAL, neutrophil gelatinase associated lipocalin.

*Table 2A.* Stability of urinary biomarkers[a]

| Collection and Processing | Mean Percentage Difference (95% confidence interval of mean % difference) when Compared to Immediate Storage at −80°C | | |
|---|---|---|---|
| | KIM-1, $n = 6$ | NAG, $n = 6$ | NGAL, $n = 6$ |
| Remain at RM[b] for 2 h, then storage at −80°C | −0.26 (−5.52 to 5.01) | 0.05 (−3.09 to 3.20) | −2.45 (−6.54 to 1.64) |
| Remain at RM for 4 h, then storage at −80°C | 1.43 (−5.39 to 8.25) | 0.48 (−3.19 to 4.15) | −5.3 (−14.89 to 4.30) |
| Remain at RM for 6 h, then storage at −80°C | −3.97 (−10.80 to 2.86) | 0.48 (−2.42 to 3.38) | 2.54 (−7.86 to 12.93) |
| Remain at RM for 24 h, then storage at −80°C | −6.52 (−11.73 to −1.30) | −0.43 (−1.46 to 0.60) | −9.78 (−22.55 to 3.00) |
| Remain at 4°C for 2 h, then storage at −80°C | 1.25 (−4.98 to 7.48) | 0.04 (−4.10 to 4.17) | −3.97 (−9.91 to 3.77) |
| Remain at 4°C for 4 h, then storage at −80°C | 0.43 (−5.37 to 6.23) | 2.38 (−1.75 to 6.51) | 6.65 (0.46 to 12.84) |
| Remain at 4°C for 6 h, then storage at −80°C | 0.16 (−5.61 to 5.92) | 2.07 (−0.69 to 4.83) | −5.19 (−13.47 to 3.09) |
| Remain at 4°C for 24 h, then storage at −80°C | −2.33 (−7.94 to 3.27) | 2.26 (−1.90 to 6.42) | −1.01 (−14.52 to 12.5) |
| Protease inhibitor *versus* no protease inhibitor | 1.23 (−3.76 to 6.23) | −0.21 (−4.53 to 4.11) | −1.51 (−7.23 to 4.20) |
| Prolonged storage at −80°C | | | |
| 1 yr with 3 repeat freeze and thaw cycles | −0.53 (−8.57 to 7.50) | 4.96 (1.51 to 8.41) | −2.43 (−13.53 to 8.66) |
| 2 yr with 3 repeat freeze and thaw cycles | NA | 2.13 (−4.47 to 8.72) | NA |

[a]Immediate storage at −80°C and place urine samples at −80°C within first 20 min after initial collection.
[b]RM, room temperature; NA, not applicable.

*Patient Characteristics for Cardiac Surgery*

The clinical and demographic characteristics of 90 adult patients are reported in Table 3. Thirty-six patients developed postoperative AKI (40%), of which 32 patients developed Stage 1 and the remaining four patients with Stage 2 AKIN criteria AKI. Early AKI was diagnosed in 16 patients within the first 24 h after surgery. Late AKI was diagnosed in 15 patients between 24 and 48 h and in the remaining 5 patients between 48 and 72 h after surgery. There were 26 (72%) of 36 AKI patients who maintained persistent elevations of Scr over 48 h after an absolute increase in Scr of ≥0.3 mg/dl from baseline. There were 14 (88%) early and 12 (60%) late postoperative AKI pa-

