Association of Elevated Urinary Concentration of Renin-Angiotensin System Components and Severe AKI

Joseph L. Alge,* Nithin Karakala,* Benjamin A. Neely,* Michael G. Janech,* James A. Tumlin,† Lakhmir S. Chawla,‡ Andrew D. Shaw,§ and John M. Arthur,*§ for the SAKInet Investigators

Summary
Background Prognostic biomarkers that predict the severity of AKI at an early time point are needed. Urinary angiotensinogen was recently identified as a prognostic AKI biomarker. The study hypothesis is that urinary renin could also predict AKI severity and that in combination angiotensinogen and renin would be a strong predictor of prognosis at the time of AKI diagnosis.

Design, setting, participants, & measurements In this multicenter, retrospective cohort study, urine was obtained from 204 patients who developed AKI after cardiac surgery from August 2008 to June 1, 2012. All patients were classified as having Acute Kidney Injury Network (AKIN) stage 1 disease by serum creatinine criteria at the time of sample collection. Urine output was not used for staging. Urinary angiotensinogen and renin were measured, and the area under the receiver-operating characteristic curve (AUC) was used to test for prediction of progression to AKIN stage 3 or in-hospital 30-day mortality. These biomarkers were added stepwise to a clinical model, and improvement in prognostic predictive performance was evaluated by category free net reclassification improvement (cfNRI) and chi-squared automatic interaction detection (CHAID).

Results Both the urinary angiotensinogen-to-creatinine ratio (uAnCR; AUC, 0.75; 95% confidence interval [CI], 0.65 to 0.85) and the urinary renin-to-creatinine ratio (uRenCR; AUC, 0.70; 95% CI, 0.57 to 0.83) predicted AKIN stage 3 or death. Addition of uAnCR to a clinical model substantially improved prediction of the outcome (AUC, 0.85; cfNRI, 0.673), augmenting sensitivity and specificity. Further addition of uRenCR increased the sensitivity of the model (cfNRI(\text{events}), 0.44). CHAID produced a highly accurate model (AUC, 0.91) and identified the combination of uAnCR >337.89 ng/mg and uRenCR >893.41 pg/mg as the strongest predictors (positive predictive value, 80.4%; negative predictive value, 90.7%; accuracy, 90.2%).

Conclusion The combination of urinary angiotensinogen and renin predicts progression to very severe disease in patients with early AKI after cardiac surgery.


Introduction
Epidemiologic studies have reported that the risk of adverse outcomes is proportional to the severity of AKI (1–4). Accurate identification of patients with severe renal injury early in the disease course could augment the efficacy of available interventions and improve patient outcomes. However, it is difficult to estimate the severity of AKI at an early time point because AKI staging is based on the magnitude of changes in serum creatinine and urine output, surrogates of GFR that do not change until after renal injury has occurred (5–7). The recent Kidney Disease Improving Global Outcomes clinical practice guideline for AKI highlighted the need for improved risk assessment for patients with established AKI (8). Biomarkers of AKI could be used to evaluate the severity of AKI at an early time point in the disease as a guide for clinical decision-making. They could also play a role in clinical trial design because they could be used to enrich the study population with patients who have severe renal injury and are more likely to benefit from an experimental therapy, increasing the statistical power of the study (9,10).

Many biomarkers have been proposed as early markers of AKI, which may be useful for the detection of AKI before increases in serum creatinine. These include neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), IL-18, cystatin C, and liver-type fatty acid–binding protein (11–16). While many studies have included an analysis of the ability of these biomarkers to predict adverse outcomes, most have done so as a secondary analysis in cohorts designed to test early diagnostic capability. Because of the inclusion of large numbers of patients without AKI, results derived from such analyses may not be generalizable to patients with established AKI. In support of this, the results of two recent studies that excluded patients without AKI have reported that neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1),...
and IL-18 are less accurate predictors of AKI progression and mortality than would be inferred from earlier studies that included patients without AKI, highlighting the need for other prognostic biomarkers (17,18).

We recently identified urinary angiotensinogen as a novel prognostic biomarker of AKI (19,20). In the current retrospective cohort study, we further evaluated the prognostic predictive power of angiotensinogen and its combination with renin. Renin was evaluated because we hypothesized that it would predict AKI severity because it cleaves angiotensinogen in the rate-limiting step of the renin-angiotensin system (RAS) (21). Because renin and angiotensinogen concentrations reflect different components within the renal RAS, the combination of the two candidate markers may improve prediction.

