# Urinary N-Acetyl-β-(D)-Glucosaminidase Activity and Kidney Injury Molecule-1 Level Are Associated with Adverse Outcomes in Acute Renal Failure

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**The role of urinary biomarkers of kidney injury in the prediction of adverse clinical outcomes in acute renal failure (ARF) has not been well described. The relationship between urinary N-acetyl-β-(D)-glucosaminidase activity (NAG) and kidney injury molecule-1 (KIM-1) level and adverse clinical outcomes was evaluated prospectively in a cohort of 201 hospitalized patients with ARF. NAG was measured by spectrophotometry, and KIM-1 was measured by a microsphere-based Luminex technology. Mean Acute Physiology, Age, Chronic Health Evaluation II (APACHE II) score was 16, 43% had sepsis, 39% required dialysis, and hospital mortality was 24%. Urinary NAG and KIM-1 increased in tandem with APACHE II and Multiple Organ Failure scores. Compared with patients in the lowest quartile of NAG, the second, third, and fourth quartile groups had 3.0-fold (95% confidence interval [CI] 1.3 to 7.2), 3.7-fold (95% CI 1.6 to 8.8), and 9.1-fold (95% CI 3.7 to 22.7) higher odds, respectively, for dialysis requirement or hospital death ($P < 0.001$). This association persisted after adjustment for APACHE II, Multiple Organ Failure score, or the combined covariates cirrhosis, sepsis, oliguria, and mechanical ventilation. Compared with patients in the lowest quartile of KIM-1, the second, third, and fourth quartile groups had 1.4-fold (95% CI 0.6 to 3.0), 1.4-fold (95% CI 0.6 to 3.0), and 3.2-fold (95% CI 1.4 to 7.4) higher odds, respectively, for dialysis requirement or hospital death ($P = 0.034$). NAG or KIM-1 in combination with the covariates cirrhosis, sepsis, oliguria, and mechanical ventilation yielded an area under the receiver operator characteristic curve of 0.78 (95% CI 0.71 to 0.84) in predicting the composite outcome. Urinary markers of kidney injury such as NAG and KIM-1 can predict adverse clinical outcomes in patients with ARF.**

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Acute renal failure (ARF) is a common occurrence in hospitalized patients (1–3) and is associated with increased morbidity and mortality (4,5). In clinical practice, ARF is detected by an incremental increase in serum creatinine over a defined and relatively short time interval. Unfortunately, changes in serum creatinine occur well after acute kidney injury has been sustained. Recent efforts have focused on the characterization of novel urinary biomarkers for the early detection of acute kidney injury (6–10). This emerging literature has improved our understanding of the value of urinary markers for the early detection of acute kidney injury, before there is a noticeable increase in serum creatinine. Notwithstanding these advances, there is limited knowledge about the prognostic utility of kidney injury markers in patients with established ARF. This is of particular clinical interest because nephrologists traditionally are consulted for ARF once the serum creatinine has already risen and presumably long after acute kidney injury was sustained. In this common clinical setting, the question of whether acute kidney injury has taken place has already been established, and other issues that pertain primarily to management and prognosis arise. In particular, predicting the risk for adverse outcomes in patients with ARF at the time of the initial nephrology consultation has remained a difficult task.

The aim of this study was to test the hypothesis that urinary N-acetyl-β-(D)-glucosaminidase (NAG) activity, a lysosomal brush border enzyme of proximal renal tubular cells and a more established urinary marker of kidney injury, and kidney injury molecule-1 (KIM-1) level, a recently described renal tubular cell dedifferentiation and injury marker (7), are associated with measures of disease severity and with adverse clinical outcomes in patients with established ARF of mixed cause and severity.

## Materials and Methods

### *Study Design*

This was a prospective cohort study of hospitalized patients with ARF, which was conducted between November 2003 and January 2006 at two tertiary care hospitals located in Boston, MA. All consecutive

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hospitalized adult patients who had ARF and for whom nephrology consultation was requested were eligible for enrollment. ARF was defined as an incremental increase in serum creatinine by 0.5, 1.0, or 1.5 mg/dl from a baseline level of ≤1.9, 2.0 to 4.9, and ≥5.0 mg/dl, respectively, as described previously (1). These criteria are graded to require a larger increase in serum creatinine according to the baseline value. Exclusion criteria were age <18 yr, current pregnancy, chronic dialysis therapy, receipt of an organ transplant within the previous year, and presence of acute obstructive uropathy. Patients who were receiving comfort measures only and those for whom therapeutic interventions were considered futile by the caregivers also were excluded. Written informed consent was obtained from all participants or their next of kin. The institutional review board of each participating center approved the study protocol.

### *Data Collection*

Medical records of study participants were reviewed prospectively to retrieve hospitalization data, including baseline demographic characteristics; coexisting conditions; and renal variables including serial serum creatinine values, presence of oliguria (as defined by urine output <400 ml/d), fractional excretion of sodium, and urinary sediment findings. Contributing causes of ARF were determined according to the notes of the nephrology consult service. A blinded investigator (O.L. or R.W.M.) who was unaware of the patient's clinical characteristics examined the urinary sediment. At the time of enrollment, two severity-of-illness scores were calculated: The Acute Physiology and Chronic Health Evaluation II (APACHE II) score (11) and the Multiple Organ Failure (MOF) score (12). The presence of sepsis was ascertained using the systemic inflammatory response syndrome criteria (13). Outcomes of interest were dialysis requirement, hospital death, and the composite outcome of dialysis requirement or hospital death.

