Urine Neutrophil Gelatinase-Associated Lipocalin Moderately Predicts Acute Kidney Injury in Critically Ill Adults

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ABSTRACT

Urine neutrophil gelatinase-associated lipocalin (uNGAL) has shown promise as a biomarker for the early detection of acute kidney injury (AKI) in fixed models of injury, but its ability to predict AKI and provide prognostic information in critically ill adults is unknown. We prospectively studied a heterogeneous population of 451 critically ill adults, 64 (14%) and 86 (19%) of whom developed AKI within 24 and 48 h of enrollment, respectively. Median uNGAL at enrollment was higher among patients who developed AKI within 48 h compared with those who did not (190 versus 57 ng/mg creatinine, \( P < 0.001 \)). The areas under the receiver operating characteristic curves describing the relationship between uNGAL level and the occurrence of AKI within 24 and 48 h were 0.71 (95% Confidence Intervals [CI]: 0.63 to 0.78) and 0.64 (95% CI: 0.57 to 0.71), respectively. Urine neutrophil gelatinase-associated lipocalin remained independently associated with the development of AKI after adjustment for age, serum creatinine closest to enrollment, illness severity, sepsis, and intensive care unit (ICU) location, although it only marginally improved the predictive performance of the clinical model alone. A Cox proportional hazards model using time to first dialysis, adjusted for APACHE II score, suggested that uNGAL independently predicts severe AKI during hospitalization [HR 2.60, 95% CI:1.55 to 4.35]. In summary, although a single measurement of uNGAL exhibited moderate predictive utility for the development and severity of AKI in a heterogeneous ICU population, its additional contribution to conventional clinical risk predictors appears limited.


Despite advances in the provision of hospitalized care, the incidence of acute kidney injury (AKI) is increasing and remains an independent predictor of morbidity and mortality.1–3 An impediment toward improving outcomes has been continued reliance on belated and unreliable markers of injury.4 Recent efforts directed toward discovery of biomarkers with early predictive and prognostic potential have yielded several candidates including neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1),5 cystatin C,6,7 Na+/H+ exchanger isoform 3 (NHE3),8 and IL-18 (IL-18).9–12 NGAL is a 25-kD protein of the lipocalin family, whose structure is defined by a calyx that modulates local iron channeling and serves as a growth and

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differentiation factor for renal tubular epithelia. Increased expression in the proximal renal tubular epithelia during ischemic injury has provided a rationale for its use as an early biomarker of AKI. Performance testing of urine NGAL (uNGAL) has yielded particular promise in patient settings with temporally defined mechanisms of injury including both pediatric and adult patients undergoing cardiopulmonary bypass, postrenal transplantation, diarrhea-associated hemolytic-uremic syndrome, and in a cohort of pediatric patients requiring mechanical ventilation.

We assessed the ability of uNGAL to predict both the development and severity of AKI in a mixed adult ICU cohort subject to heterogeneous patterns, timing, and causes of injury. The added and conjoint predictive ability of uNGAL beyond a panel of a priori selected clinical predictors for AKI was also quantified. Data were obtained from subjects enrolled in the ongoing NIH-sponsored Validation of biomarkers in Acute Lung Injury Diagnosis (VALID) study, a single-center, multi-ICU prospective cohort whose primary purpose is to investigate panels of new and existing plasma, serum, or urine protein biomarkers to both diagnose Acute Lung Injury/Acute Respiratory Distress Syndrome (ALI/ARDS) in at-risk patients and identify patients with ALI/ARDS early who are at highest risk for adverse clinical outcomes (Figure 1).

RESULTS

Subject Characteristics

Of 451 subjects, AKI was detected in 86 (19.1%) within 48 h of enrollment, defined as a minimum 50% or 0.3 mg/dl increase in serum creatinine by Acute Kidney Injury Network (AKIN) consensus criteria from the value drawn closest to enrollment. Baseline characteristics were compared with 305 subjects who had serum creatinine available at both 24 and 48 h and did not develop AKI (Table 1). Subjects developing AKI were more likely to carry a diagnosis of chronic kidney disease (CKD), diabetes mellitus (DM), a higher degree of illness severity, meet sepsis and severe sepsis criteria, and require vasoressor support than patients who did not develop AKI (P < 0.05). In addition, the surgical ICU experienced a higher rate of AKI than other ICUs. There were no statistically significant differences in ethnicity, admission rates of ALI/ARDS, number of potential nephrotoxic medications, or the frequency of iodinated contrast administration at enrollment between subjects who did and did not subsequently develop AKI.