*Table 2B.* Stability of urinary biomarkers at prolonged storage at −20°C

| Collection and Processing | Mean Percentage Difference (95% confidence interval of mean % difference) | | |
|---|---|---|---|
| | KIM-1, $n = 32$ | NAG, $n = 32$ | NGAL, $n = 32$ |
| Prolonged storage at −20 and −80°C | | | |
| 2 yr with 3 repeat freeze and thaw cycles (−20 *versus* −80°C) | −15.08 (−21.26 to −8.91) | −8.88 (−16.03 to −1.74) | −40.24 (−52.67 to −27.82) |
| compared with initial biomarker levels 2 yr before at −20°C | −6.75 (−16.99 to 3.49) | −6.11 (−14.44 to 2.22) | NA |

tients who maintained persistent elevations of Scr over 48 h after an absolute increase in Scr of ≥0.3 mg/dl from baseline. No differences were noted between patients with and without AKI with respect to age or sex. CPB time was significantly longer in patients with AKI ($P < 0.001$). The increases in Scr on postoperative days were statistically significantly different from the baseline and non-AKI groups ($P < 0.05$). One early AKI patient required continuous renal replacement therapy. Five patients with postoperative AKI died in the hospital.

### *Quantitation of Serial Urinary KIM-1, NAG, and NGAL Proteins*

Comparisons of median and mean biomarker levels among patients with and without postoperative AKI are shown in Table 4 and Figure 1. Urinary KIM-1 levels were significantly elevated immediately after surgery and 3 h later ($P < 0.005$) and remained elevated 24 h later ($P < 0.05$) in the early postoperative AKI group. However, urinary KIM-1 levels were not higher in the late postoperative AKI group compared with the non-AKI group. Urinary NGAL and NAG levels peaked immediately after operation and then decreased in both AKI and non-AKI groups at later time points. Urinary NGAL levels were higher in the late postoperative AKI group ($P < 0.05$), but not in the early postoperative AKI group. Urinary NAG levels were elevated without statistical significance in both early and late postoperative AKI groups.

We observed a decrease of Scr immediately after operation in most AKI and non AKI patients. None of the AKI patients had an absolute increase in Scr of ≥0.3 mg/dl (or ≥30% delta Scr) from baseline immediately after surgery.

### *ROC Analysis of Urinary NAG, KIM-1, and NGAL Independently and Combined as Early Biomarkers for Detection of Postoperative AKI*

The performance of KIM-1, NAG, and NGAL in diagnosing postoperative AKI is illustrated in Table 5. Immediately after surgery and 3 h after the operation, the AUCs for the diagnosis of postoperative AKI using KIM-1 were 0.68 and 0.65; 0.61 and 0.63 for NAG; 0.59 and 0.65 for NGAL; and 0.75 and 0.78, respectively, for the combination of the three biomarkers. Subgroup analysis of patients with early postoperative AKI, immediately and 3 h after operation, showed AUCs for the diagnosis of AKI using KIM-1 were 0.79 and 0.73; 0.60 and 0.59 for NAG; 0.51 and 0.58 for NGAL; and 0.80 and 0.84, respectively, for the combination of the three biomarkers. Table 6 demonstrates the sensitivity, specificity, and positive and negative likelihood ratio immediately and 3 h after operation for diagnosis of AKI.

The performance difference between the three combined biomarkers and some of the individual biomarkers by AUC for diagnosis of early AKI was statistically significantly different at immediately and 3 h after operation ($P < 0.05$), but there was no added value for diagnosis of late AKI.

## Discussion

It is critical to detect AKI in a timely fashion to initiate rapid interventions. Previous animal studies clearly demonstrated that the treatment of AKI should be started well before the rise of Scr and very early after the insult (31-34). Sensitive biologic markers of renal tubular injury are needed to detect early AKI because current AKI diagnosing and staging criteria are entirely based on an increase in Scr or decrease in urine output. Scr is unacceptably insensitive and increases too slow, and urine output is affected by the use of diuretic or prerenal azotemia. In this study, we demonstrate that: (1) urinary KIM-1, NAG, and NGAL are elevated well before an increase in Scr in adult patients undergoing cardiac surgery; (2) combining biomarkers allows early detection of postoperative AKI before a rise of Scr with improved sensitivity; and (3) prolonged duration of storage at −80°C and several freeze-thaw cycles do not have a significant effect on the stability of urinary KIM-1, NAG, and NGAL proteins.

