Urinary Angiotensinogen Level Predicts AKI in Acute Decompensated Heart Failure: A Prospective, Two-Stage Study

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ABSTRACT
A major challenge in prevention and early treatment of acute cardiorenal syndrome (CRS) is the lack of high-performance predictors. To test the hypothesis that urinary angiotensinogen (uAGT) is an early predictor for acute CRS and 1-year prognosis in patients with acute decompensated heart failure (ADHF), we performed a prospective, two-stage, multicenter cohort study in patients with ADHF. In stage I (test set), 317 patients were recruited from four centers. In stage II (validation set), 119 patients were enrolled from two other centers. Daily uAGT levels were analyzed consecutively. AKI was defined according to Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guidelines. In stage I, 104 (32.8%) patients developed AKI during hospitalization. Daily uAGT peaked on the first hospital day in patients who subsequently developed AKI. After multivariable adjustment, the highest quartile of uAGT on admission was associated with a 50-fold increased risk of AKI compared with the lowest quartile. For predicting AKI, uAGT (area under the receiver-operating characteristic curve [AUC]=0.84) outperformed urinary neutrophil gelatinase-associated lipocalin (AUC=0.78), the urinary albumin/creatinine ratio (AUC=0.71), and the clinical model (AUC=0.77). Survivors in stage I were followed prospectively for 1 year after hospital discharge. The uAGT level independently predicted the risk of 1-year mortality (adjusted odds ratio, 4.5; 95% confidence interval, 2.1 to 9.5) and rehospitalization (adjusted odds ratio, 3.6; 95% confidence interval, 1.6 to 5.7). The ability of uAGT in predicting AKI was validated in stage II (AUC=0.79). In conclusion, uAGT is a strong predictor for acute CRS and 1-year prognosis in ADHF.


Acute heart failure is the leading cause of hospitalization worldwide and represents a significant economic burden.1 In patients with acute decompensated heart failure (ADHF), the incidence of AKI is high, ranging from 25% to 51%.2–4 Coexistence of acute cardiac and renal dysfunction, known as acute (or type 1) cardiorenal syndrome (CRS),5 is a serious clinical state associated with significantly increased morbidity and mortality.6,7

A major barrier to improved clinical outcomes in patients with ADHF is the lack of ability to identify

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patients at high risk of AKI early enough to initiate beneficial interventions. It is well recognized that serum creatinine is not an early marker of AKI in patients with ADH. In recent years, efforts have been directed toward the development of biomarkers for the early detection of AKI, and have yielded several biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1, and IL-18. To date, only a few small clinical studies have tested the utility of these biomarkers, mostly NGAL, in predicting acute CRS and have yielded only modest performance in general. In addition, there is a particular lack of predictors for longer-term prognosis in patients with acute CRS. In each of these circumstances, the early detection of patients at high risk of developing AKI, before a detectable change in serum creatinine, would afford clinicians a critical time window to halt or reverse the ongoing renal injury.

The underlying causes of acute CRS are multifactorial, but are likely related to the derangement in cardiorenal interactions as well as renal hypoperfusion as the result of inappropriate use of diuretics or other strategies to alleviate congestion. Increasing evidence has revealed that the intrarenal renin-angiotensin system (RAS) plays a vital role in maintaining hemodynamic balance and cardiorenal interaction, which are often disrupted in patients with acute heart failure. In animal studies, activation of the intrarenal RAS is an initial response to hypoperfusion in cardiac and renal injury, and is also an important contributor to the progression of the disease. Intraparenal angiotensinogen (AGT), a principal substrate of the local RAS, is mainly formed in the proximal tubule cells and secreted into the tubule lumen. Urinary AGT (uAGT) level correlates with intrarenal AGT and angiotensin II, and has been shown to be an indicator of intrarenal RAS activity in both animal and clinical studies.