Materials and Methods

Patients and Urine Samples

Urine samples were obtained from 204 consecutively enrolled patients who had cardiac surgery at one of the SAKINet institutions between August 1, 2008, and June 1, 2012. This cohort involved 74 samples included in our previously described study (19). Informed consent was obtained in accordance with the institutional review board-approved protocol at each institution. Samples were collected and stored using a standard operating procedure that included centrifugation, addition of protease inhibitors, and storage at −80°C. Urine samples were collected as early as possible after Acute Kidney Injury Network (AKIN) serum creatinine criteria were met, and all were collected within the first 72 hours after surgery. Inclusion criteria were surgery of the heart or ascending aorta and development of AKIN stage 1 AKI by creatinine criteria within 3 days of surgery. Patients with ESRD, baseline serum creatinine >3.0 mg/dl, or AKIN stage 2 or higher AKI at the time of collection were excluded. Patients were followed until time of death or hospital discharge and were staged according to the maximum increase in serum creatinine using the AKIN classification system (6). Urine output criteria were not used in diagnosis or staging because these data were not available.

Measurement of Biomarkers

Urine samples were thawed at 37°C, and sandwich ELISAs were used to measure urinary angiotensinogen (Immunobiologic Laboratories) and renin (R and D Systems) according to the manufacturer’s protocol. Urine protein was measured using the QuantTest Red Total Protein Assay kit (Quantimetrix). All biomarker concentrations were corrected for urine creatinine (biomarker-to-creatinine ratio). Urine creatinine was measured using the Jaffe assay.

Statistical Analyses

Patients were grouped according to maximum AKIN stage, and biomarker concentrations were compared using the Kruskal-Wallis test with post hoc pairwise comparison. Univariate receiver-operating characteristic (ROC) curve analysis was performed to determine whether Cleveland Clinic score, percentage increase in serum creatinine from baseline, urinary angiotensinogen-to-creatinine ratio (uAnCR), and urinary renin-to-creatinine ratio (uRenCR) predicted the composite outcome development of AKIN stage 3 or in-hospital mortality within 30 days of surgery (AKIN stage 3 or death). The Cleveland Clinic score is a perioperative risk score developed by Thakar et al. to predict AKI that requires dialysis after cardiac surgery (22). The score includes the following variables: sex, congestive heart failure, left ventricular ejection fraction <35%, preoperative use of intraaortic balloon pump, chronic obstructive pulmonary disease, insulin-requiring diabetes, previous cardiac surgery, emergency surgery, type of cardiac surgery, and preoperative serum creatinine. Variables were considered predictive if the area under the ROC curve (AUC) differed statistically from 0.5. Cleveland Clinic score and percentage increase in serum creatinine were combined in a multivariable logistic regression model (clinical model) to predict the outcome. Biomarker concentrations were log_{10}-transformed and added individually to the clinical model; category free net reclassification improvement (cNRI) and integrated discrimination improvement were used to determine whether the addition of biomarkers improved prognostic predictive performance (23,24). The Wilcoxon signed-rank test was used to test the significance of the difference in the average calculated risk of patients who met the outcome after addition of a biomarker to the model. A classification tree was created using the four inputs (uAnCR, uRenCR, Cleveland Clinic score, and percentage change in serum creatinine at collection) to determine optimal cutoffs to be used together to identify patients at high risk of meeting the outcome AKIN stage 3 or death. The tree was grown using chi-squared automatic interaction detection (CHAID), specifying at least 10 cases per parent node and 5 cases per daughter node. Nodes were split using the Pearson chi-squared test if $P < 0.05$. Bonferroni correction was applied to the $P$ values to adjust for multiple comparisons. Twenty-five-fold cross-validation was performed. Statistical tests were performed in SPSS software, version 21, and SigmaPlot, version 11.0.

Results

Patient Characteristics

Urine samples were retrospectively analyzed from 204 patients who underwent cardiac surgery. Samples were obtained after surgery at the time of diagnosis with AKI. All patients were classified as having AKIN stage 1 AKI at the time of urine sample collection. Twenty-six patients progressed to AKIN stage 2, and 22 progressed to AKIN stage 3. Twenty-six patients met the primary outcome of AKIN stage 3 or death. When patients were grouped by the primary outcome, there were no statistically significant differences in demographic variables, time of sample collection, or operative variables (Table 1). However, compared with those who did not meet the outcome, patients who met the outcome had elevated preoperative serum creatinine (median, 1.1 versus 1.3 mg/dl; $P = 0.02$), serum creatinine (sCr) at collection (median, 1.6 versus 1.9 mg/dl; $P < 0.001$), and percentage increase in sCr from baseline that had occurred at the time of collection (median, 41% versus 64%; $P = 0.003$).