Table 2 displays enrollment uNGAL and outcome measures grouped according to AKI status. Both uncorrected and corrected (for urine creatinine) uNGAL measurements at enrollment were significantly higher in the AKI group compared with subjects without AKI (P < 0.001). AKI patients were more likely to die during hospitalization, require renal replacement therapy (RRT), and have fewer dialysis-free and ventilator-free days than non-AKI patients (P < 0.001).

Association between uNGAL and AKI Development

Figure 2 shows uNGAL concentrations (corrected for urine creatinine) in subjects grouped according to AKI status within 24 and 48 h of enrollment. Within 24 h of enrollment, 64 patients were diagnosed as having AKI (median uNGAL 313 [interquartile range (IQR): 59 to 2355] ng/mg) and were compared with 386 subjects without AKI (median uNGAL 56 [IQR: 16 to 194] ng/mg) (P < 0.001). By 48 h after enrollment, 86 subjects had been diagnosed as having AKI (median uNGAL 190 [IQR: 32 to 995] ng/mg) compared with 305 subjects who had serum creatinine available within both 24- and 48-h windows of detection and did not develop AKI within that time (median uNGAL 57 [IQR: 17 to 203] ng/mg) (P < 0.001).

The areas under the receiver operating characteristic curve (AUC-ROC) of uNGAL for the prediction of AKI occurrence within 24 and 48 h were 0.71 (95% CI: 0.63 to 0.78) and 0.64 (95% CI: 0.57 to 0.71), respectively (Figure 3A).

Figure 3B examines the prediction of sustained AKI within 24 and 48 h. Corrected uNGAL levels for 47 patients with AKI detected within 24 h of enrollment that persisted into the next 24-h window of detection were 275 [IQR: 73 to 2463] ng/mg compared with 57 [IQR:17 to 203] ng/mg in the 305 subjects without AKI. Median corrected uNGAL levels were 247 [IQR: 62 to 2289] ng/mg for 52 patients developing AKI any time within 48 h that persisted into the next 24-h window of detection compared with 70 [IQR: 18 to 248] ng/mg in the 251 patients who did not have injury at any time during the 72 h follow-up period. The AUC-ROCs were 0.70 (95% CI: 0.61 to 0.78) for sustained injury occurring within 24 h and 0.66 (95% CI: 0.58 to 0.75) within 48 h.

Sensitivity Analyses of Baseline Renal Function

A frequent problem confronting AKI research and management in ICU patients is a lack of information regarding baseline renal function. Consequently, the inclusion of subjects whose renal function has already deteriorated may reduce the predictive ability of candidate biomarkers for AKI development. In our study, baseline renal function was unavailable in...

Figure 1. VALID study scheme.
Table 1. Baseline clinical data grouped according to AKI status within 48 hours of enrollment

<table>
<thead>
<tr>
<th>Variable</th>
<th>No AKI (n = 305)</th>
<th>AKI (n = 86)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52 [37 to 63]</td>
<td>55 [43 to 68]</td>
<td>0.064</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>170 (56%)</td>
<td>56 (63%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Caucasian, n (%)</td>
<td>263 (86%)</td>
<td>73 (85%)</td>
<td>0.78</td>
</tr>
<tr>
<td>DM, n (%)</td>
<td>54 (18%)</td>
<td>30 (35%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CKD, n (%)</td>
<td>27 (9%)</td>
<td>23 (27%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ICU Type, n (%)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Medical</td>
<td>151 (49%)</td>
<td>47 (55%)</td>
<td></td>
</tr>
<tr>
<td>Surgical</td>
<td>54 (18%)</td>
<td>29 (34%)</td>
<td></td>
</tr>
<tr>
<td>Trauma</td>
<td>92 (30%)</td>
<td>7 (8%)</td>
<td></td>
</tr>
<tr>
<td>Cardiac</td>
<td>8 (3%)</td>
<td>3 (3%)</td>
<td></td>
</tr>
<tr>
<td>APACHE II</td>
<td>22 [18 to 28]</td>
<td>30 [23 to 34]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>modAPACHE II</td>
<td>21 [17 to 26]</td>
<td>26 [20 to 31]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SAPS</td>
<td>44 [32 to 55]</td>
<td>57 [41 to 69]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sepsis, n (%)</td>
<td>114 (37%)</td>
<td>47 (55%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Severe sepsis, n (%)</td>
<td>109 (36%)</td>
<td>46 (53%)</td>
<td>0.003</td>
</tr>
<tr>
<td>ALI/ARDS, n (%)</td>
<td>42 (14%)</td>
<td>16 (19%)</td>
<td>0.27</td>
</tr>
<tr>
<td>Severe exposure Preceding 48 h, n (%)</td>
<td>41 (13%)</td>
<td>9 (10%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Vasopressor use, n (%)</td>
<td>115 (38%)</td>
<td>44 (51%)</td>
<td>0.025</td>
</tr>
<tr>
<td>Number of nephrotoxins at time of enrollment</td>
<td>0 [0 to 1]</td>
<td>0 [0 to 1]</td>
<td>0.45</td>
</tr>
<tr>
<td>Dialysis following admission to enrollment (extrapolated to 24 h)</td>
<td>2.0 [0.7 to 4.8]</td>
<td>2.9 [0.7 to 7.8]</td>
<td>0.036</td>
</tr>
<tr>
<td>Serum creatinine closest to enrollment</td>
<td>0.9 [0.7 to 1.2]</td>
<td>1.5 [1.0 to 2.2]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