Recently, urinary NGAL, NAG, and KIM-1 emerged as sensitive biomarkers capable of detecting injury early in pediatric patients. Mishra *et al.* (15) reported that urinary NGAL was found to have an AUC-ROC of 0.99 at 2 h and 1.00 at 4 h after operation in 71 children undergoing CPB. A subsequent study using all of the AKI cases and under half of the non-AKI cases showed urinary KIM-1 and NAG had an AUC-ROC of 0.83 and 0.69 at 12 h, respectively (14). There were, however, no patients who required dialysis or died. Bennet *et al.* (19) reported that urine NGAL was maximally induced at 6 h after cardiac sur-

*Table 3.* Patient's characteristic and clinical outcome[a]

| Characteristic | AKI ($n$ = 36) | Early AKI[b] ($n$ = 16) | Late AKI ($n$ = 20) | Non-AKI ($n$ = 54) |
|---|---|---|---|---|
| Age (yr) | 68.31 ± 2.30 | 65.41 ± 4.16 | 70.64 ± 2.45 | 60.39 ± 1.98 |
| Gender (male/female) | 23/13 | 9/7 | 14/6 | 38/16 |
| Scr (mg/dl) | | | | |
| baseline (pre-OP) | 1.06 ± 0.06 | 1.10 ± 0.11 | 1.02 ± 0.05 | 1.02 ± 0.04 |
| post-OP (immediately) | 0.99 ± 0.05[d] | 1.09 ± 0.10[d] | 0.93 ± 0.05 | 0.84 ± 0.04 |
| post-OP day 1 | 1.28 ± 0.08[c] | 1.59 ± 0.13[ce] | 1.04 ± 0.05 | 0.91 ± 0.04 |
| post-OP day 2 | 1.51 ± 0.10[c] | 1.66 ± 0.19[c] | 1.40 ± 0.08[c] | 0.92 ± 0.04 |
| post-OP day 3 | 1.52 ± 0.11[c] | 1.55 ± 0.21[c] | 1.50 ± 0.10[c] | 0.91 ± 0.05 |
| post-OP day 4 | 1.54 ± 0.14[c] | 1.50 ± 0.21[c] | 1.57 ± 0.21[c] | 0.93 ± 0.05 |
| post-OP day 5 | 1.57 ± 0.19[c] | 1.39 ± 0.21[d] | 1.76 ± 0.32[c] | 0.92 ± 0.06 |
| Delta Scr change (%) | | | | |
| post-OP (immediately) | −7.94 ± 2.60[d] | −5.71 ± 4.41[d] | −9.50 ± 3.20[d] | −17.41 ± 2.07 |
| post-OP day 1 | 22.78 ± 4.95[c] | 49.38 ± 4.70[ce] | 1.50 ± 3.72[d] | −10.37 ± 2.27 |
| post-OP day 2 | 45.83 ± 5.54[c] | 56.25 ± 10.04[c] | 37.50 ± 5.47[c] | −11.30 ± 2.48 |
| post-OP day 3 | 44.24 ± 6.51[c] | 44.67 ± 10.59[c] | 43.89 ± 8.33[c] | −11.60 ± 3.11 |
| post-OP day 4 | 46.56 ± 11.06[c] | 40.00 ± 10.69[c] | 52.35 ± 18.80[c] | −15.20 ± 4.09 |
| post-OP day 5 | 48.89 ± 15.69[c] | 31.43 ± 10.73[c] | 67.69 ± 30.28[c] | −14.58 ± 4.26 |
| Mean duration of AKI (days) | 3.31 ± 0.40 | 4.13 ± 0.64 | 2.65 ± 0.46 | NA |
| Type of heart operation | | | | |
| CABG-CPB | 6 | 3 | 3 | 11 |
| OPCABG | 4 | 3 | 1 | 7 |
| single valve | 10 | 4 | 6 | 18 |
| multiple valve | 1 | 0 | 1 | 1 |
| CABG + valve | 11 | 3 | 8 | 4 |
| aneurysm (or aneurysm + valve) | 1 | 1 | 0 | 7 |
| other | 3 | 2 | 1 | 6 |
| Intraoperative | | | | |
| number of patients with CPB | 31 (86%) | 12 (75%) | 19 (95%) | 47 (87%) |
| CPB (min) | 147.1 ± 11.6[d] | 151.7 ± 21.1[d] | 144.3 ± 13.9[d] | 113.9 ± 6.7 |
| aortic cross clamp time (min) | 92.2 ± 6.3 | 100.4 ± 12.6 | 86.8 ± 6.4 | 79.6 ± 5.0 |
| Postoperative | | | | |
| ICU-free days within 30 d | 23.7 ± 1.4[c] | 21.3 ± 2.7[c] | 25.6 ± 1.2[d] | 27.8 ± 0.3 |
| hospital-free days within 30 d | 15.5 ± 1.6[c] | 14.2 ± 2.5[c] | 16.6 ± 2.0[d] | 21.5 ± 0.9 |
| renal replacement therapy | 1 | 1 | 0 | 0 |
| mortality | 5 | 3 | 2 | 0 |