Biomarker Concentrations by AKIN Stage and According to Primary Outcome

The uAnCR was correlated with both maximum sCr and the maximum percentage increase in sCr (rho = 0.38 and
that a patient achieved (Table 2), and a statistically significant difference in uAnCR and uRenCR between patients who developed AKIN stage 3 AKI compared with those who only developed AKIN stage 1 AKI. When patients were grouped according to the primary outcome, development of AKIN stage 3, or death, those who met the outcome had higher uAnCR than those who did not (median, 280.72 [IQR, 638.96–1189.98] compared with 894.71 [IQR, 335.43–2894.06] pg/mg; P<0.001).

Univariate Prediction of AKIN Stage 3 or Death
Selected clinical variables and putative prognostic biomarkers were tested for the ability to predict the outcome AKIN stage 3 or death. Cleveland Clinic score (Figure 1A) and the percentage change in sCr at the time of sample collection (Figure 1B) both predicted the outcome, with an AUC of 0.72 (95% CI, 0.62 to 0.83) and 0.68 (95% CI, 0.55 to 0.82), respectively. In comparison, angiotensinogen and renin were also moderately strong predictors (Figure 1, C and D), whereas the urine protein-to-creatinine ratio did not predict AKIN stage 3 or death (AUC, 0.58; 95% CI, 0.44 to 0.72). The AUC of the uAnCR was 0.75 (95% CI, 0.65 to 0.85). The AUC of the uRenCR was 0.70 (95% CI, 0.57 to 0.83). Additionally, the prognostic predictive power of these variables was evaluated in the subset of the cohort (n=81) that was classified as RIFLE (Risk, Injury, Failure, Loss, and ESRD) stage R at the time of collection because it has been reported that this criterion has a lower false-positive rate than AKIN stage 1 for diagnosis of AKI. Compared with the entire cohort, this analysis found little difference in the ability of uAnCR (AUC, 0.73; 95% CI, 0.60 to 0.87), uRenCR (AUC, 0.71; 95% CI, 0.55 to 0.87), and Cleveland Clinic score (AUC, 0.74; 95% CI, 0.61 to 0.88) to predict AKIN stage 3 or death, whereas the predictive power of the percentage increase in serum creatinine (AUC, 0.77; 95% CI, 0.67 to 0.88) was substantially improved.

Multivariable Prediction of AKIN Stage 3 or Death
Multivariable logistic regression was used to create a model that included relevant clinical variables and biomarkers. Cleveland Clinic score and percentage change in sCr were combined into a multivariable logistic regression model. This clinical model had an AUC of 0.79 (95% CI, 0.69 to 0.88) for the outcome of AKIN stage 3 or death.