We conducted a prospective, two-stage, multicenter observational study in 436 patients with ADHF who were enrolled on the first day of admission. In stage I (test set), 317 patients with ADHF were recruited from four centers. In stage II (validation set), 119 patients were enrolled from two other centers. Our primary objective was to test and validate the hypothesis that daily measures of uAGT during the first week of admission predict the development of AKI and 1-year prognosis in these patients. This is the first study in patients with ADHF to demonstrate the utility of uAGT as a predictive biomarker of AKI and 1-year mortality, rehospitalization, and recovery of renal function.

RESULTS

Cohort Description

Stage I (Test Set)

There were 423 patients with ADHF (all Chinese) enrolled from four centers who were screened for potential participation. Of these patients, 106 were excluded according to the exclusion criteria. A total of 317 patients, 47.7% with newly diagnosed ADHF, were included in the study (Supplemental Figure 1).

Stage II (Validation Set)

There were 149 patients with ADHF from the other two centers who were screened according to a protocol exactly the same as that used in stage I. Of these patients, 30 were excluded. A total of 119 patients, 42.9% with newly diagnosed ADHF, were enrolled (Supplemental Figure 1).

uAGT as a Predictor for the Primary End Point

In the stage I study, AKI occurred in 104 (32.8%) patients, with 90% (n=94) occurring within 5 days after admission. We grouped patients into those with and without AKI. Compared with those who did not develop AKI, patients who developed AKI were older, with more comorbidity and more severe acute heart failure. AKI was more frequent in those with preexisting CKD (Table 1). However, no differences were found between the two groups with respect to the use of renin-angiotensin-aldosterone system inhibitors or diuretics (Supplemental Table 1).

Patients enrolled in the stage II study exhibited characteristics similar to those in stage I (Supplemental Table 2).

In the test set, uAGT values on admission were significantly higher in patients who subsequently developed AKI during hospitalization compared with those who did not develop AKI and healthy volunteers (Figure 1A). Figure 1B displays serial measures of uAGT over the first 7 days of hospital admission. Compared with patients with ADHF who did not develop AKI, those who developed AKI had a marked rise in uAGT at all-time points (P<0.001). The pattern of uAGT was characterized by a peak on the first day of hospital admission; by contrast, the peak of serum creatinine rise occurred on the fourth day of hospital admission. The increase in serum creatinine lasted for >3 days in 81.7% of patients with AKI. The ELISA results were further confirmed by Western blot (Supplemental Figure 2A). Elevation of uAGT was noted in patients with AKI both with and without preexisting CKD, but the levels were significantly higher in patients with prior CKD (Figure 1C).

Unlike uAGT, plasma AGT levels on admission were similar between patients who developed AKI and those who did not develop AKI. There was no significant change in plasma AGT in patients with ADHF over the first 7 days of hospital admission (Supplemental Figure 2, B and C).

uAGT levels were associated with incident AKI in patients with and without preexisting CKD in the stage I cohort (Supplemental Figure 3). This was further supported by the multivariate analyses. After adjustment for centers and for clinical variables including the use of RAS inhibitors or diuretics, uAGT was the most powerful predictor of AKI, which was true for patients with and without preexisting CKD. The highest quartile of uAGT on the first day of admission was associated with increased risk for AKI by 50-fold compared with the lowest quartile (Table 2). When uAGT was analyzed as a continuous variable, higher uAGT was also associated with the development of AKI (odds ratio per SD, 5.32; 95% confidence interval [95% CI], 3.32 to 8.52; P<0.001) in a multivariable model.

Furthermore, changes in uAGT (baseline levels minus levels at days 2–7), when used as time-dependent covariates, were
significantly associated with increased risk of AKI after adjustment for other known risk factors (hazard ratio [HR], 1.26; 95% CI, 1.13 to 1.41; P<0.001).

Performance of uAGT for Predicting AKI in Subgroup Analyses

For predicting AKI, the area under the receiver-operating characteristic (ROC) curve (AUC) of uAGT on admission for all participants in the test set was 0.84. A cutoff of 55 μg/g creatinine yielded good sensitivity (0.80) and specificity (0.78) (Figure 2A). The AUCs of uAGT, in subgroups with and without preexisting CKD, were greater than those of urinary NGAL, the urinary albumin to creatinine ratio (UACR), and the clinical model, in particular, among patients with prior CKD (Figure 2, A–C). The best cutoff value of uAGT for predicting AKI was significantly higher in patients with prior CKD (96.6 μg/g creatinine) compared with those with preserved eGFR on admission (50.0 μg/g creatinine).