### *Processing and Storage of Urinary Samples*

Fresh urinary samples were obtained at the time of enrollment and were centrifuged immediately to remove insoluble elements after routine test-strip urinalysis. The urine sediment was examined by light microscopy and evaluated for the presence or absence of granular casts. The supernatant was treated with a protease inhibitor cocktail tablet (Complete, Mini; Roche Diagnostics, Mannheim, Germany) and stored at −80°C until assayed. This cocktail tablet inhibits a broad spectrum of serine, cysteine, and metalloproteases as well as calpains that are present in mammalian tissues.

### *Measurement of Urinary NAG Activity*

NAG activity was measured in the urine by a colorimetric assay (Boehringer Mannheim, Mannheim, Germany). In brief, this method uses the substrate 3-cresolsulfonphthaleinyl-N-acetyl-β-D-glucosaminidine-sodium, which is hydrolyzed by NAG when present in the urinary sample. This reaction releases 3-cresolsulfonphthalein-sodium, which is measured by spectrophotometry. According to the manufacturer's instructions, 1 ml of the substrate solution was incubated for 5 min at 37°C. A 50-μl aliquot of the urinary sample then was added to the substrate solution, mixed, and incubated for 15 min at 37°C. After incubation, 2 ml of the stop reagent solution that contained sodium carbonate was added to the sample mixture and allowed to stand for 10 min at room temperature. The absorbance then was measured by a spectrophotometer (Beckman Coulter, Fullerton, CA) set at 580 nm. A single measurement was performed per sample. The inter- and intra-assay coefficients of variation were 4.3 and 6.0%, respectively. Results were normalized to urinary creatinine values and expressed in mU/mg creatinine.

### *Measurement of Urinary KIM-1 Level*

Urinary KIM-1 measurements were performed using microsphere-based Luminex xMAP technology with polyclonal antibodies raised against the human KIM-1 ectodomain. This technique is an adaptation of the sandwich ELISA assay, as described previously (7,14,15). For measurements, 30 μl of urine samples were analyzed in duplicate.

The lowest limit of detection for this assay is 0.125 ng/ml. The inter- and intra-assay variability was <10%. The urinary KIM-1 level was expressed in absolute terms and also normalized to the urinary creatinine concentration. Three blinded investigators (M.C.P, W.K.H., and V.S.V.) who were unaware of the patients' clinical characteristics performed all of the urinary biomarker measurements.

### *Statistical Analyses*

Comparisons between urinary NAG and KIM-1 quartiles were made by the Kruskal-Wallis or ANOVA tests, as appropriate, for continuous variables and by $\chi^2$ test for categorical variables. Logistic regression analysis was used to examine the association of urinary NAG activity and KIM-1 level with the composite outcome of dialysis requirement or hospital death. This composite end point was chosen because it takes into consideration hospital survival bias for dialysis requirement. The models were adjusted for either the APACHE II score or the MOF score. These baseline covariates were chosen because the APACHE II score represents a composite illness-severity score that takes into consideration several demographic, physiologic, and laboratory variables and the presence of sepsis, whereas the MOF score represents a composite organ failure score that is characterized by physiologic and laboratory criteria that indicate severe vital organ dysfunction. In a separate model, the analysis was adjusted for the four covariates: Sepsis, oliguria, cirrhosis, and mechanical ventilation. These four variables were selected on the basis of the strength of their univariate associations with the composite outcome. In this model, a backward selection approach was adopted with a $P < 0.2$ as the criterion to retain covariates in the final model.

Restricted cubic splines functions with four knots (*i.e.*, piece-wise cubic polynomials) were used to explore the functional form of the urinary NAG activity and KIM-1 level and graphically display their univariate relationship with the composite outcome of dialysis requirement or hospital death (16). Ninety-five percent confidence intervals for the area under the curves were calculated.

The performance characteristics of urinary NAG activity and KIM-1 level in predicting the composite outcome were described using the area under a receiver operator characteristic (ROC) curve and compared with the performance of more traditional clinical severity indices of kidney injury, including serum creatinine, urine output (using the negative value because of its inverse association with adverse outcomes), and APACHE II score. The serum creatinine at enrollment was chosen to serve as a standard for comparison to test formally whether various models (*i.e.*, differences in the areas under the models' ROC curves) are statistically more or less discriminating, using the nonparametric method of DeLong *et al.* (17).

Results are expressed as means (with SD) or percentages. Figures are graphically displayed as box and whisker plots. Differences were considered statistically significant at $P < 0.05$. All statistical procedures were performed using SAS version 9.1 (SAS Institute, Cary, NC), except for Figures 1 and 2, which were generated using the SPSS version 12.0 (Chicago, IL).