### Table 1. Characteristics of cohort of post–cardiac surgery patients enrolled in study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AKIN Stage 1 or 2 and Survived</th>
<th>AKIN Stage 3 or Death</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>178</td>
<td>26</td>
<td>0.97</td>
</tr>
<tr>
<td>Age (yr)(^{,b})</td>
<td>68 (59.0–76.0)</td>
<td>65.5 (58.0–79.0)</td>
<td>0.71</td>
</tr>
<tr>
<td>Women, % (n)</td>
<td>32.6 (58)</td>
<td>38.5 (10)</td>
<td>0.9</td>
</tr>
<tr>
<td>White, % (n)</td>
<td>70.2 (125)</td>
<td>69.2 (18)</td>
<td>0.9</td>
</tr>
<tr>
<td>Surgical variables(^{a})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CABG, % (n)</td>
<td>46.6 (83)</td>
<td>34.6 (9)</td>
<td>0.35</td>
</tr>
<tr>
<td>Valve replacement, % (n)</td>
<td>28.7 (51)</td>
<td>26.9 (7)</td>
<td>0.96</td>
</tr>
<tr>
<td>CABG + valve, % (n)</td>
<td>16.9 (30)</td>
<td>23.1 (6)</td>
<td>0.62</td>
</tr>
<tr>
<td>Other, % (n)</td>
<td>7.9 (14)</td>
<td>15.4 (4)</td>
<td>0.37</td>
</tr>
<tr>
<td>Bypass, % (n)</td>
<td>86.0 (153)</td>
<td>88.5 (23)</td>
<td>0.97</td>
</tr>
<tr>
<td>Bypass time (min)(^{b})</td>
<td>141.0 (83.0–192.0)</td>
<td>159.5 (62.0–203.0)</td>
<td>0.66</td>
</tr>
<tr>
<td>Collection time (hr postop)(^{b})</td>
<td>21.8 (19.2–43.0)</td>
<td>21.6 (19.2–33.6)</td>
<td>0.69</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative(^{b})</td>
<td>1.1 (0.9–1.3)</td>
<td>1.3 (1.0–1.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>At collection(^{b})</td>
<td>1.6 (1.3–1.9)</td>
<td>1.9 (1.6–3.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Increase at collection (%)(^{b})</td>
<td>41 (30–56)</td>
<td>64 (35–80)</td>
<td>0.003</td>
</tr>
<tr>
<td>Outcomes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to maximum creatinine (d)(^{b,}(^c)</td>
<td>2.0 (1.0–3.0)</td>
<td>5.0 (3.75–8.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time to discharge or death (d)(^{b,}(^c)</td>
<td>7.0 (6.0–10.0)</td>
<td>14.0 (9.75–24.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AKIN stage 3, % (n)</td>
<td>0 (0)</td>
<td>84.6 (22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AKIN stage 3 or death, % (n)</td>
<td>0 (0)</td>
<td>100 (26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Death, % (n)</td>
<td>0 (0)</td>
<td>34.6 (9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Renal replacement therapy, % (n)</td>
<td>0 (0)</td>
<td>50.0 (13)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Statistical significance was determined by the chi-squared test for categorical variables and the Mann-Whitney U test for continuous variables. AKIN, Acute Kidney Injury Network; CABG, coronary artery bypass grafting.

\(^{a}\)Type of surgery is reported as CABG only, valve replacement only, CABG + valve replacement, and other procedures.

\(^{b}\)Continuous variables are reported as median (interquartile range).

\(^{c}\)Days are reported as the number of days after surgery.
Table 2. Distribution of urinary biomarker concentrations by maximum Acute Kidney Injury Network stage

<table>
<thead>
<tr>
<th>Variable</th>
<th>AKIN Stage 1</th>
<th>AKIN Stage 2</th>
<th>AKIN Stage 3</th>
<th>P Value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>156</td>
<td>26</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>uAnCR (ng/mg)</td>
<td>29.22 (10.72–82.42)</td>
<td>36.39 (14.56–163.54)</td>
<td>96.7&lt;sup&gt;b&lt;/sup&gt; (38.23–457.34)</td>
<td>0.002</td>
</tr>
<tr>
<td>uRenCR (pg/mg)</td>
<td>257.28 (113.88–564.34)</td>
<td>406.79 (144.06–922.47)</td>
<td>894.71&lt;sup&gt;b&lt;/sup&gt; (335.43–2894.06)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Biomarker concentrations are reported as median (interquartile range). AKIN, Acute Kidney Injury Network; uAnCR, urinary angiotensinogen-to-creatinine ratio; uRenCR, urinary renin-to-creatinine ratio.

<sup>a</sup>Kruskal-Wallis test.

<sup>b</sup>P<0.05 in post hoc pairwise comparison with AKIN stage 1 group.

Figure 1. Univariate receiver-operating characteristic (ROC) curves for the outcome of Acute Kidney Injury Network stage 3 AKI or death. Clinical variables Cleveland Clinic score (A) and percentage increase in serum creatinine (sCr) (B) at the time of sample collection, as well as the biomarkers urinary angiotensinogen-to-creatinine ratio (C) and urinary renin-to-creatinine ratio (D) were tested for the ability to predict the outcome. The diagonal gray line shows the line of identity for between the true-positive (sensitivity) and false-positive (1-specificity) rates of the test and has an area under the ROC curve (AUC) of 0.5. Variables were considered predictive if the AUC was >0.5 and the 95% confidence interval (CI) did not overlap 0.5.
When uAnCR was added to the clinical model, the AUC improved to 0.85 (95% CI, 0.78 to 0.92; \( P = 0.01 \) compared with clinical model) (Figure 2A). We used cfNRI and integrated discrimination improvement to assess the incremental improvement in prognostic accuracy gained by addition of uAnCR to the clinical model (Table 3). The cfNRI for addition of uAnCR to the model was 0.67 (95% CI, 0.26 to 1.09; \( P = 0.001 \)), and classification improved for both patients who did (events) and did not (nonevents) meet the outcome (cfNRIevents, 0.28; cfNRInonevents, 0.39) (Figure 2, B and C). The integrated discrimination improvement was 0.06 (\( P = 0.09 \)). Although the integrated discrimination improvement did not reach statistical significance, the calculated risk for the event group increased significantly when uAnCR was added to the model (median risk, 0.22 versus 0.26 for the clinical model and the clinical model plus uAnCR, respectively; \( P = 0.04 \)). Addition of uAnCR increased in both sensitivity and specificity (Figure 2, F and G). A final model was created that included the clinical model, uAnCR, and uRenCR. The addition of uRenCR did not further improve the AUC (AUC, 0.85; 95% CI, 0.77 to 0.92) (Figure 2A). However, uRenCR had a cfNRI of 0.55 (95% CI, 0.14 to 0.96; \( P = 0.01 \)) (Table 3), which was primarily driven by an improvement in the