[a]Values are means (±SEM).
[b]Early AKI, development of AKI within first 24 h after operation; Late AKI, development of AKI between 24 and 72 h after operation; CPB, cardiopulmonary bypass; CABG-CPB, coronary artery bypass grafting with CPG; OPCABG, off-pump coronary artery bypass grafting; ICU, intensive care unit; Scr, serum creatinine concentration; Delta Scr change, % change in Scr from baseline.
[c]$P < 0.005$ *versus* non-AKI.
[d]$P < 0.05$ *versus* non-AKI.
[e]$P < 0.005$ *versus* late AKI.

gery and declined after that in 196 children undergoing CPB, with an AUC-ROC of 0.99 at 6 h after CPB. There were 4 of 99 AKI patients who required dialysis support. Both studies demonstrated great performance of NGAL for early detection of AKI; however, these studies are restricted to children undergoing cardiac surgery. Patients with chronic kidney disease, diabetes mellitus, and peripheral vascular disease—common comorbidities in adult patients undergoing cardiac surgery—were excluded.

The study presented here showed less impressive performance of urinary NAG, KIM-,1 and NGAL for early detection of AKI when compared with previous studies involving pediatric patients; however, our urinary NGAL finding is similar to existing published studies among adult patients. In a single-

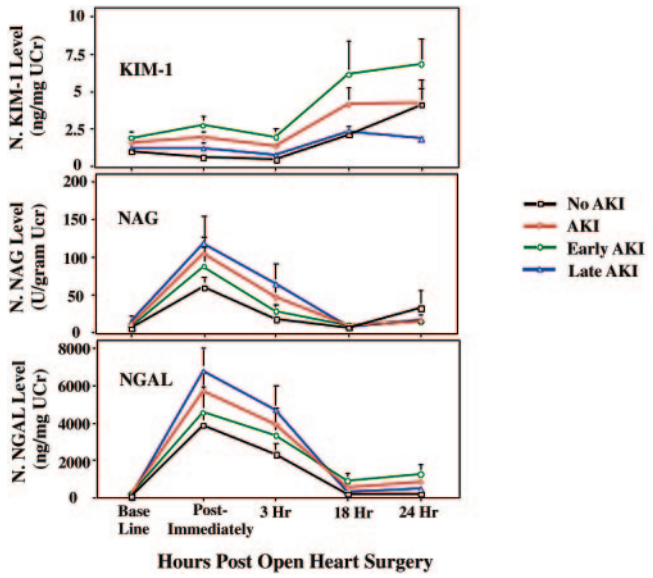


*Figure 1.* Pattern of urinary biomarker expression. Graphs show mean normalized urinary kidney injury molecule-1 (KIM-1), N-acetyl-β-D-glucosaminidase (NAG), and neutrophil gelatinase associated lipocalin (NGAL) concentrations at multiple time points after cardiac surgeries. Error bars are SEM. AKI, acute kidney injury; early AKI, development of acute kidney injury within first 24 h after operation; late AKI, development of acute kidney injury between 24 and 72 h after operation; Ucr, urine creatinine concentration.