## Results

### *Overall Characteristics of the Cohort*

A total of 201 patients were enrolled. Mean age was 65 yr, 91% were white, and 45% were women. Mean APACHE II

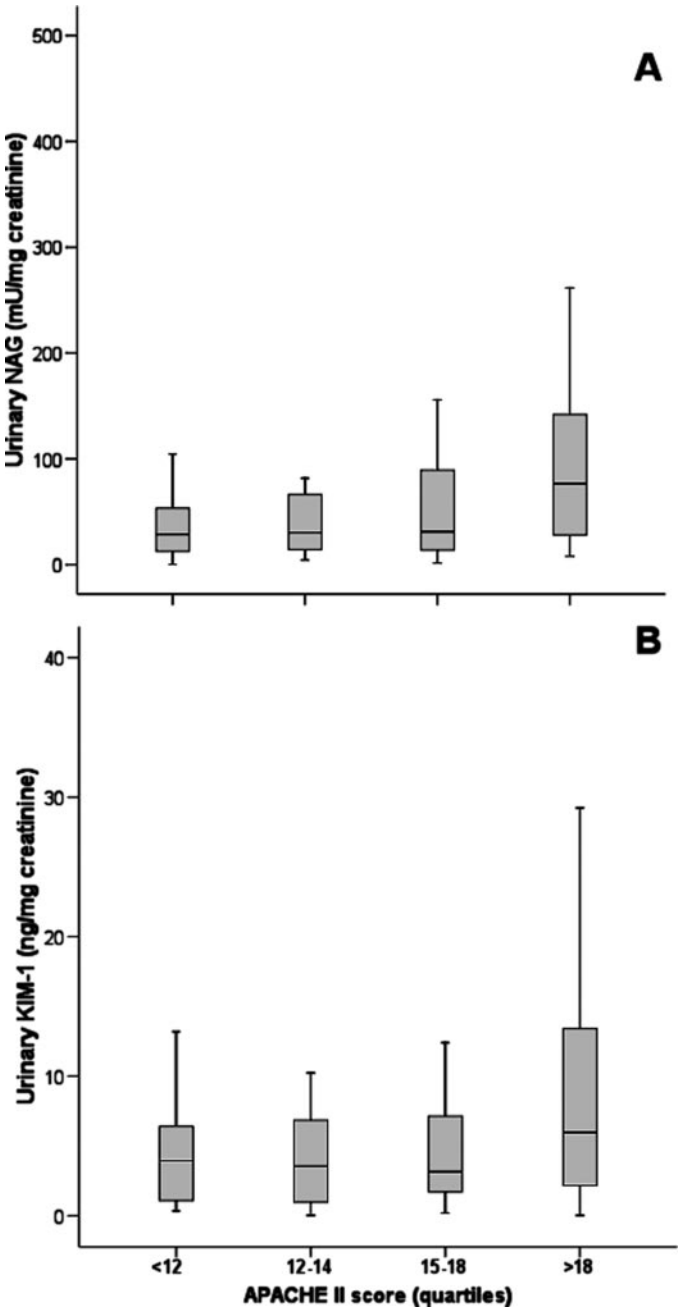


*Figure 1.* Urinary N-acetyl-β-(D)-glucosaminidase (NAG) activity (A) and kidney injury molecule-1 (KIM-1) level (B) stratified by the Acute Physiology and Chronic Health Evaluation (APACHE) II score quartiles. $P < 0.001$ for NAG activity and $P = 0.052$ for KIM-1 level, by the Kruskal-Wallis test. The box and whisker plots show the 10th, 25th, 50th (median), 75th, and 90th percentile values.