DM, Diabetes Mellitus; CKD, Chronic Kidney Disease; ICU, Intensive Care Unit; APACHE II, Acute Physiology and Chronic Health Evaluation II score; modAPACHE II, Modified APACHE II score; SAPS, Simplified Acute Physiology Score; ALI, Acute Lung Injury; ARDS, Acute Respiratory Distress Syndrome. Potential nephrotoxins included radiocontrast within 48 h prior to enrollment or aminoglycosides, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers (ACE/ARB), nonsteroidal anti-inflammatory agents (NSAIDs), cyclooxygenase-2 inhibitors (COX-2), trimethoprim-sulfamethoxazole (TMP-SMX), calcineurin inhibitors, or acyclovir at the time of enrollment. Values are presented as either proportions or median [interquartile range]. P values <0.05 denote statistical significance.

Table 2. Biomarker values and outcomes for subjects grouped according to AKI status within 48 hours of enrollment

<table>
<thead>
<tr>
<th>Variable</th>
<th>No AKI (n = 305)</th>
<th>AKI (n = 86)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine NGAL (ng/mg)</td>
<td>48.1 [12.7 to 179.3]</td>
<td>126.6 [32.1 to 623.1]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urine creatinine (mg/ml)</td>
<td>0.88 [0.47 to 1.35]</td>
<td>0.82 [0.52 to 1.37]</td>
<td>0.9</td>
</tr>
<tr>
<td>Urine NGAL/Cr (ng/mg)</td>
<td>57 [17 to 203]</td>
<td>190 [32 to 995]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log$_{10}$(NGAL/Cr)</td>
<td>1.83 ± 0.86</td>
<td>2.31 ± 1.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dialysis following enrollment</td>
<td>3 (1%)</td>
<td>14 (16%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dialysis-free days</td>
<td>28 [28 to 28]</td>
<td>27 [5 to 28]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ventilator-free days</td>
<td>24 [19 to 26]</td>
<td>11 [0 to 25]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ICU length of stay</td>
<td>9 [5 to 17]</td>
<td>7 [4 to 23]</td>
<td>0.36</td>
</tr>
<tr>
<td>Hospital length of stay following enrollment</td>
<td>12 [8 to 20]</td>
<td>12 [6 to 27]</td>
<td>0.81</td>
</tr>
<tr>
<td>28-day hospital mortality</td>
<td>31 (10%)</td>
<td>33 (38%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Variables are presented as either mean ± standard deviation or median [IQR]. P values <0.05 denote statistical significance.

52% of the subjects. We performed two sensitivity analyses to further explore this issue. We first restricted analysis to patients whose eGFR at enrollment was ≥ 75 ml/min/1.73 m$^2$, thereby reducing the possibility of previously established AKI. Eighteen subjects subsequently developed AKI within 24 h of measurement and were compared with the 257 patients without AKI within 24 h. Median uNGAL for subjects developing AKI was 203 [IQR: 102 to 401] ng/mg and 44 [IQR: 14 to 102] ng/mg for the controls (P < 0.001). In this subgroup, the AUC for uNGAL to predict the occurrence of AKI within 24 h improved to 0.77 (95% CI: 0.64 to 0.90) (Figure 3A – red line).