To further validate the predictive value of uAGT for AKI, the AUC for predicting AKI was analyzed in an independent validation cohort recruited from the other centers (stage II study). As shown in Figure 2D, uAGT was validated in predicting AKI in the stage II cohort (AUC=0.79). Moreover, using the optimal cutoff of uAGT obtained from the test cohort (55 μg/g), the predictive performance of uAGT in the validation set yielded similar sensitivity (0.72) and specificity (0.76) compared with those in the test set (0.80 and 0.78, respectively).

uAGT as a Predictor for Secondary End Points

Of the 317 participants in the stage I study, 55 (17.3%) died within 1 year after admission. A level of uAGT≥55 μg/g creatinine on admission was associated with a significantly increased probability of all-cause mortality (HR, 4.7; 95% CI, 2.7 to 8.2) and rehospitalization (HR, 3.7; 95% CI, 2.2 to 6.6) over the 1-year follow-up period (Supplemental Figure 4, A and B). Because of sample size constraints, we were unable to conduct subgroup analyses. Figure 3A shows the mortality of participants as a function of uAGT level and brain natriuretic peptide (NT-proBNP)
concentration on admission. Patients with ADHF with a 
 uAGT level $\geq 55 \mu g/g$ creatinine and an NT-proBNP level 
 above the median had the highest overall mortality. Patients 
 with an elevated NT-proBNP but without an increase in uAGT 
 had a prognosis comparable to those with lower NT-proBNP. 
 For predicting 1-year mortality, the AUC of uAGT was 0.77, 
 which was greater than that of NT-proBNP (0.63) and of the 
 clinical model alone (0.75) (Figure 3B).

Compared with those with uAGT $<55 \mu g/g$ creatinine, 
 patients with AKI with uAGT $\geq 55 \mu g/g$ creatinine on admission 
 exhibited a significantly higher rate of failure in renal 
 recovery at discharge (26.5% versus 70.6%). Among patients 
 who developed AKI in the absence of preexisting CKD, a 
 higher incidence of progression to CKD (48.0%) was found 
 in those with uAGT $\geq 55 \mu g/g$ creatinine compared with 
 patients with uAGT $<55 \mu g/g$ creatinine (16.0%). After multivariate adjustment, uAGT $\geq 55 \mu g/g$ creatinine was the most 
powerful predictor for 1-year mortality and rehospitalization 
(Table 3), as well as failure in renal recovery (Table 4).

In addition to uAGT levels on admission, uAGT concentrations 
at discharge also predicted postdischarge mortality 
(AUC, 0.71, 95% CI, 0.62 to 0.81) and failure in renal recovery 
(AUC, 0.64; 95% CI, 0.51 to 0.77).

**Effect of uAGT on Risk Reclassification of AKI and 1-Year Mortality**

To determine whether uAGT materially improved risk reclassification, we used the net reclassification index (NRI) and the integrated discrimination improvement (IDI) in the stage I study. As shown in Table 5, the addition of uAGT significantly improved the risk reclassification of AKI over urinary NGAL, the clinical model, and the combination of urinary NGAL and the clinical model. The addition of uAGT also significantly improved the prognostic prediction over NT-proBNP and the clinical model alone.

**DISCUSSION**

A major challenge in prevention and early treatment of acute CRS has been a lack of high-performance predictive biomarkers. The current use of serum creatinine as an indicator of CRS often leads to diagnostic delays and potential misclassification of actual injury status, and produces little information regarding the underlying cause.27

In a stage I study of 317 patients with ADHF, we found that uAGT measured on the first day of hospital admission is a powerful predictor for AKI. Elevation of uAGT predicted acute CRS 2–4 days before rises in serum creatinine. The performance of uAGT is superior to previously reported biomarkers such as NGAL and UACR, as well as the clinical predictive model. The value of uAGT for predicting acute CRS was further demonstrated in a validation cohort of 119 patients in the stage II study. These data suggest that uAGT might be a novel and strong biomarker for identifying patients at high risk of CRS in the setting of ADHF. Supporting our findings, previous reports identified an association between elevated uAGT levels and the deterioration of renal function in patients with CKD.26

The question of whether the uAGT level specifically predicts CRS or whether it also serves as a biomarker for AKI from other causes remains to be addressed. Active investigations are underway.