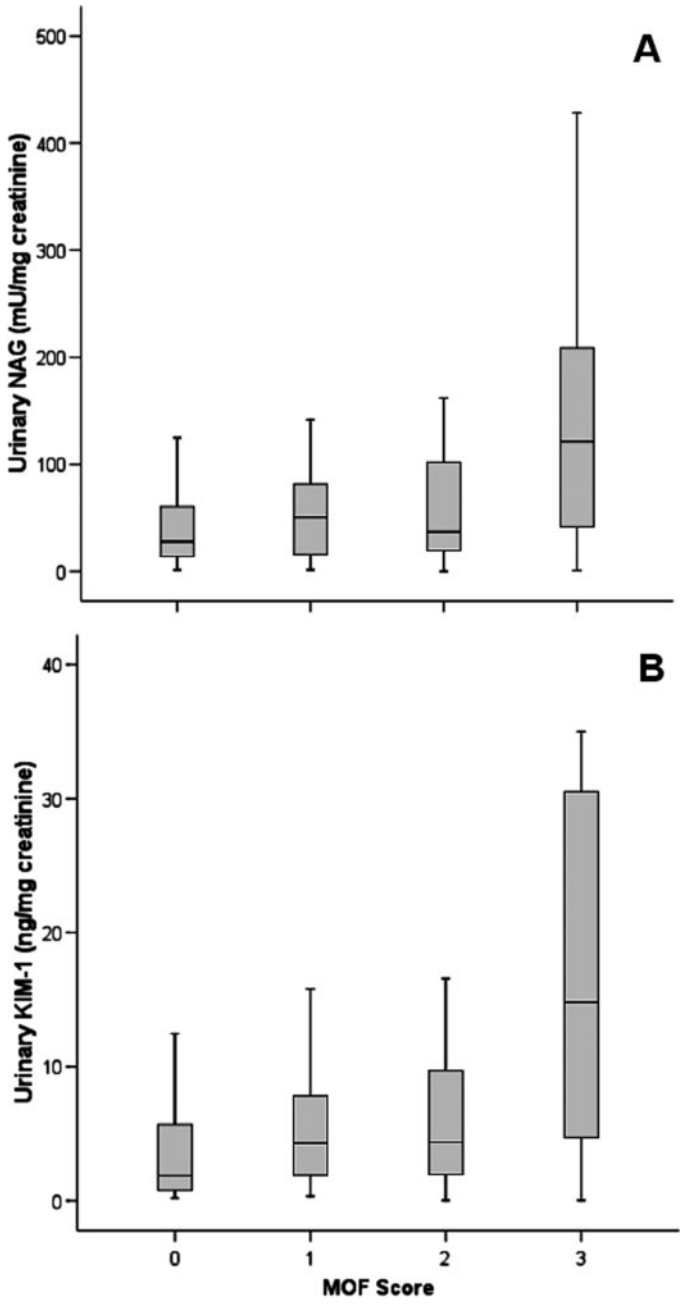


*Figure 2.* Urinary NAG activity (A) and KIM-1 level (B) stratified by the Multiple Organ Failure (MOF) score. $P = 0.003$ for NAG activity and $P = 0.001$ for KIM-1 level, by the Kruskal-Wallis test. The box and whisker plots show the 10th, 25th, 50th (median), 75th, and 90th percentile values.

score was 16, 73% were in the intensive care unit, 43% had sepsis, and 24% required mechanical ventilation. ARF often was the result of multifactorial causes, and 18% of patients were oliguric. Thirty-nine percent required dialysis, and hospital mortality was 24%. Among the survivors, 13% were dialysis dependent at the time of hospital discharge.

### *Characteristics of the Cohort Stratified by Urinary NAG Activity and KIM-1 Level*

Table 1 displays the characteristics of the cohort according to the urinary NAG activity quartiles. At baseline, patients in the highest urinary NAG quartile had a significantly higher prevalence of sepsis, higher APACHE II score, higher MOF score, a higher likelihood of mechanical ventilation, higher prevalence of oliguria, and fractional excretion of sodium >1% as compared with those with lower urinary NAG. Urinary NAG

*Table 1.* Characteristics of the cohort according to the urinary NAG activity quartiles[a]

| Characteristic | Quartile | | | | *P* |
|---|---|---|---|---|---|
| | First ($n = 50$) | Second ($n = 50$) | Third ($n = 51$) | Fourth ($n = 50$) | |
| Age (yr) | 66 (16) | 68 (14) | 64 (14) | 62 (16) | 0.196 |
| Female gender (%) | 34 | 50 | 41 | 54 | 0.180 |
| White ethnicity (%) | 86 | 90 | 90 | 96 | 0.396 |
| Serum creatinine (mg/dl) | | | | | |
| baseline value | 1.5 (0.5) | 1.6 (0.6) | 1.5 (0.6) | 1.6 (0.7) | 0.645 |
| enrollment value | 3.4 (1.7) | 3.7 (1.6) | 3.6 (1.5) | 4.1 (3.1) | 0.320 |
| peak value | 4.0 (2.2) | 4.5 (2.2) | 4.5 (2.1) | 5.1 (4.5) | 0.290 |
| discharge value | 2.6 (2.1) | 2.5 (1.5) | 2.6 (1.9) | 2.5 (1.6) | 0.988 |
| Sepsis (%) | 30 | 46 | 35 | 62 | 0.007 |
| Mechanical ventilation (%) | 16 | 16 | 16 | 50 | <0.001 |
| APACHE II score | 14 (5) | 15 (6) | 16 (6) | 20 (7) | <0.001 |
| MOF score (%) | | | | | |
| 0 | 36 | 38 | 28 | 16 | 0.002 |
| 1 | 44 | 36 | 57 | 40 | |
| 2 | 16 | 22 | 14 | 22 | |
| 3 | 4 | 4 | 2 | 22 | |
| Cause of ARF (%) | | | | | |
| multifactorial | 40 | 34 | 49 | 22 | 0.113 |
| ischemic | 32 | 42 | 28 | 36 | |
| nephrotoxic | 16 | 10 | 18 | 20 | |
| sepsis | 8 | 6 | 6 | 18 | |
| atheroembolic disease | 4 | 8 | 0 | 4 | |
| Oliguria (%) | 10 | 8 | 24 | 29 | 0.017 |
| Urinary FeNa >1% (%) | 43 | 44 | 44 | 75 | 0.003 |
| Urinary granular casts (%) | 66 | 85 | 71 | 82 | 0.126 |
| Dialysis requirement (%) | 14 | 38 | 45 | 60 | <0.001 |
| Hospital death (%) | 12 | 16 | 26 | 42 | 0.002 |
| Dialysis requirement or hospital death (%) | 22 | 46 | 51 | 72 | <0.001 |

[a]Urinary N-acetyl-$\beta$ -(D)-glucosaminidase (NAG) was measured in 201 patients. Data are means (SD) or percentages. APACHE II, Acute Physiology and Chronic Health Evaluation II; ARF, acute renal failure; FeNa, fractional excretion of sodium; KIM-1, kidney injury molecule-1; MOF, Multiple Organ Failure.

activity increased in tandem with APACHE II score quartiles ($P < 0.001$; Figure 1A) and MOF scores ($P < 0.003$; Figure 2A). Dialysis requirement, hospital death, and the composite outcome of dialysis requirement or hospital death also were significantly higher in the urinary NAG fourth quartile patient group as compared with the other quartile groups (Table 1).