Secondly, we used multiple imputation to estimate baseline GFR in subjects with missing data (52% of the cohort). The Acute Dialysis Quality Initiative (ADQI) has previously recommended imputing (replacing missing values with) an eGFR of 75 ml/min/1.73 m$^2$ for all patients without known baseline GFR data. Given that this type of overall mean imputation tends to potentially underestimate baseline renal function (median serum creatinine 1.1 [IQR: 0.91 to 1.28] mg/dl) compared with subjects with known baselines who tended to be older and sicker (median creatinine 0.8 [0.6 to 1.0] mg/dl). Given that this type of overall mean imputation potentially increases, rather than reduce, bias, we used multiple imputation methodology to more adequately estimate baseline renal function in patients with missing baseline data. This method accounts for other known patient data or predictors such as age, diabetes, coronary artery disease, peripheral vascular disease, chronic congestive heart failure, the diagnosis of CKD, and known baseline serum creatinine values. After multiple imputation, the median serum creatinine in the group without known baseline function was estimated to be 0.7 [IQR: 0.6 to 0.9] mg/dl. Taking all subjects with known and estimated baseline values, we subsequently...
filtered out patients with preexisting AKI (an increase of 0.3 or 50% in serum creatinine between known or estimated baseline to enrollment). Of the 228 subjects remaining, 16 (7.0%) developed subsequent AKI within 24 h of enrollment with an AUC of 0.61 (95% CI: 0.46 to 0.76).

**Association between uNGAL and Dialysis or Mortality**

To assess whether uNGAL predicted clinical outcomes, we examined the association between uNGAL and the provision of dialysis or hospital mortality within 28 d of enrollment. Median enrollment uNGAL levels were significantly higher in the 83 subjects who died (223 [IQR:36 to 1074] ng/mg) compared with the 407 subjects who survived (56 [IQR:16 to 195] ng/mg)(P < 0.001) as well as in the 17 subjects receiving acute dialysis (548 [IQR:156 to 4665] ng/mg) versus the 473 not receiving acute dialysis (61 [IQR:17 to 232] ng/mg)(P < 0.001) (Figure 4). Cox proportional hazards model was performed to assess the effect of uNGAL to predict time-to-first dialysis event within 28 d. Death was treated as a competing risk, and the model was adjusted for APACHE II score. The adjusted HR for log₁₀ transformed uNGAL was 2.60 (95% CI: 1.55 to 4.35).

We also explored the association between uNGAL and AKIN staging. Of subjects with AKI, 61 (70.9%) met AKIN stage I, 7 (8.2%) met AKIN stage II, and 18 (20.9%) met AKIN stage III criteria by change in serum creatinine or having received RRT within 48 h of enrollment. Given the paucity of subjects that met stage II injury criteria, we combined them with individuals meeting stage III injury. Supplemental Figure 1 shows the distribution of enrollment uNGAL values for stage I (median 158 [IQR: 27 to 430] ng/mg) compared with stage II + III combined (median 390 [IQR: 39 to 4317] ng/mg). The AUC of uNGAL to predict the occurrence of AKIN stage I was 0.62 (95% CI: 0.54 to 0.70) and for the combined AKIN stages II and III, the AUC was 0.71 (95% CI: 0.59 to 0.83) (Supplemental Figure 2).

Figure 2. Enrollment uNGAL values grouped by AKI status within 24 and 48 h of measurement. Values are displayed as box-plot summaries with p-values <0.05 denoting statistical significance.

Figure 3. (A) AUC-ROCs for AKI development within 24 h (black line) and 48 h (gray line) of enrollment. A sensitivity analysis restricted to patients with an estimated GFR at enrollment of ≥75 ml/min/1.73m² was also performed (red line). The AUC values and 95% CIs are listed within the figure. (B) The AUC-ROCs for sustained AKI within 24 h (black line) and 48 h (gray line) of enrollment. The AUC values and 95% CIs are listed within the figure.
Relative Contribution of uNGAL to a Clinical Prediction Model

To determine the added contribution of uNGAL to predict the occurrence of AKI within 24 h beyond known clinical predictors, we added uNGAL to a multivariable logistic regression including age, the presence of sepsis, modified APACHE II score, serum creatinine closest to the time of enrollment (in general, the routine morning lab value draw on enrollment day), and ICU location (Table 3). Given these predictors, uNGAL remained independently predictive for the subsequent development of AKI [OR 1.71, 95% CI (1.12 to 2.60)]. However, when assessed by AUC-ROC, the addition of uNGAL improved the clinical model AUC 0.81 (95% CI: 0.74 to 0.87) only marginally to 0.82 (95% CI: 0.75 to 0.88) (Figure 5).