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**Figure 2.** Urinary angiotensinogen and renin improve prediction of a clinical model for the outcome of Acute Kidney Injury Network stage 3 AKI or death. (A) Receiver-operating characteristic (ROC) curves are shown for the clinical model (includes Cleveland Clinic score and percentage change in serum creatinine at the time of sample collection), the clinical model plus angiotensinogen (uAnCR), and the clinical model plus uAnCR plus urinary renin (uRenCR). ROC curves were considered statistically significant if the 95% CI of the area under the ROC curve (AUC) did not overlap 0.5. (B–E) Scatterplots show the improvement in risk prediction gained by adding (B and C) uAnCR and (D and E) uRenCR to the multivariate clinical model. The diagonal gray line represents the line of identity, which indicates no change in the calculated risk between the model before and after addition of the biomarker. Data points represent the calculated risks for individual patients using the two models being compared. If the data point lies below the line of identity, addition of the biomarker lowers this patient’s calculated risk, whereas if the data point is above the line of identity, the addition of the biomarker increases the calculated risk. Addition of uAnCR to the clinical model resulted in a net lower calculated risk for (B) patients who did not meet the combined outcome (nonevents; category free net reclassification improvement [cfNRI] nonevents, 0.39) and a net higher calculated risk for (C) patients who did meet the outcome (events; cfNRI events, 0.28). Addition of uRenCR to the clinical model plus uAnCR resulted in a net higher calculated risk for (E) patients who met the outcome (events; cfNRI events, 0.44) and a modest net lower risk for (D) patients who did not meet the outcome (nonevents; cfNRI nonevents, 0.11). The integrated sensitivity and specificity plots (F and G) show the improvement in sensitivity and specificity gained by addition of the biomarkers. Addition of uAnCR to the clinical model resulted in a gain of both sensitivity (F) and specificity (G), while addition of uRenCR to the clinical model plus uAnCR increased sensitivity (F) but did not alter specificity (G).
In this retrospective cohort study, we measured angiotensinogen and renin in urine samples that had been obtained from patients who had undergone cardiac surgery early after AKI diagnosis had been made on the basis of AKIN serum creatinine criteria. Of these patients, only 81 had an increase in sCr >50% and none had an increase ≥100% at the time of collection. Both urinary angiotensinogen and renin predicted the composite outcome of AKIN stage 3 or death. Although this study is limited by the use of a composite outcome that relatively few (26 of 204) patients met, we chose this outcome because our objective was to identify patients at high risk of severe adverse outcomes. Selection bias cannot be entirely ruled out because of the retrospective design of this study. We demonstrated that urinary angiotensinogen improved the predictive power of a clinical model by using the net reclassification improvement and integrated discrimination improvement. These data confirm our previous findings regarding the prognostic predictive power of urinary angiotensinogen, although it is not a true

### Table 3. Incremental improvement in prognostic predictive power by addition of angiotensinogen and renin to a clinical model

<table>
<thead>
<tr>
<th>Reference Model</th>
<th>New Model</th>
<th>cfNRI_events</th>
<th>cfNRI_nonevents</th>
<th>cfNRI (95% CI)</th>
<th>P Value</th>
<th>IDI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical modelż</td>
<td>Clinical modelż + uAnCR</td>
<td>0.28</td>
<td>0.39</td>
<td>0.67 (0.26 to 1.09)</td>
<td>0.001</td>
<td>0.06</td>
<td>0.09</td>
</tr>
<tr>
<td>Clinical modelż + uAnCR</td>
<td>Clinical modelż + uAnCR + uRenCR</td>
<td>0.44</td>
<td>0.11</td>
<td>0.55 (0.14 to 0.96)</td>
<td>&lt;0.01</td>
<td>0.01</td>
<td>0.38</td>
</tr>
</tbody>
</table>

cfNRI, category free net reclassification improvement; CI, confidence interval; IDI, integrated discrimination improvement; uAnCR, urinary angiotensinogen-to-creatinine ratio; uRenCR, urinary renin-to-creatinine ratio.