Table 2 displays the characteristics of the cohort according to the urinary KIM-1 level quartiles. At baseline, the urinary KIM-1 fourth quartile patient group had significantly higher MOF scores and a higher prevalence of oliguria as compared with the other quartile groups. Urinary KIM-1 level also increased in tandem with APACHE II score quartiles ($P = 0.052$; Figure 1B) and MOF scores ($P = 0.001$; Figure 2B). The composite outcome of dialysis requirement or hospital death also was significantly higher in the urinary KIM-1 fourth quartile patient group as compared with the other quartile groups.

*Association of Urinary NAG Activity and KIM-1 Level with the Composite Outcome of Dialysis Requirement or Hospital Death*

Results of the logistic regression analyses are shown in Table 3. Compared with the NAG first quartile group, the second, third, and fourth quartile groups were associated with 3.0-, 3.7-, and 9.1-fold higher odds for dialysis requirement or hospital death on univariate analysis, respectively. This graded association persisted after adjustment for the APACHE II score; the MOF score; or the combined covariates cirrhosis, sepsis, oliguria, and mechanical ventilation.

Compared with the urinary KIM-1 first quartile group, the second, third, and fourth quartile groups were associated with 1.4-, 1.4-, and 3.2-fold higher odds for dialysis requirement or hospital death on univariate analysis, respectively. This association was attenuated after adjustment for the APACHE II score; the MOF score; or the combined covariates cirrhosis, sepsis, oliguria, and mechanical ventilation.

*Table 2.* Characteristics of the cohort according to urinary KIM-1 level quartiles[a]

| Characteristic | Quartile | | | | *P* |
|---|---|---|---|---|---|
| | First ($n = 49$) | Second ($n = 50$) | Third ($n = 50$) | Fourth ($n = 49$) | |
| Age (yr) | 67 (13) | 70 (13) | 64 (16) | 60 (15) | 0.005 |
| Female gender (%) | 37 | 42 | 44 | 57 | 0.215 |
| White ethnicity (%) | 92 | 84 | 86 | 100 | 0.032 |
| Serum creatinine (mg/dl) | | | | | |
| baseline value | 1.6 (0.6) | 1.6 (0.6) | 1.5 (0.6) | 1.5 (0.7) | 0.540 |
| enrollment value | 3.2 (1.9) | 3.7 (4.7) | 3.7 (1.4) | 4.2 (2.9) | 0.116 |
| peak value | 3.7 (2.4) | 4.3 (1.7) | 4.4 (1.8) | 5.6 (4.6) | 0.016 |
| discharge value | 2.0 (1.1) | 2.6 (1.4) | 2.5 (1.6) | 3.0 (2.6) | 0.051 |
| Sepsis (%) | 31 | 36 | 50 | 53 | 0.069 |
| Mechanical ventilation (%) | 25 | 16 | 24 | 33 | 0.291 |
| APACHE II score | 15 (7) | 16 (5) | 17 (6) | 17 (7) | 0.259 |
| MOF score (%) | | | | | |
| 0 | 53 | 28 | 20 | 16 | <0.001 |
| 1 | 27 | 56 | 52 | 43 | |
| 2 | 14 | 16 | 20 | 23 | |
| 3 | 6 | 0 | 8 | 18 | |
| Cause of ARF (%) | | | | | |
| multifactorial | 35 | 48 | 38 | 27 | 0.311 |
| ischemic | 37 | 24 | 36 | 41 | |
| nephrotoxic | 12 | 18 | 16 | 16 | |
| sepsis | 10 | 6 | 4 | 16 | |
| atheroembolic disease | 6 | 4 | 6 | 0 | |
| Oliguria (%) | 13 | 13 | 12 | 33 | 0.012 |
| Urinary FeNa >1% (%) | 48 | 58 | 39 | 60 | 0.177 |
| Urinary granular casts (%) | 73 | 74 | 80 | 74 | 0.854 |
| Dialysis requirement (%) | 29 | 34 | 40 | 53 | 0.076 |
| Hospital death (%) | 14 | 22 | 24 | 37 | 0.074 |
| Dialysis requirement or hospital death (%) | 37 | 44 | 44 | 65 | 0.029 |

[a]Urinary KIM-1 was measured in 198 patients. Data are means (SD) or percentages.