To date, only small-scale clinical studies have been conducted to examine the utility of the biomarkers, mostly NGAL,
in predicting AKI in ADHF, which only yielded modest performance. NGAL is a well established biomarker for renal injury and is the most tested marker in patients with ADHF.\textsuperscript{13–19} Consistent with previous reports,\textsuperscript{19,28} the performance of urinary NGAL in this cohort had an AUC of 0.78. uAGT improved the AUC to 0.84. The predictive performance of uAGT was also better than UACR (0.84 versus 0.71), a marker that is associated with postoperative AKI in patients undergoing cardiac surgery.\textsuperscript{29} Although uAGT level was reported to be correlated with proteinuria in patients with renal impairment,\textsuperscript{30} elevation of uAGT was an independent predictor of CRS even after adjusting UACR. The risk reclassification, as measured by NRI and IDI, was significantly improved through the addition of uAGT to the clinical model and to the combination of urinary NGAL and the clinical model, supporting the concept that a single biomarker is not sufficient for the evaluation of a complex clinical setting such as acute CRS. A multimarker approach is therefore more likely to be of greater use.

In patients who developed acute CRS, those with preexisting CKD had a higher risk of mortality and morbidity than those without.\textsuperscript{4} However, prediction of AKI in patients with ADHF with preexisting CKD has not been well investigated in previous biomarker studies. In this study, we recruited patients with ADHF whose serum creatinine records over the 6-month period before admission were available. This design allowed us to determine the predictive performance of uAGT in patients with and without prior CKD. Our results showed that elevation of uAGT on admission was more prominent in patients with preexisting CKD compared with those without, and that the best cutoff value was

### Table 2. Multivariate logistic regression analyses of uAGT as a predictor for AKI in the stage I cohort

<table>
<thead>
<tr>
<th>uAGT (μg/g Cr) on Admission</th>
<th>Unadjusted OR</th>
<th>Adjusted OR*</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All study participants (N=317; 79–80 per quartile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (&lt;10)</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 2 (10–35)</td>
<td>3.67</td>
<td>2.23</td>
<td>1.05 to 4.38</td>
<td>0.04</td>
</tr>
<tr>
<td>Quartile 3 (36–148)</td>
<td>22.92</td>
<td>11.31</td>
<td>4.58 to 27.99</td>
<td>0.00</td>
</tr>
<tr>
<td>Quartile 4 (&gt;148)</td>
<td>51.64</td>
<td>50.01</td>
<td>12.32 to 203.23</td>
<td>0.00</td>
</tr>
<tr>
<td>Patients without preexisting CKD (n=235; 58–59 per quartile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (&lt;9)</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 2 (9–30)</td>
<td>8.27</td>
<td>1.44</td>
<td>0.60 to 3.48</td>
<td>0.42</td>
</tr>
<tr>
<td>Quartile 3 (31–120)</td>
<td>42.52</td>
<td>13.68</td>
<td>3.90 to 47.92</td>
<td>0.00</td>
</tr>
<tr>
<td>Quartile 4 (&gt;120)</td>
<td>71.61</td>
<td>73.27</td>
<td>6.96 to 777.12</td>
<td>0.00</td>
</tr>
<tr>
<td>Patients with preexisting CKD (n=82; 20–21 per quartile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (&lt;12)</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 2 (12–71)</td>
<td>1.50</td>
<td>2.50</td>
<td>0.42 to 14.82</td>
<td>0.31</td>
</tr>
<tr>
<td>Quartile 3 (72–221)</td>
<td>12.50</td>
<td>10.67</td>
<td>1.94 to 58.69</td>
<td>0.01</td>
</tr>
<tr>
<td>Quartile 4 (&gt;221)</td>
<td>65.00</td>
<td>68.00</td>
<td>8.50 to 541.80</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Cr, creatinine.