*cfNRI is a means of calculating the effect of adding a new variable to a predictive model on the overall accuracy of the model. cfNRI is the sum of cfNRI\_events and cfNRI\_nonevents, cfNRI\_events and cfNRI\_nonevents are the proportion of patients who met the outcome (events) or those who did not, respectively, which are correctly reclassified by the new model minus the proportion of patients who are incorrectly reclassified. Correct reclassification is defined as a calculated risk of meeting the outcome that is higher for events and lower for nonevents compared with the reference model. If all events and nonevents were correctly reclassified, the cfNRI\_events and cfNRI\_nonevents would be +1, and the cfNRI would be 2.

*IDI is a means of quantifying the effect of addition of a new variable to a predictive model on the magnitude of the change in the difference between the average calculated risk of patients who met the outcome compared with those who did not. The mean risk of the two groups is calculated using the reference model and the new model, and IDI is simply the difference between the discrimination slopes of the two models.

*Clinical model is a multivariate logistic regression model including the Cleveland Clinic score and the percentage increase in serum creatinine from baseline at the time of sample collection.

### Discussion

In this retrospective cohort study, we measured angiotensinogen and renin in urine samples that had been obtained from patients who had undergone cardiac surgery early after AKI diagnosis had been made on the basis of AKIN serum creatinine criteria. Of these patients, only 81 had an increase in sCr >50% and none had an increase ≥100% at the time of collection. Both urinary angiotensinogen and renin predicted the composite outcome of AKIN stage 3 or death. Although this study is limited by the use of a composite outcome that relatively few (26 of 204) patients met, we chose this outcome because our objective was to identify patients at high risk of severe adverse outcomes. Selection bias cannot be entirely ruled out because of the retrospective design of this study. We demonstrated that urinary angiotensinogen improved the predictive power of a clinical model by using the net reclassification improvement and integrated discrimination improvement. These data confirm our previous findings regarding the prognostic predictive power of urinary angiotensinogen, although it is not a true

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**Classification Tree**

Multivariate logistic regression is a powerful technique for evaluating the predictive power of biomarkers. However, because of its complexity it is unlikely to be clinically useful in the setting of acute illness, where the decision to intervene is especially time sensitive. Additional limitations include the inability to identify subsets of patients in whom biomarkers underperform and insensitivity to potentially important nonlinear interactions between covariates in the model, both of which could be informative from a mechanistic perspective. Therefore, we sought to create a simple algorithm that identifies patients at high risk of meeting the outcome AKIN stage 3 or death, which could be used to guide decision-making. To accomplish this we chose to use CHAID to grow a classification tree that assigned patients to risk groups by identifying interactions among the same variables that were previously used in multivariate logistic regression. In this analysis, uAnCR, uRenCR, Cleveland Clinic score, and percentage change in serum creatinine were all statistically significant classifiers for the outcome (Supplemental Figure 1). However, the model selected only urinary angiotensinogen and renin for use in prediction of the outcome (Figure 3). Using a cutoff of >337.89 ng/mg for uAnCR, the model divided patients into low-risk (n=184) and intermediate-risk (n=20) groups, in which 9.8% and 40.0% of the patients met the outcome, respectively. The intermediate-risk group was then further subdivided into a low- and high-risk group using the cutoff of uRenCR >893.41 pg/mg. Applying the combination of these two cutoffs for uAnCR and uRenCR resulted in a group of 10 patients, 8 of whom met the outcome, yielding a sensitivity of 30.8% and specificity of 98.9%. Because specificity is the primary determinant of PPV and NPV when the prevalence of the outcome of interest is low, as was the case in our cohort, the model achieved high PPV and NPV (80.4% and 90.7%, respectively). Overall, the model correctly classified 90.2% of patients, and the 25-fold cross-validation estimate of the risk of misclassification of events (± SEM) was 0.13±0.02. This model had an AUC of 0.91 and compared favorably with the multivariate logistic regression model (Table 4 and Figure 4).
validation study because approximately one third of the patients included in this cohort (74 patients, 10 of whom met the outcome) were included in our previous study (19). We also found that elevated urinary renin is associated with severe AKI, and addition of renin to the multivariate model appeared to improve sensitivity. This finding indicates that the interaction between urinary angiotensinogen and renin concentration is an important prognostic indicator. However, a limitation of this study is that we did not compare these biomarkers to other novel AKI biomarkers, such as NGAL, KIM-1, IL-18, and liver-type fatty acid–binding protein, although we did compare them with the urinary protein-to-creatinine ratio.