We also assessed the ability of uNGAL to “reclassify” the degree of risk for AKI within 24 h as assessed by our clinical model (Table 4). Based on our clinical prediction model, subjects were categorized into prespecified “low,” “intermediate,” and “high” risk groups using cutoffs of <30%, 30 to 60%, and >60%, respectively. We compared the proportions of reclassified subjects across these three risk groups when uNGAL was added to the clinical model. Among 64 patients who subsequently developed AKI, six (9.4%) were correctly reclassified to be at higher risk when uNGAL was added to the clinical model; however, six (9.4%) subjects were also incorrectly reclassified to be at lower risk. Among the 386 subjects who did not develop AKI at 24 h, eight (2.1%) were correctly reclassified to be at lower risk using uNGAL, while 14 (3.6%) were incorrectly reclassified to be at higher risk. The net reclassification improvement for uNGAL added to the clinical prediction model was −1.5% (P = 0.78).

DISCUSSION

Urine NGAL has performed well as a biomarker for the early detection of AKI in discrete types of injury and study populations. We prospectively examined the predictive ability of uNGAL in a high-risk setting where AKI is described by diverse etiologies and patterns of injury. Single-point measurement of uNGAL within 48 h demonstrated moderate discrimination for the prediction of subsequent AKI with performance opti-
nized most proximal to the time of clinical detection (i.e., within 24 h of serum creatinine elevation). The latter has been observed in other urine biomarker studies of critically ill adults, perhaps reflecting increased expression as evolving tubular injury becomes more pronounced. We further demonstrated an independent association between uNGAL and other important clinical outcomes including the provision of acute dialysis and hospital death within 28 d.

The performance of uNGAL assessed in this manner was less robust compared with more fixed mechanisms of injury such as in postcardiopulmonary bypass or delayed graft function. This observation, in part, likely reflects the complexity of the mixed ICU patient population studied. Although better established in ischemia-reperfusion, it is unlikely that a single biomarker such as uNGAL can alone explain all observed causes of serum creatinine elevations in an ICU population. For example, while enrollment on ICU day 2 allowed for aggressive volume resuscitation typical within the first 24 h of ICU admission (supported by the overall positive fluid balance in our cohort), we cannot rule out that some subjects may have remained under-resuscitated, although analysis of those with sustained injury did not substantially augment performance. In addition, while attempting to control for sepsis and illness severity in our multivariable model, systemic expression of NGAL in this high acuity cohort may have also conferred some loss of specificity. Finally, it remains possible that the consensus AKIN criteria employed as our a priori definition of AKI may be overly sensitive, especially in those with chronically impaired GFR where small increases in serum creatinine reflect a proportionally smaller change in GFR. This may explain both the improved performance of uNGAL when restricting the population to those with a relatively preserved GFR at enrollment and when examining more severe injury (AKIN II and III). It may also partially account for the relatively large effect of enrollment serum creatinine in our multivariate model. These findings underscore the role of serum creatinine as a suboptimal gold standard and the need to examine prediction of renal outcomes beyond changes in serum creatinine.

While the critically ill are likely to derive maximal benefit from novel biomarker panels with diagnostic and prognostic potential, they also present unique challenges for study. As a high-risk cohort, for example, some subjects may have already developed AKI at the time of presentation. Proper identification of these subjects is predicated upon knowledge of the subjects’ baseline renal function, information often lacking in the critical care setting. Our principal analysis included all-comers to the ICU using serum creatinine closest to enrollment as the reference value. While potentially limiting prediction of de novo injury, it assessed the ability of uNGAL to indicate evolving or subsequent injury in a setting with realistic demands where baseline function is often unavailable. However, our attempts at limiting the inclusion of subjects with pre-existing injury by restricting analysis to those with relatively preserved renal function at enrollment (eGFR ≥ 75 ml/min/1.73 m²) resulted only in a modest improvement in performance. We also attempted to provide an estimation of baseline GFR by multiple imputation in those with missing data. After excluding those with pre-existing AKI at the time of enrollment, the predictive performance of uNGAL did not improve. It is important to note that these sensitivity analyses were exploratory in nature and should be interpreted with caution given the reduction in case number resulting from the restrictions applied.