* Adjusted for age, NT-proBNP, serum creatinine, serum albumin, hypertension, diabetes, UACR, hemoglobin, centers, treatment with diuretics, and treatment with RAS inhibitors. Cr, creatinine; OR, odds ratio.

**Figure 2. ROC analyses for predicting AKI. (A–C) uAGT, uNGAL, UACR, and clinical model for predicting AKI in all participants (A), in patients without preexisting CKD (B), and in patients with preexisting CKD (C). (D) ROC analysis for the test and validation sets. Cr, creatinine.**
higher in patients with prior CKD. In contrast with urinary NGAL, which best identifies AKI in patients with an eGFR in the range of 90 to 120 ml/min, the predictive performance of uAGT was better in individuals with eGFR<60 ml/min (patients with preexisting CKD). This unique characteristic of uAGT makes it a useful predictor for AKI, in particular, among those with preexisting CKD. This may have considerable implications for patients with ADHF, 30%–45% of whom present with preexisting CKD. Early prediction of AKI in these patients remains a challenge and previously reported biomarkers have shown only modest performance in this situation.

Our data also suggest that uAGT could be used at the time of hospital admission to assess 1-year prognosis of ADHF. ADHF is often associated with poor in-hospital and postdischarge outcomes, with a 25%–35% mortality rate at 12 months. Identification of those at higher risk of poor prognosis remains a challenge in clinical practice. Results of the study showed that a level of uAGT≥55 pg/g creatinine independently predicted 1-year mortality and rehospitalization in patients with ADHF. Elevation of uAGT also predicted an increased risk for progression to CKD in acute CRS, supporting the finding that a marked increase in uAGT is associated with severe AKI.

Importantly, we compared the prognostic performance of uAGT with a recently established clinical model and NT-proBNP, a validated marker for prognosis of acute heart failure. uAGT performed substantially better than NT-proBNP in predicting mortality (AUC=0.77 versus AUC=0.63). Furthermore, the addition of uAGT significantly improved risk reclassification of mortality over the clinical model and NT-proBNP alone as measured by NRI and IDI.

We simultaneously measured both urine and plasma AGT in this study. Unlike uAGT, plasma AGT did not significantly change in patients with ADHF with or without AKI. uAGT is an indicator reflecting the activity of intrarenal RAS, a hormonal cascade that has pleiotropic effects in the kidney, including the regulation of hemodynamics, sodium reabsorption, cellular proliferation, and apoptosis. It is now apparent that intrarenal RAS is regulated independently with circulating RAS. Renal AGT produced in proximal tubular cells seems to be secreted directly into the tubular lumen. In rats infused with human AGT, circulating AGT was not detectable in the urine, suggesting limited glomerular permeability and/or tubular degradation. These findings support the concept that uAGT originates from that secreted by renal tubule cells. In keeping with our findings, a previous study reports activation of intrarenal RAS in a renal ischemic model in the absence of change in circulating RAS.

Our study has the following strengths. First, we measured biomarkers from admission for 7 consecutive days, and compared the predictive performance of uAGT to previously reported biomarkers or clinical models in the setting of ADHF. We demonstrated that uAGT is a powerful predictor of AKI in ADHF and outperforms NGAL, UACR, NT-proBNP, and clinical models. Using a two-stage design, we showed consistent results in both the test and validation cohorts. The study participants were recruited from six centers, representing patients with ADHF in various clinical settings. In addition, the stage I study prospectively followed the patients with ADHF for 1 year after discharge, which allowed us to assess the utility of uAGT as a predictor of 1-year prognosis. Another strength of this study was that serum creatinine before admission was available for the study patients, which allowed us to determine the predictive performance of uAGT in subgroups with and without prior CKD. Finally, the level of uAGT was measured and validated by an ELISA kit and by Western blot in a central laboratory, which ensured high-quality uAGT measurements.