CHAID was used to grow a classification tree to identify risk subgroups. Others have reported CHAID to be less accurate than multivariate logistic regression in ROC curve analysis (25). We chose to use CHAID because of its relative simplicity and the ease of graphic representation of the results. Additionally, CHAID offers some analytical advantages compared with logistic regression. Whereas logistic regression identifies independent predictors, CHAID is adept at identifying interactions among the variables, including nonlinear relationships, and it can be used to identify subgroups in which covariates exert the greatest influence in a predictive model (25–27). Thus, CHAID can be used to generate or test hypotheses regarding the potential role of the relationship between variables.
The CHAID classification tree model was the best predictor (Figure 2A, where it is titled clinical model plus uAnCR plus uRenCR. for the multivariate logistic regression (MLR) is also displayed in creatinine ratio, and urinary renin-to-creatinine ratio. The ROC curve atinine from baseline that had occurred, urinary angiotensinogen-to-creatinine ratio (uAnCR), and urinary renin-to-creatinine ratio (uRenCR). AUC, area under the receiver-operating characteristic curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; CHAID, chi-squared automatic interaction detection.

Variables: Cleveland clinic score, percentage increase in serum creatinine at the time of sample collection, urinary angiotensinogen-to-creatinine ratio (uAnCR), and urinary renin-to-creatinine ratio (uRenCR). The ROC curves of two multivariate models, a chi-squared automatic interaction detection (CHAID) classification tree, and a multivariate logistic regression model are shown. Both models included four interaction detection (CHAID) classification tree, and a multivariate logistic regression model were generated using the following variables: Cleveland Clinic score, percentage increase in serum creatinine at the time of sample collection, urinary angiotensinogen-to-creatinine ratio (uAnCR), and urinary renin-to-creatinine ratio (uRenCR). AUC, area under the receiver-operating characteristic curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; CHAID, chi-squared automatic interaction detection.

<table>
<thead>
<tr>
<th>Model</th>
<th>AUC (95% CI)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multivariate logistic regression$^a$</td>
<td>0.85 (0.77 to 0.92)</td>
<td>76.9</td>
<td>79.1</td>
<td>35.0</td>
<td>76.5</td>
</tr>
<tr>
<td>CHAID$^b$</td>
<td>0.91 (0.87 to 0.96)$^c$</td>
<td>30.8</td>
<td>98.9</td>
<td>80.4</td>
<td>90.7</td>
</tr>
</tbody>
</table>

Multivariate logistic regression and CHAID models were generated using the following variables: Cleveland Clinic score, percentage increase in serum creatinine at the time of sample collection, urinary angiotensinogen-to-creatinine ratio (uAnCR), and urinary renin-to-creatinine ratio (uRenCR). AUC, area under the receiver-operating characteristic curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; CHAID, chi-squared automatic interaction detection.

$^a$Cutoff specific performance characteristics shown are from the point on the receiver-operating characteristic curve closest to the point of 100% sensitivity and specificity.

$^b$Cutoff specific performance characteristics shown are for the node representing uAnCR >337.89 ng/mg and uRenCR >893.41 pg/mg.

$^c$P=0.02 compared with multivariate logistic regression model.