Zappitelli et al. also recently examined the predictive accuracy of uNGAL in a cohort of critically ill children for the early prediction and prognosis of AKI. Even in this cohort, where baseline function was well-defined, analysis of a single-point urine specimen for the subsequent detection of AKI over 48 h yielded only slightly better performance than observed in our study—AUC 0.78 (95% CI 0.62 to 0.95). Potential reasons for these observed differences include variations in the spectrum of critical illness and co-morbidity burden in a pediatric cohort with a mean age of six years compared with over fifty years. In addition, our study included all-comers from several different ICU populations (medical, surgical, trauma) and was not restricted to those requiring mechanical ventilation. Nonetheless, similar differences in the predictive accuracy of uNGAL between pediatric and adult patients have been previously observed, such as in cardiopulmonary bypass, reflecting the need for validation in both groups.

The overall contribution of uNGAL to the performance of our clinical model of prediction as assessed by the area under the receiver operating curve was modest. This finding has also been observed within large population-based cohorts using...
more established biomarker panels in well-characterized illnesses such as cardiovascular disease. Wang et al. recently demonstrated that the addition of multiple biomarkers resulted only in incremental benefit when added to conventional risk factors for the prediction of future cardiovascular events or death in the Framingham Offspring Study. It has been estimated that meaningful additions to the discriminative performance by a biomarker to a reasonable set of clinical predictors requires an extremely robust association between the biomarker and outcome. Minimization of such overlap in the distribution of uNGAL within a critically ill cohort between cases and controls defined by modest creatinine elevations may be difficult to achieve. Accordingly, receiver operating curves are relatively insensitive to such predictive improvements as they provide a global assessment of discrimination. However, as it may be the case that the added predictive value of AKI biomarker(s) may be most appreciated in patients with intermediate risk, based on clinical predictors rather than those at extreme ends of the spectrum, we applied the recently introduced net reclassification improvement method to quantify the change in risk when adding a biomarker to an existing prediction model. While empiric, our prespecified choice of risk cutoffs were based on an underlying goal to identify patients at moderately high risk for AKI for enrollment in clinical trials. Using these cutoffs, however, uNGAL still did not provide substantial added predictive value over the clinical prediction model alone.

Limitations of this study include that creatinine measurements were made based on clinical decision making and were not protocol-driven. Although the high acuity of these patients dictated that single, daily assessments of creatinine were made in most subjects, our ability to detail the pattern of injury more precisely (hours rather than days) was limited. These prediction models were also based on a single-point time assessment of uNGAL rather than serial measurements over the first few hours, the latter of which would have provided information on how changes in biomarker relation to the development and prognosis of AKI. As uNGAL expression has also been observed to peak transiently approximately 4 to 6 h after injury in cardiopulmonary bypass, serial urine collection may have increased the likelihood of capturing that point of maximal discrimination. However, unlike cardiopulmonary bypass or contrast nephropathy, AKI in a complex critically ill patient is less likely to be a discrete event limited to a few hours and more often is described by an accumulation of multiple insults over time.

In summary, we found uNGAL to be independently associated with and exhibit moderate discrimination for the detection of subsequent AKI within a heterogeneous ICU population. However, as a stand-alone marker using modest creatinine-based cutoffs, uNGAL seems to have limited utility beyond a conventional clinical risk-prediction model. As single biomarkers have also been unable to provide sufficient discriminating or prognostic power in other complex disease states in the critically ill, such as ALI/ARDS, the relative and combined predictive capacity of uNGAL within a “panel” of other promising candidate markers in a similarly complex disease such as AKI is warranted. Furthermore, the role of uNGAL in predicting more important measures of AKI severity, including dialysis provision or mortality, remains to be fully explored in larger cohorts.