center prospective study, a cohort of 426 adult patients, urinary NGAL levels were increased within 1 h after CPB and found to have AUC-ROCs of 0.60 at 3 h and 0.61 at 18 h after CPB (17). Koyner *et al.* (20) also reported that urinary NGAL has AUC-ROCs of between 0.61 and 0.70 at various time points after cardiac surgery in adult patients. For the study presented here, urine samples were diluted up to 1:5000 before performing an NGAL ELISA assay. It is therefore possible that a lack of standardization for the measurement of urinary NGAL biomarker may contribute to the discrepancy between the studies.

How to combine multiple biomarkers for clinical use remains a challenge. In this study, we used multiple logistic regressions to combine three biomarkers and showed added value for early detection of AKI. Thus, the usefulness of this approach remains to be validated in future studies. We are currently testing in a prospective study involving a larger number of adult patients whether a panel of urinary and serum biomarkers enhances the detection of AKI and prognosis for prediction of renal replacement therapy and mortality.

There are several limitations to our observational study. Currently, there is no standard definition for AKI that is not based on a change in Scr. The relatively poor performance of each urinary biomarker for early detection of AKI may have been affected by the definition of AKI. It is possible that some patients developed AKI because of prerenal etiologies or mild acute tubular injury given the rapid reversibility of Scr to baseline in the AKI group. However, persistent increase in Scr alone cannot exclude prerenal azotemia. There were more cases of rapid reversibility of Scr in late postoperative AKI than early postoperative AKI. This may explain why combination of the three biomarkers has better diagnostic performance for detection of AKI in early postoperative AKI when compared with late postoperative AKI. Additional urine collections are needed beyond the first 24 h after operation to validate the role of urinary biomarkers for early detection of late postoperative AKI. A direct comparison of Scr with urinary biomarkers for the detection of AKI is difficult in our study because the Scr was measured two times, whereas urinary markers were measured at up to five time points for the first 24 h after operation. We observed a decrease in Scr right after open-heart surgery due to plenty of fluid infusion in most of AKI and non-AKI patients. However, urinary KIM-1, NAG, and NGAL were already elevated immediately after operation when Scr for all AKI patients ($n = 36$) had not yet changed from baseline. None of the AKI patients had an absolute increase in Scr of ≥0.3 mg/dl from baseline immediately after operation. The clinical importance of elevated levels of KIM-1, NAG, and NGAL during the postoperative period remains uncertain in the non-AKI group. Subclinical alterations in renal function detected by urinary biomarkers have been reported previously without overt changes in Scr (35-37). Because there was no pathologic evaluation of the kidney we cannot relate the biomarkers to injury directly. Our findings indicate that there is a need for a new standard definition of AKI not based on a change in Scr but preferably based on multiple biomarkers and clinical parameters that are able to detect initial renal injury within minutes to hours. Urine samples from 6 and 12 h after cardiac operations were not collected in our study, at which time points a previous study showed KIM-1 and NAG reached peak levels after cardiac surgery in pediatric patients (14). Mishra *et al.* (15) and Bennett *et al.* (19) showed that urine NGAL was maximally induced at 4 to 6 h after cardiac surgery and declined after that in pediatric patients. Comparisons among the different study should be made with caution for a variety of reasons. There is no accepted standard timing of urine collections for the detection of these biomarkers. Our study is based on adult patients with different characteristics compared with pediatric patients. The incidence of comorbidities is much higher in adult cardiac surgery patients, and adult patients undergo a higher variability of surgeries compared with pediatric patients. We recognize that normalization of urinary KIM-1, NAG, and NGAL concentrations to urine creatinine concentration is less than ideal for several reasons, including the nonsteady state of creatinine balance in patients with AKI. However, we analyzed our data using both absolute and normalized values but found no marked difference.