The results of the restricted cubic splines functions that explored the univariate relationship between urinary NAG activity and KIM-1 level, respectively, with the composite outcome of dialysis requirement or hospital death are displayed in Figure 3. Increasing levels of both NAG and KIM-1 were associated with a heightened probability for the composite outcome but differed in their curve characteristics. Increasing urinary NAG activity at levels <50 mU/mg was associated with steeper increases in probability for the outcome as compared with levels >50 mU/mg, where the direct relationship persisted but in an attenuated manner (Figure 3A). In comparison, KIM-1 levels were associated with the outcome in the pattern of an S-shaped curve with increasing slope below 10 ng/mg, then decreasing slope between 10 and 15 ng/mg, and asymptotic flattening of the slope above 15 ng/mg (Figure 3B). Accordingly, urinary KIM-1 levels >15 ng/mg were not associated with large increases in the predicted probability of the outcome.

### *Prognostic Performance Characteristics of Urinary Biomarkers Compared with Selected Clinical Variables*

Table 4 displays the area under the ROC curve (AUC) of selected clinical and urinary predictor variables for the composite outcome of dialysis requirement or hospital death. Urinary NAG activity performed better than serum creatinine or urine output, two traditional clinical parameters. The APACHE II score, a composite score of 15 clinical variables, provided an AUC of 0.78, which was improved marginally by combining this score with urinary NAG activity (AUC 0.79) or KIM-1 level (AUC 0.80). The combination of urinary KIM-1 level or NAG activity with the four variables hepatic cirrhosis, sepsis, oliguria, and mechanical ventilation performed as well as the 15-variable APACHE II score for prediction of the composite outcome (AUC 0.78). The combination of urinary markers with APACHE II score or the four-variable model yielded areas under the ROC curves that were statistically significantly more discriminating than serum creatinine at enrollment alone (Table 4).

## Discussion

The present study describes the relationship of urinary NAG activity and urinary KIM-1 level with clinical measures of disease severity in patients with ARF. This report also examines the utility of these kidney injury markers for risk stratification

*Table 3.* Association of urinary markers with the composite outcome of dialysis requirement or hospital death[a]

| Predictor Variable | Urinary NAG Activity (OR [95% CI]) | *P* | Urinary KIM-1 Level (OR [95% CI]) | *P* |
|---|---|---|---|---|
| Unadjusted | | | | |
| second quartile group (*versus* first quartile group) | 3.0 (1.3 to 7.2) | <0.001 | 1.4 (0.6 to 3.0) | 0.034 |
| third quartile group (*versus* first quartile group) | 3.7 (1.6 to 8.8) | | 1.4 (0.6 to 3.0) | |
| fourth quartile group (*versus* first quartile group) | 9.1 (3.7 to 22.7) | | 3.2 (1.4 to 7.4) | |
| Adjusted for APACHE II score | | | | |
| second quartile group (*versus* first quartile group) | 3.3 (1.3 to 8.5) | 0.004 | 1.2 (0.5 to 3.0) | 0.113 |
| third quartile group (*versus* first quartile group) | 3.5 (1.4 to 9.0) | | 1.0 (0.4 to 2.6) | |
| fourth quartile group (*versus* first quartile group) | 5.4 (2.0 to 14.6) | | 2.8 (1.0 to 7.4) | |
| Adjusted for MOF score | | | | |
| second quartile group (*versus* first quartile group) | 3.4 (1.3 to 8.7) | <0.001 | 1.2 (0.5 to 2.9) | 0.327 |
| third quartile group (*versus* first quartile group) | 4.3 (1.7 to 10.8) | | 0.9 (0.4 to 2.2) | |
| fourth quartile group (*versus* first quartile group) | 7.2 (2.7 to 19.0) | | 2.0 (0.8 to 4.9) | |
| Adjusted for cirrhosis, sepsis, oliguria, and mechanical ventilation | | | | |
| second quartile group (*versus* first quartile group) | 3.4 (1.3 to 8.9) | 0.009 | 1.3 (0.5 to 3.3) | 0.311 |
| third quartile group (*versus* first quartile group) | 3.2 (1.3 to 8.3) | | 0.9 (0.4 to 2.4) | |
| fourth quartile group (*versus* first quartile group) | 4.8 (1.7 to 13.1) | | 2.1 (0.8 to 5.7) | |

[a]CI, confidence interval; OR, odds ratio.

at a clinically relevant point in the course of ARF, the time of nephrology consultation. The results indicate that in patients with established ARF, urinary NAG activity and KIM-1 level are useful surrogates for the severity of ARF and have a prognostic utility that is similar to or better than conventionally used severity markers, such as the urine output and serum creatinine level. Both urinary NAG activity and KIM-1 level also are associated with the degree of concurrent disease severity, as determined by the general clinical severity measures of APACHE II and MOF score. Moreover, urinary NAG and KIM-1, measured at the time of nephrology consultation, both were associated with the composite outcome of dialysis requirement or hospital death. These findings are of particular relevance because in clinical practice, nephrologists often encounter patients with established ARF, namely after a rise in serum creatinine. A more precise estimation of the severity of ARF and ability to predict adverse clinical outcomes potentially can facilitate the targeted introduction of conventional and novel biologic therapies to patients who are more likely to benefit.