The study also had limitations. First, uAGT, but not blood AGT, was predictive of AKI in patients with ADHF. Although urinary markers have several advantages, including the non-invasive nature of sample collection and few interfering
proteins, some disadvantages also exist, including difficulty in collecting samples from patients with severe oliguria and potential changes in urinary biomarker levels induced by fluid status and diuretic therapy. Second, the diagnosis of AKI was based on an increase in serum creatinine, which may introduce the conundrum of using a flawed outcome variable to analyze the performance of novel biomarkers.† Evidence of AKI on renal biopsy would be the gold standard but was not feasible in our large cohort. As is true in the case of most AKI studies, we were not able to use urine output for AKI diagnosis because an indwelling urinary catheter was not present in most of the patients.

In summary, this study showed that uAGT can serve as an early predictor for the development of AKI and 1-year prognosis in the setting of ADHF. If further confirmed, use of uAGT as a predictive biomarker may improve clinicians’ ability to assess ADHF patients’ risk of developing acute CRS and prognosis, which in turn would help clinicians to plan and initiate the most appropriate management strategies during admission and after discharge for patients with ADHF.

**CONCISE METHODS**

**Patients**

This is a prospective, two-stage, multicenter cohort study approved by the Institutional Review Board of the National Clinical Research Center of Kidney Disease. All of the study participants provided written informed consent. The stage I study (test set) was conducted in four academic medical centers in three cities (Guangzhou, Shenzhen, and Guiyang) between September 2011 and September 2013. Sample collection for the stage II study (validation set) was performed in two centers in Beijing and Zhanjiang from February 2013 to February 2014. The patients in two stage study were enrolled according to the same inclusion and exclusion criteria described below.

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**Table 3. Multivariate logistic regression analyses: Predictors of mortality (n=317) and rehospitalization in the stage I cohort (n=286)**

<table>
<thead>
<tr>
<th>Variables on Admission</th>
<th>Unadjusted OR</th>
<th>Adjusted OR*</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality from admission to 1-yr follow-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (per 10-yr increase)</td>
<td>1.83</td>
<td>1.71</td>
<td>1.25 to 2.35</td>
<td>0.004</td>
</tr>
<tr>
<td>Treatment with diuretics (yes versus no)</td>
<td>2.53</td>
<td>2.45</td>
<td>1.11 to 5.41</td>
<td>0.03</td>
</tr>
<tr>
<td>NT-proBNP ≥ median (yes versus no)</td>
<td>2.92</td>
<td>2.59</td>
<td>1.22 to 5.52</td>
<td>0.02</td>
</tr>
<tr>
<td>uAGT≥55 μg/g Cr (yes versus no)</td>
<td>5.19</td>
<td>4.47</td>
<td>2.10 to 9.54</td>
<td>0.001</td>
</tr>
<tr>
<td>Rehospitalization from discharge to 1-yr follow-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes (yes versus no)</td>
<td>2.20</td>
<td>1.94</td>
<td>1.00 to 3.80</td>
<td>0.05</td>
</tr>
<tr>
<td>Preexisting CKD (yes versus no)</td>
<td>2.86</td>
<td>2.41</td>
<td>1.04 to 5.60</td>
<td>0.02</td>
</tr>
<tr>
<td>uAGT≥55 μg/g Cr (yes versus no)</td>
<td>3.98</td>
<td>3.61</td>
<td>1.63 to 7.74</td>
<td>0.00</td>
</tr>
</tbody>
</table>

OR, odds ratio; Cr, creatinine.
*Adjusted for age, hypertension, diabetes, preexisting CKD, serum albumin, NT-proBNP, hemoglobin, UACR, treatment with an angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, treatment with diuretics, and centers. Hosmer-Lemeshow goodness-of-fit test: for mortality, chi-squared value=6.035 (P=0.64); and for rehospitalization, chi-squared value=1.837 (P=0.87).