In conclusion, we have demonstrated that a panel of urinary biomarkers may improve the early detection of postoperative AKI. Larger prospective studies are necessary to validate the temporal expression pattern of various urinary biomarkers for early detection of AKI, how to combine multiple biomarkers for early detection of AKI, and how this temporal course relates to the onset, severity, and outcome of AKI. There is also an urgent need for a new standard definition of AKI that is not based on a change in Scr.

*Table 4.* Comparison of various normalized urinary marker levels in patients with AKI and non-AKI[a]

| | Pre-OP | Immediately Post-OP | 3 h Post-OP | 18 h Post-OP | 24 h Post-OP |
|---|---|---|---|---|---|
| Normalized KIM-1 (ng/mg Ucr)[b] | | | | | |
| AKI | 1.1 (0.7 to 1.4) ($n$ = 36) | 1.4 (0.1 to 2.4)[c] ($n$ = 35) | 0.7 (0.0 to 2.1)[d] ($n$ = 28) | 2.6 (1.2 to 4.0) ($n$ = 34) | 2.1 (1.4 to 3.9) ($n$ = 34) |
| early AKI | 1.3 (0.8 to 1.7)[d] ($n$ = 16) | 2.5 (0.2 to 4.5)[cf] ($n$ = 15) | 1.5 (0.0 to 3.6)[d] ($n$ = 12) | 4.3 (1.1 to 8.2)[d] ($n$ = 15) | 4.2 (1.5 to 10.2)[de] ($n$ = 15) |
| late AKI | 0.8 (0.5 to 1.4) ($n$ = 20) | 0.5 (0.0 to 2.0) ($n$ = 20) | 0.4 (0.0 to 1.5) ($n$ = 16) | 2.5 (1.1 to 3.3) ($n$ = 19) | 1.6 (1.1 to 2.2) ($n$ = 19) |
| non-AKI | 0.8 (0.6 to 1.0) ($n$ = 54) | 0.0 (0.0 to 0.6) ($n$ = 54) | 0.0 (0.0 to 0.3) ($n$ = 51) | 1.7 (1.3 to 2.7) ($n$ = 48) | 1.7 (1.0 to 2.7) ($n$ = 42) |
| Normalized NAG (U/gram Ucr) | | | | | |
| AKI | 4.7 (2.7 to 9.6) ($n$ = 36) | 47.9 (28.5 to 93.9) ($n$ = 35) | 19.8 (6.1 to 27.9) ($n$ = 28) | 6.4 (5.3 to 9.2)[d] ($n$ = 34) | 7.6 (4.4 to 13.9) ($n$ = 34) |
| early AKI | 7.6 (1.0 to 16.3) ($n$ = 16) | 37.8 (18.0 to 132.3) ($n$ = 15) | 20.9 (3.7 to 43.6) ($n$ = 12) | 7.7 (5.1 to 12.5) ($n$ = 15) | 7.3 (3.6 to 22.5) ($n$ = 15) |
| late AKI | 4.3 (2.4 to 9.6) ($n$ = 20) | 51.9 (26.2 to 168.8) ($n$ = 20) | 17.7 (5.3 to 97.8) ($n$ = 16) | 5.6 (5.2 to 10.7) ($n$ = 19) | 8.0 (3.5 to 13.9) ($n$ = 19) |
| non-AKI | 4.2 (2.1 to 5.6) ($n$ = 54) | 24.9 (14.5 to 55.6) ($n$ = 54) | 9.2 (5.5 to 16.5) ($n$ = 51) | 4.0 (3.2 to 7.2) ($n$ = 48) | 7.5 (4.7 to 10.2) ($n$ = 42) |
| Normalized NGAL (ng/mg Ucr) | | | | | |
| AKI | 6.1 (2.8 to 12.0) ($n$ = 36) | 4579.0 (1544.8 to 8074.2) ($n$ = 35) | 2071.4 (455.4 to 6211.0)[d] ($n$ = 28) | 71.2 (31.7 to 255.6)[c] ($n$ = 34) | 69.6 (9.9 to 323.1) ($n$ = 34) |
| early AKI | 9.5 (1.4 to 85.1) ($n$ = 16) | 2044.1 (36.0 to 7247.3) ($n$ = 15) | 1153.3 (1.1 to 8000.0) ($n$ = 12) | 70.8 (6.4 to 1418.2)[d] ($n$ = 15) | 73.3 (8.0 to 4752.1) ($n$ = 13) |
| late AKI | 5.4 (2.4 to 13.1) ($n$ = 20) | 7151.1 (1704.2 to 9810.8)[d] ($n$ = 20) | 3041.7 (324.5 to 7457.0)[d] ($n$ = 16) | 75.6 (26.5 to 251.9)[d] ($n$ = 19) | 70.1 (9.1 to 621.6) ($n$ = 19) |
| non-AKI | 3.8 (2.9 to 6.7) ($n$ = 54) | 2308.5 (344.1 to 5044.8) ($n$ = 54) | 130.6 (35.9 to 601.9) ($n$ = 51) | 15.8 (9.0 to 22.0) ($n$ = 48) | 20.9 (13.8 to 31.6) ($n$ = 42) |
| Delta Scr (%) | | | | | |
| AKI | | −10.00 (−10.00 to 0.00)[d] ($n$ = 34) | | | −20.00 (10.00 to 31.45)[c] ($n$ = 36) |
| early AKI | | 0.00 (−20.00 to 7.60)[d] ($n$ = 14) | | | 40.00 (30.00 to 70.00)[c] ($n$ = 16) |
| late AKI | | −10.00 (−20.00 to 0.00) ($n$ = 20) | | | 5.00 (−10.00 to 15.50)[d] ($n$ = 20) |
| non-AKI | | −20.00 (−20.00 to −10.00) ($n$ = 54) | | | −10.00 (−20.00 to 0.00) ($n$ = 54) |