The utility of urinary NAG activity as a marker of acute or chronic kidney injury was described more than 30 yr ago (18). In subsequent years, urinary NAG activity was measured to detect mild, subclinical renal tubular damage (19,20). More recently, tubular enzymuria, including the excretion of urinary NAG, was shown to be of diagnostic value for the early detection of acute kidney injury (21,22). NAG is the most active glycosidase found in proximal tubular epithelial cell lysosomes, and an increase in the urinary activity of this enzyme is a sensitive and reasonably specific measure of renal tubular damage, because its relatively large molecular weight (>130 kD) precludes filtration by the glomerulus (23). Urinary NAG activity remains elevated during the course of active renal disease. On the basis of these attributes, earlier reports proposed that urinary NAG might serve as an early detection and prognostic marker (23). In more recent years, urinary NAG activity also has been shown to be elevated in chronic kidney disease as a result of diabetes (24) and chronic lithium exposure (25); therefore, it might lack specificity for the purpose of risk stratification in ARF. In addition, milder forms of acute kidney injury already might be associated with urinary excretion of this renal tubular epithelial brush border enzyme (26), thus leading to false-positive results.

Urinary KIM-1, however, is a more recently described type 1 cell membrane glycoprotein that is expressed in humans and rodents when the injured renal proximal tubule assumes a dedifferentiated phenotype (27). As previously reported, KIM-1 is strongly upregulated in proximal tubular epithelial cells during various states that are characterized by epithelial cell dedifferentiation, including ischemia, toxic renal injury, polycystic kidney disease, and renal cell carcinoma (27–30). Preliminary data from humans have shown that KIM-1 is a sensitive and specific marker for the early detection of acute kidney injury after cardiopulmonary bypass surgery and, in that setting, is superior to urinary NAG activity (31).

To our knowledge, this is the first large study to test the hypothesis of whether urinary markers of kidney injury are associated with adverse clinical outcomes in patients with established ARF. In addition, because the timing of enrollment depended on nephrology consultation, there likely was considerable heterogeneity in the time lapsed between the occurrence of acute kidney injury, development of ARF, and urine sampling. This also suggests that the association of the urinary markers with disease severity and adverse outcomes is rela-

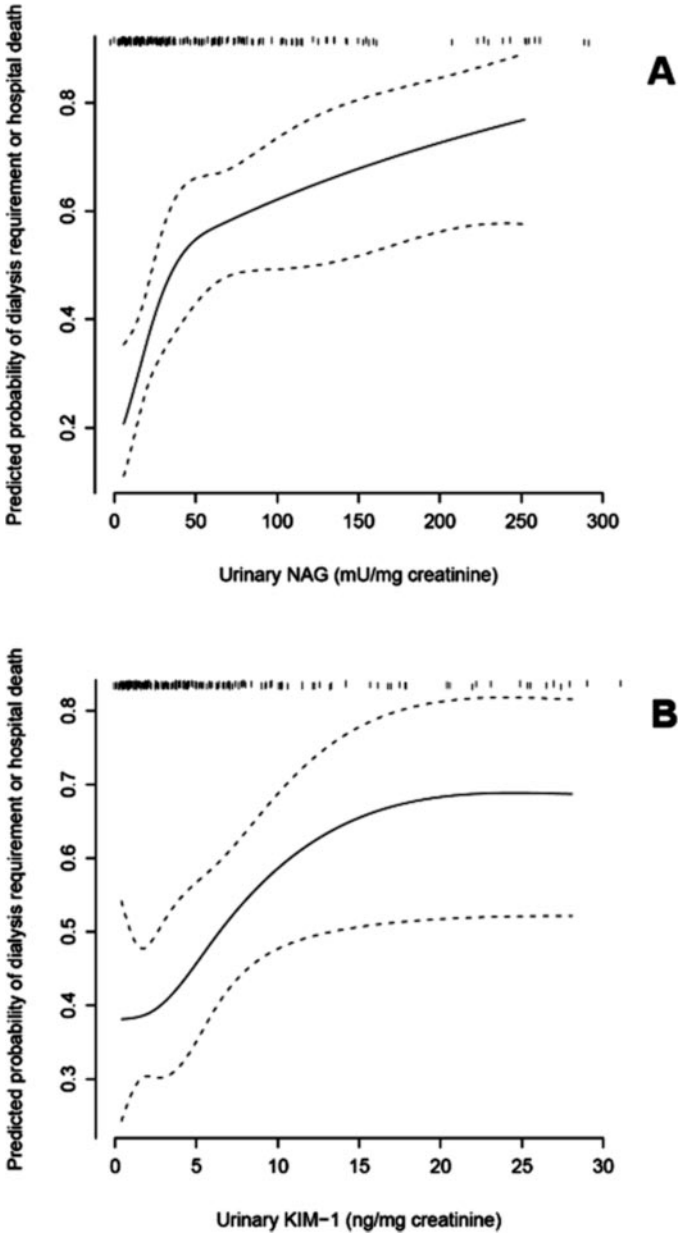


*Figure 3.* Univariate relationship of urinary NAG activity (A) and KIM-1 level (B) with the predicted probability for the composite adverse clinical outcome of dialysis requirement or hospital death, using restricted cubic splines functions (16). The solid line represents the point estimate, and the hatched lines represent the 95% confidence interval of the predicted probability. Markings at the top of the graph indicate individual observation points.