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**Table 4. Multivariate logistic regression analyses: Predictors of failure in renal recovery in the stage I cohort (n=82)**

<table>
<thead>
<tr>
<th>Variables on Admission</th>
<th>Unadjusted OR</th>
<th>Adjusted OR*</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uAGT≥55 μg/g Cr (yes versus no)</td>
<td>6.96</td>
<td>6.59</td>
<td>2.14 to 20.30</td>
<td>0.001</td>
</tr>
<tr>
<td>Age (per 10-yr increase)</td>
<td>1.20</td>
<td>1.11</td>
<td>0.78 to 1.57</td>
<td>0.58</td>
</tr>
<tr>
<td>Preexisting CKD (yes versus no)</td>
<td>1.81</td>
<td>2.81</td>
<td>1.05 to 7.47</td>
<td>0.04</td>
</tr>
<tr>
<td>Diabetes (yes versus no)</td>
<td>1.94</td>
<td>1.76</td>
<td>0.68 to 4.55</td>
<td>0.25</td>
</tr>
<tr>
<td>Hypertension (yes versus no)</td>
<td>1.14</td>
<td>1.30</td>
<td>0.05 to 3.33</td>
<td>0.60</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uAGT≥55 μg/g Cr (yes versus no)</td>
<td>6.96</td>
<td>4.35</td>
<td>1.20 to 15.70</td>
<td>0.03</td>
</tr>
<tr>
<td>Serum albumin&lt;35 g/L (yes versus no)</td>
<td>4.16</td>
<td>2.63</td>
<td>0.59 to 11.75</td>
<td>0.20</td>
</tr>
<tr>
<td>NT-proBNP ≥ median (yes versus no)</td>
<td>1.45</td>
<td>1.37</td>
<td>0.49 to 3.55</td>
<td>0.59</td>
</tr>
<tr>
<td>Hemoglobin&lt;110 g/L (yes versus no)</td>
<td>1.64</td>
<td>1.31</td>
<td>0.25 to 7.58</td>
<td>0.71</td>
</tr>
<tr>
<td>UACR (mg/g Cr)</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01 to 1.03</td>
<td>0.12</td>
</tr>
<tr>
<td>Model 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uAGT≥55 μg/g Cr (yes versus no)</td>
<td>6.96</td>
<td>8.30</td>
<td>2.34 to 29.41</td>
<td>0.001</td>
</tr>
<tr>
<td>Treatment with diuretics (yes versus no)</td>
<td>4.41</td>
<td>4.72</td>
<td>1.58 to 14.14</td>
<td>0.01</td>
</tr>
<tr>
<td>Treatment with ACEI/ARB (yes versus no)</td>
<td>0.80</td>
<td>1.40</td>
<td>0.47 to 4.18</td>
<td>0.55</td>
</tr>
</tbody>
</table>

OR, odds ratio; Cr, creatinine; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.
*Hosmer-Lemeshow goodness-of-fit test: model 1, chi-squared value=6.524 (P=0.59); model 2, chi-squared value=6.610 (P=0.58); and model 3, chi-squared value=2.356 (P=0.67).
Table 5. NRI and IDI analyses for risk reclassification of AKI and 1-year mortality in the stage I cohort

<table>
<thead>
<tr>
<th>Outcome</th>
<th>AUC Biomarker</th>
<th>Biomarker+Clinical Model</th>
<th>Clinical Modelb</th>
<th>P Valuec</th>
<th>Value (SEM)</th>
<th>P Valuec</th>
<th>Value (SEM)</th>
<th>P Valuec</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKI</td>
<td>uAGT</td>
<td>0.84</td>
<td>0.86</td>
<td>0.77</td>
<td>0.01</td>
<td>0.06 (0.02)</td>
<td>0.003</td>
<td>0.13 (0.06)</td>
</tr>
<tr>
<td></td>
<td>uNGAL</td>
<td>0.78</td>
<td>0.84</td>
<td></td>
<td>0.04</td>
<td>0.05 (0.02)</td>
<td>0.03</td>
<td>0.08 (0.04)</td>
</tr>
<tr>
<td></td>
<td>uAGT+uNGAL</td>
<td>0.86</td>
<td>0.90</td>
<td></td>
<td>&lt;0.001</td>
<td>0.09 (0.03)</td>
<td>&lt;0.001</td>
<td>0.19 (0.09)</td>
</tr>
<tr>
<td>Mortality</td>
<td>uAGT</td>
<td>0.77</td>
<td>0.81</td>
<td>0.75</td>
<td>0.03</td>
<td>0.06 (0.02)</td>
<td>0.01</td>
<td>0.16 (0.07)</td>
</tr>
<tr>
<td></td>
<td>NT-proBNP</td>
<td>0.63</td>
<td>0.77</td>
<td></td>
<td>0.22</td>
<td>0.02 (0.01)</td>
<td>0.08</td>
<td>0.07 (0.03)</td>
</tr>
<tr>
<td></td>
<td>uAGT+NT-proBNP</td>
<td>0.79</td>
<td>0.82</td>
<td></td>
<td>0.02</td>
<td>0.09 (0.03)</td>
<td>&lt;0.001</td>
<td>0.16 (0.07)</td>
</tr>
</tbody>
</table>