CONCISE METHODS

Patients

This study was performed on the first 588 patients enrolled between February 2006 and January 2007 in the ongoing NIH-sponsored Validation of biomarkers in Acute Lung Injury Diagnosis (VALID) study for whom data were available. VALID is a single-center, multi-ICU prospective cohort study with an anticipated total enrollment of 2550 patients, whose primary purpose is to investigate panels of new and existing plasma, serum, or urine protein biomarkers to both diagnose ALI/ARDS in at-risk patients and to identify patients with ALI/ARDS who are at highest risk for adverse clinical outcomes. All adult (>18 yr of age) patients admitted to one of four ICUs (Medical, Cardiac, Surgical, Trauma) at Vanderbilt University Medical Center (VUMC) who remained in the ICU at day 2 were eligible for enrollment. Informed consent was obtained from the patient or surrogate, whenever possible; however, given the minimally invasive nature of the study, a waiver of consent was permissible under our Institutional Review Board. Patients were excluded if they experienced a cardiac arrest before enrollment, had transfer orders written or anticipated within 4 h, died or were discharged within 48 h of ICU admission, were admitted for uncomplicated overdose, or were in the ICU for more than 3 d before enrollment. Patients with chronic lung disease requiring oxygen supplementation or pulmonary fibrosis were also excluded. Secondary exclusion criteria applied for the current study included 49 subjects with a history of ESRD or renal transplant and 15 subjects who received acute dialysis at or before enrollment. Of the 524 remaining patients, 490 urine specimens were available for analysis. Twenty-five subjects were without serum creatinine data within 24 and 48 h of enrollment, usually due to anticipated death, discharge, or nonrelated condition resulting in decreased monitoring of renal function (e.g., isolated trauma). An additional 14 subjects had creatinine missing within 24 h of enrollment and did not have AKI at 48 h. These patients could not definitively be classified as controls for the within 24- or 48-h analyses and were therefore also not included in the primary analyses. All 490 subjects were included in the secondary analysis examining death and dialysis provision. The study protocol and consent forms were approved by the VUMC Human Subjects Institutional Review Board before study initiation and were in accordance with the Declaration of Helsinki.

Clinical Data Collection

The general study schematic is shown in Figure 1. Demographic data including: age, gender, weight, ethnicity, and ICU admission diagnoses were collected at the time of enrollment. General severity-of-illness scoring systems including APACHE II34 and SAPS II55 were collected on enrollment day. A modified APACHE II score was calcu-
Multiple imputation has been shown to be preferred over single imputation 25–27 to estimate missing baseline eGFR using available predictors of renal function, including age, history of hypertension, diabetes, peripheral vascular disease, coronary artery disease, chronic congestive heart failure, and the lowest serum creatinine available within 1 yr of enrollment (from those subjects in whom it was available).

Urine for NGAL and creatinine measurements was collected the morning of enrollment from the proximal meter reservoir of the Foley catheter, immediately placed on ice, pipetted into 400 microliter aliquots and frozen at −80°C within 1 h of collection. NGAL levels were measured in urine using the human NGAL Enzyme-linked immunosorbent assay kit (Antibody Shop, Gentofte, Denmark, no. 036). Samples were run in duplicate and lab personnel were blinded to the injury status of each patient. The mean Intra-assay coefficient of variation in our laboratory was 8.0%. Urine creatinine was measured at the VUMC General Clinical Research Center core laboratory facility using a quantitative colorimetric assay (VITROS Chemistry Products; Ortho-Clinical Diagnostics Inc., Rochester, NY).

**Statistical Analyses**

Measurements at enrollment of patients grouped according to injury status achieved over 48 h were compared. Continuous data were described as median with IQR and compared using the Wilcoxon rank sum test. Categorical variables were expressed as proportions and compared using the Pearson χ².

Examination of the association between uNGAL measurement and AKI status at enrollment was performed separately for 24 and 48 h using logistic regression. For each time period analysis, controls were selected based on having data for that time period and having no intermission missing data. For example, to be a control for detection within 48 h, subjects needed to have data both at 24 h and 48 h and remain a control at both time periods to avoid potential misclassification as a case. For prediction within 24 h, subjects must have had serum creatinine data within 24 h but did not have to have data at 48 h to be considered. Controls at 24 h that subsequently became cases or had no serum creatinine data available between 24 and 48 h were not allowed to serve as controls for the 48-h analysis but were allowed to remain controls for the 24 h analyses. The goal of this approach was to apply an unbiased use of serum creatinine as a gold-standard (albeit, an imperfect one) and to minimize potential survivor-bias. Goodness-of-fit of each logistic regression was assessed using the Hosmer-Lemeshow test. The ability of uNGAL to discriminate between cases and noncases of subsequent injury using these criteria was determined using ROC curves providing sensitivity and specificity at different cutoff values of uNGAL to detect AKI and the AUC. To assess the additive predictive ability of NGAL to a priori specified traditional predictors of AKI such as age, modified APACHE II score, the presence of sepsis, serum creatinine closest to enrollment, and ICU location, multivariable logistic regression modeling was used. Adjusted and nonadjusted effects of NGAL were presented as odds ratios with 95% confidence intervals.