tively robust in a variety of clinical settings. Although neither urinary NAG nor KIM-1 was superior to the APACHE II score in predicting adverse outcomes, areas under the ROC curve for the more traditional and clinically commonly used severity indices, including serum creatinine and urine output, were of inferior prognostic value as compared with NAG and KIM-1. The combination of APACHE II with a urinary marker seemed to provide a marginally better area under the ROC curve than APACHE II alone, but this was not statistically significant. However, when urinary NAG activity or KIM-1 level was combined with the four dichotomous variables indicating presence of hepatic cirrhosis, sepsis, oliguria, and mechanical ventilation at enrollment, an area under the ROC curve equal to the multivariable APACHE II score for the detection of the composite outcome of dialysis requirement or death was achieved. This suggests that the use of urinary markers in combination with a limited set of simple clinical variables may perform well for the prediction of adverse outcomes in patients with ARF and may be easier to use than a more complex, generic disease severity score such as APACHE II. Of note, a previous single-center study of 73 nonoliguric patients with ARF as a result of acute tubular necrosis evaluated the prognostic value of several urinary low molecular weight proteins, including NAG, for the prediction of a subsequent need for dialysis but not for the prediction of mortality (32).

In this study, we found some variability in the association of the urinary markers with severity of illness and adverse outcomes, which is intriguing and merits further discussion. Urinary NAG activity showed a stronger association with APACHE II score than KIM-1 level, but the association of the urinary KIM-1 level with the MOF score was stronger than that of the NAG activity. Furthermore, when plotted against the predicted probability for the development of the composite outcome, dialysis requirement or death, NAG showed a continuous increase in the probability for the outcome even at the higher end of its range (Figure 3A), whereas no further increase in the probability for the outcome was observed at higher KIM-1 levels (Figure 3B). This phenomenon might be related to the characteristics of KIM-1 as a proximal tubular cell dedifferentiation marker, which persists during the injury and recovery phase. In later stages of ARF, KIM-1 expression might not always be associated with a deleterious outcome. In addition, adjustment for MOF score as well as adjustment for sepsis, hepatic cirrhosis, mechanical ventilation, and oliguria attenuated the association of urinary KIM-1 level with the composite adverse outcome more than that of urinary NAG activity.

In this study, we normalized urinary NAG activity and KIM-1 level to the urinary creatinine level to account for differences in relative amounts of water extracted along the nephron. We obtained similar results when absolute values for these markers were used in the analyses (data not shown).

This study supports the hypothesis that urinary markers can be used to predict adverse outcomes in hospitalized patients with ARF of mixed severity and cause. However, the present data are not sufficient to conclude that urinary NAG activity or KIM-1 level should be used routinely for clinical decision making. Nevertheless, our data argue that measurement of urinary markers in patients with established ARF for prognostic stratification can be a promising concept and, also if used in combination, might alleviate well-described limitations of existing clinical prognostic tools (33–35). Further research should be focused on clarification of the diagnostic and prognostic capabilities of individual and multiple biomarkers, ideally using technologies that will permit bedside determinations. Specific combinations of markers then could be tailored to certain clin-

*Table 4.* AUC of several variables for the prediction of the composite outcome of dialysis requirement or hospital death[a]

| Predictor Variable | AUC (95% CI) |
|---|---|
| Serum creatinine at enrollment | 0.60 (0.52 to 0.68) |
| Urine output[b] | 0.65 (0.57 to 0.73) |
| Urinary KIM-1 level | 0.61 (0.53 to 0.69) |
| Urinary NAG activity | 0.71 (0.63 to 0.78) |
| Urinary KIM-1 level and NAG activity | 0.71 (0.63 to 0.78) |
| APACHE II score | 0.78 (0.71 to 0.84)[c] |
| APACHE II score and urinary NAG activity | 0.79 (0.73 to 0.85)[c] |
| APACHE II score and urinary KIM-1 level | 0.80 (0.74 to 0.86)[c] |
| APACHE II score, urinary KIM-1 level, and NAG activity | 0.83 (0.77 to 0.88)[c] |
| Cirrhosis, sepsis, oliguria, mechanical ventilation, and urinary NAG activity | 0.78 (0.71 to 0.84)[c] |
| Cirrhosis, sepsis, oliguria, mechanical ventilation, and urinary KIM-1 level | 0.78 (0.71 to 0.84)[c] |
| Cirrhosis, sepsis, oliguria, mechanical ventilation, urinary KIM-1 level, and NAG activity | 0.80 (0.73 to 0.86)[c] |

[a]AUC, area under the ROC curve; ROC, receiver operating characteristic.
[b]The negative value of the urine output was used for the analysis.
[c]Statistically significant difference compared with serum creatinine at enrollment.

ical conditions (*e.g.*, sepsis, hemorrhagic shock). In addition, prognostic tools that are based on clinical findings could be combined with one or several urinary biomarkers to increase their prognostic precision, as demonstrated in our analysis.

## Conclusion

Although urinary NAG and KIM-1 seem to be promising prognostic markers in patients with ARF, further investigation is required to establish the temporal factors that govern their excretion. Finally, the utility of NAG and KIM-1 relative to other emerging markers for ARF needs to be determined.

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## Disclosures

None.

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