[a]Values are medians (95% confidence interval for median).
[b]Ucr, urine creatinine concentration.
[c]$P < 0.005$ *versus* non-AKI.
[d]$P < 0.05$ *versus* non-AKI.
[e]$P < 0.005$ *versus* late AKI.
[f]$P < 0.05$ *versus* late AKI.

*Table 5.* Areas under the curve (AUCs) for normalized urinary biomarkers at various time points[a]

| | Time after CPB | | | | |
|---|---|---|---|---|---|
| | Pre-OP | Immediately After | 3 h | 18 h | 24 h |
| AKI ($n = 36$) | | | | | |
| KIM-1 | 0.61 (0.51 to 0.72) | 0.68 (0.58 to 0.78) | 0.65 (0.54 to 0.75) | 0.60 (0.49 to 0.71) | 0.59 (0.47 to 0.70) |
| NAG | 0.56 (0.46 to 0.67) | 0.61 (0.50 to 0.71) | 0.63 (0.51 to 0.73) | 0.64 (0.52 to 0.74) | 0.56 (0.44 to 0.67) |
| NGAL | 0.55 (0.45 to 0.66) | 0.59 (0.48 to 0.69) | 0.65 (0.54 to 0.76) | 0.70 (0.59 to 0.80) | 0.59 (0.47 to 0.70) |
| Combined | 0.63 (0.52 to 0.73) | 0.75 (0.65 to 0.84)[b] | 0.78 (0.68 to 0.87)[c] | 0.70 (0.58 to 0.79) | 0.68 (0.56 to 0.78) |
| Delta Scr (%) | | 0.67 (0.56 to 0.77) | | | 0.83 (0.74 to 0.90) |
| Early AKI ($n = 16$) | | | | | |
| KIM-1 | 0.72 (0.60 to 0.82) | 0.79 (0.67 to 0.88)[d] | 0.73 (0.60 to 0.83) | 0.69 (0.56 to 0.80) | 0.71 (0.57 to 0.83) |
| NAG | 0.58 (0.45 to 0.69) | 0.60 (0.47 to 0.71) | 0.59 (0.46 to 0.71) | 0.65 (0.52 to 0.77) | 0.54 (0.40 to 0.68) |
| NGAL | 0.55 (0.42 to 0.67) | 0.51 (0.38 to 0.63) | 0.58 (0.45 to 0.70) | 0.68 (0.56 to 0.79) | 0.64 (0.50 to 0.77) |
| Combined | 0.75 (0.63 to 0.85) | 0.80 (0.69 to 0.89)[b] | 0.84 (0.72 to 0.92)[e] | 0.83 (0.72 to 0.92)[c] | 0.78 (0.65 to 0.88)[f] |
| Delta Scr (%) | | 0.72 (0.60 to 0.82) | | | 1.00 (0.95 to 1.00) |
| Late AKI ($n = 20$) | | | | | |
| KIM-1 | 0.54 (0.42 to 0.65) | 0.61 (0.49 to 0.72) | 0.60 (0.47 to 0.71) | 0.54 (0.41 to 0.66) | 0.54 (0.40 to 0.67) |
| NAG | 0.55 (0.43 to 0.67) | 0.62 (0.51 to 0.74) | 0.65 (0.52 to 0.76) | 0.62 (0.50 to 0.74) | 0.54 (0.41 to 0.67) |
| NGAL | 0.56 (0.44 to 0.67) | 0.66 (0.54 to 0.76) | 0.71 (0.59 to 0.82) | 0.72 (0.60 to 0.82) | 0.62 (0.48 to 0.74) |
| Combined | 0.54 (0.42 to 0.66) | 0.72 (0.60 to 0.82) | 0.74 (0.62 to 0.84) | 0.59 (0.46 to 0.71) | 0.65 (0.52 to 0.77) |
| Delta Scr (%) | | 0.64 (0.52 to 0.75) | | | 0.70 (0.58 to 0.80) |