A Cox proportional hazards model was used to analyze the role of uNGAL in predicting the independent risk for receiving acute dialysis. In the Cox regression model, death was treated as a competing risk.39 Patients were followed during hospitalization for the events of first

**Laboratory Data Collection**

The highest and lowest serum creatinine values were obtained via routine care of the primary team and recorded daily from enrollment until the end of the 72-h follow-up period. The presence of AKI was assessed daily by calculating the change in serum creatinine from the value closest to the time of enrollment (in general, the routine morning lab value draw on enrollment day) to the maximum serum creatinine recorded on each subsequent day. AKI was defined as a 0.3 mg/dl or 50% increase in serum creatinine according to the Acute Kidney Injury Network (AKIN) criteria for serum creatinine.22 Data on hospital admission serum creatinine and the lowest serum creatinine recorded at VUMC within a 12-mo period preceding hospital admission were collected retrospectively, if available. We used this relatively conservative definition to increase our efficiency in filtering out patients with pre-enrollment AKI used in our sensitivity analyses. Multiple imputation has been shown to be preferred over single imputation.27 Thus, for our sensitivity analysis, we used multiple

lateral based on the total APACHE II score minus the points derived from the serum creatinine value. The presence of the systemic inflammatory response syndrome (SIRS), sepsis, or severe sepsis was determined on a daily basis according to the American College of Chest Physicians/Society of Critical Care Medicine Consensus definition. Information on vasopressor use, blood product transfusion, 24 h urine output, and fluid balance were collected during the ensuing 72-h follow-up period. Fluid balance between ICU admission and enrollment (i.e., enrollment day balance) was the total recorded fluid balance recorded during this time interval extrapolated to 24 h. In addition, data on potential nephrotoxin exposure and anti-inflammatory medication was collected on ICU admission and daily, including: intravenous contrast, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (ACE/ARB), trimethoprim-sulfamethoxazole, nonsteroidal anti-inflammatory agents (NSAIDS), cyclooxygenase-2 (COX-2) inhibitors, aminoglycosides, amphotericin, acyclovir, calcineurin inhibitors, systemic corticosteroids, statins, activated protein C, and diuretics. A history of CKD was defined by its record on the admission history or by a GFR of <60 ml/min/1.73 m² estimated by the abbreviated Modification of Diet and Renal Disease (MDRD) equation \[ \text{GFR (ml/min/1.73 m²)} = 186 x (S_c)_{-1.154} x (\text{Age})^{-0.203} x (0.742 \text{ if female}) x (1.210 \text{ if African American}) \text{ (conventional units)} \] derived from the lowest recorded serum creatinine within a year of enrollment, if available.

General clinical outcomes, including ICU and hospital length of stay and hospital mortality, were recorded. Renal-specific outcomes included need for nephrology consultation, indication and type of RRT, and dialysis-dependence at discharge. We also recorded dialysis-free survival over a 28-d period beginning at ICU admission, defined as 28 minus the total number of dialysis days. Dialysis days included all days patient underwent dialysis beginning on the initial dialysis day and recorded each day until the last dialysis was performed. Any day between dialysis days counted as a day. A period of 96 h between dialysis sessions was considered recovery in this acute setting. If a patient died before 28 d, the patient was only credited dialysis-free days for the days the patient was alive and not receiving dialysis.
dialysis and death. Patients discharged alive without dialysis were censored at 28-d follow-up because of the low likelihood of outpatient dialysis initiation within 28 d. To prevent over-fitting, models were adjusted for APACHE II (unmodified) score alone, a validated severity of disease classification system that includes enrollment serum creatinine. Log-log plots of the survival function and Schoenfeld residuals versus log-time plots were used to assess the proportional hazards assumption.

The net reclassification improvement (NRI) was calculated as a measure to estimate any overall improvement in reclassification with clinical variables and NGAL instead of clinical variables alone.28 The NRI was estimated by combining proportions of improved reclassification among patients with and without AKI. Among patients with AKI, improved reclassification was obtained by subtracting proportions of patients who were reclassified being in a lower risk group from that in a higher risk group. Similarly among patients without AKI, improved reclassification is obtained by subtracting proportions of patients who were reclassified being in a higher risk group from that in a lower risk group. The NRI estimates the net proportion of cases that appropriately move to higher or lower risk strata. Reclassification analyses were computed with AKI risk at 24 h.

The statistical software package R version 2.7.2 (www.r-project.org) and SAS version 9 were used for analyses, and two-sided P values of less than 0.05 were required to achieve statistical significance.

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DISCLOSURES

None.

REFERENCES