Figure 4. | Receiver-operating characteristic curves (ROCs) for prediction of Acute Kidney Injury Network stage 3 AKI or death. The ROC curves of two multivariate models, a chi-squared automatic interaction detection (CHAID) classification tree, and a multivariate logistic regression model are shown. Both models included four variables: Cleveland Clinic score, percentage increase in serum creatinine from baseline that had occurred, urinary angiotensinogen-to-creatinine ratio, and urinary renin-to-creatinine ratio. The ROC curve for the multivariate logistic regression (MLR) is also displayed in Figure 2A, where it is titled clinical model plus uAnCR plus uRenCR. The CHAID classification tree model was the best predictor (P=0.02 compared with the multivariate logistic regression model).

and the outcome of interest, which is a particularly intrigu- ing feature for testing combinations of biomarkers (26). The CHAID classification tree identified patients with con- comitant elevation of both angiotensinogen and renin as the group with the highest risk for the outcome of AKIN stage 3 or death. We interpret these results as an indication that activation of the renin-angiotensin system could mod- ulate AKI severity. Importantly, this analysis found that angiotensinogen was a stronger predictor than renin, and renin was not a useful predictor when angiotensinogen was <337.89 ng/mg. This is congruent with our hypothesis that renin improves the predictive accuracy of angio- tensinogen based on the biologic relationship between the two proteins. Angiotensinogen is the only known natural substrate for renin (21). The CHAID model is also an in- formative guide for the potential use of the combination of these biomarkers in the event of discordance between the two. A limitation of the results of the CHAID model is that it identified only 30.8% of patients who met the outcome. Thus, if used to direct clinical decision-making, it would miss many patients who might benefit from intervention. However, it ruled out patients who did not meet the outcome with high accuracy, which could be clinically valu- able. By identifying patients who will not develop the outcome and therefore do not require intervention, this test could reduce unnecessary exposure of low-risk pa- tients to therapies that could have adverse effects.

Our findings suggest a role for the RAS in the pathobi- ology of AKI, which is in agreement with data from animal models of AKI. Angiotensin II increases and angiotensin 1–7 decreases in kidney tissue after ischemia reperfusion injury in rats (28,29). Interestingly, intrarenal angiotensin II concentration strongly correlates with urinary angioten- sinogen concentration but is not correlated with plasma angiotensinogen, which implies that elevated urinary an- giotensinogen reflects activation of the intrarenal RAS (30). Increased amounts of renin would also contribute to RAS activation because the conversion of angiotensinogen to angiotensin I by renin is the rate-limiting step of the RAS. Inhibition of angiotensin-converting enzyme and the angio- tensin II type 1 receptor with captopril and losartan, respec- tively, reduce renal inflammation and mitigate the severity of AKI in rats subjected to renal ischemia-reperfusion in- jury (31–37). Therefore, patients with higher concentrations of urinary angiotensinogen and renin could have a more severe inflammatory response during AKI. Importantly, the source of urinary angiotensinogen and renin during AKI remains unclear. However, both of these genes are expressed in both the proximal and distal tubule, and both proteins have been detected in the proximal tubule (38,39).

Urinary angiotensinogen and renin appear to be elevated during the early extension phase of AKI (most patients in our cohort were classified as having AKIN stage 1 but not
as meeting RIFLE-R criteria), and this biomarker combination could be used to guide clinical trial enrollment. Although therapies that prevent or mitigate injury, and those that reverse early injury, would be unlikely to be effective at this time point, interventions that prevent extension of the injury by attenuating inflammation and those that promote renal recovery could still be useful. Indeed, angiotensinogen and renin could be ideal for selectively enriching the study population in patients who could potentially benefit from these types of therapies. Although it correctly identified only 30.8% of the patients who met the outcome of AKIN stage 3 or death, the combination of uAnCR >337.89 ng/mg and uRenCR >893.41 pg/mg had a PPV of 80.4%, a 6.3-fold enrichment for the endpoint of stage 3 AKI or death (compared with 12.8% prevalence). The large PPV relative to sensitivity was due to the combination of a low prevalence of the outcome in our cohort and a high specificity (98.9%), which resulted in a test that ruled out low-risk patients with high accuracy. Using the combination of uAnCR and uRenCR to screen patients for enrollment in a clinical trial would increase the effect size of an intervention and result in an improved statistical power and a reduction in the number of patients who would need to be enrolled. However, the tradeoff is that a large number of patients would need to be screened. Using our cohort as an example, the ratio of enrolled to screened patients would be approximately 1:19 if the results from the CHAID model were used as inclusion criteria enrollment because only 4.9% of patients had uAnCR and uRenCR values above these cutoffs. Therefore, the cost of screening would need to be weighed against the potential benefits of enrichment (increased power and reduced enrollment), taking into account the assumed effect size of the intervention.

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Disclosures
J.M.A., M.G.J., and J.L.A. are named inventors on a patent application filed by the Medical University of South Carolina for angiotensinogen as a kidney disease biomarker.

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