[a]Values are AUC (95% confidence interval for AUC).
[b]$P < 0.05$ *versus* NGAL, NAG.
[c]$P < 0.05$ *versus* KIM-1, NAG.
[d]$P < 0.05$ *versus* NGAL.
[e]$P < 0.05$ *versus* KIM-1, NAG, NGAL.
[f]$P < 0.05$ *versus* NAG.

*Table 6.* Performance of urinary KIM-1, NAG, and NGAL for diagnosis of AKI at various time points

| AKI ($n = 36$) | Sensitivity | Specificity | Positive Likelihood Ratio | Negative Likelihood Ratio |
|---|---|---|---|---|
| Urinary KIM-1 at Post Immediately | | | | |
| >1.2 ng/mg Ucr | 51.43 | 77.78 | 2.31 | 0.62 |
| >1.8 ng/mg Ucr | 42.86 | 88.89 | 3.86 | 0.64 |
| Urinary KIM-1 at 3 h Post-OP | | | | |
| >1.2 ng/mg Ucr | 35.71 | 90.20 | 3.64 | 0.71 |
| >1.8 ng/mg Ucr | 32.14 | 96.08 | 5.46 | 0.72 |
| Urinary NAG at Post Immediately | | | | |
| >18.5 U/gram Ucr | 82.86 | 44.44 | 1.49 | 0.39 |
| >25.0 U/gram Ucr | 74.29 | 50.00 | 1.49 | 0.51 |
| Urinary NAG at 3 h Post-OP | | | | |
| >18.5 U/gram Ucr | 53.57 | 70.59 | 1.82 | 0.66 |
| >25.0 U/gram Ucr | 28.57 | 80.39 | 1.46 | 0.89 |
| Urinary NGAL at Post Immediately | | | | |
| >456.0 ng/mg Ucr | 71.43 | 38.89 | 1.17 | 0.73 |
| >1900.0 ng/mg Ucr | 65.71 | 50.00 | 1.31 | 0.69 |
| Urinary NGAL at 3 h Post-OP | | | | |
| >456.0 ng/mg Ucr | 71.43 | 62.75 | 1.92 | 0.46 |
| >1900.0 ng/mg Ucr | 50.00 | 70.59 | 1.70 | 0.71 |

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## Disclosures

None.

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