LVEF, left ventricular ejection fraction.

*The NRI is calculated through two-way category by using the event rate of AKI and mortality in the stage I cohort as thresholds.

bThe clinical model for predicting AKI is composed of age, hypertension, diabetes, preadmission eGFR, LVEF, NT-proBNP, hemoglobin, and UACR. The clinical model for predicting mortality is composed of age, sex, hypertension, preadmission eGFR, LVEF, serum Na, and hemoglobin.

*Biomarker+clinical model versus clinical model.

Eligible participants were patients with ADHF (1) aged 18–80 years who were admitted to the four participating hospitals, and (2) who had at least three measurements of serum creatinine over a 6-month period before admission. Exclusion criteria included exposure to nephrotoxin within 4 weeks before admission or during their hospital stay, preexisting advanced CKD (CKD, chronic dialysis, or preadmission eGFR<30 ml/min per 1.73 m²), urinary tract infection or obstruction, cancer, a concurrent diagnosis of an acute coronary syndrome, cardiogenic shock or need for inotropes, a history of cardiac transplantation and/or ventricular assist devices, and heart failure after cardiac surgery. To test the predictive ability of the biomarkers for incident AKI, patients who had AKI on admission (i.e., those with a 50% increase in serum creatinine from preadmission level on the day of hospitalization) were also excluded.

Procedures
This was an observational study in which all of the study patients received the standard of care for ADHF.41 Sample collection in two stages was as follows.

Spot urine and blood samples were collected immediately after admission before any in-hospital treatment and every 24 hours for the first 7 days during hospitalization. The remaining urine and blood samples were obtained at the time of routine morning sample collection for clinical care purposes until hospital discharge. We also took urine and blood samples from age- and sex-matched healthy volunteers to establish normal AGT values. The urine samples were centrifuged at 3000×g for 10 minutes and the supernatants were stored at -80°C. Serum creatinine was measured on admission and at least twice a day during the first 3 days and daily thereafter. All survivors in stage I were followed-up after discharge every 3 months in a designated outpatient clinic or by phone for up to 12 months. Serum creatinine was measured to determine changes in renal function. Death status and date of death were obtained through initiating phone calls to patients’ homes, searching the local Death Index, and reviewing the hospital records.

Laboratory Measurements
Urine and blood samples collected from the participating hospitals were shipped by commercial cold chain transportation. All of the biomarkers were measured in a central laboratory using a standard protocol, and all of the samples were labeled using study identification numbers without personal identifiers or clinical conditions.

ELISA for AGT Quantification
We used a sandwich ELISA kit (Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan) for quantifying AGT in urine and plasma in both stage I and stage II studies according to the manufacturer’s protocol. Values for intra- and inter-assay variability were 3.0% and 7.0%, respectively.

Western Blot Analyses for uAGT
The samples were boiled for 10 minutes in denaturing buffer and subjected to standard Western blot analyses with an affinity-purified goat polyclonal antibody against human AGT (R&D Systems). Simultaneous blots under identical conditions with known quantities of recombinant human AGT (Immuo-Biological Laboratories) were included as standards for quantification of uAGT.

Other Biomarker Measurements
To compare the predictive performance of uAGT and reported renal injury markers, urinary NGAL was measured with an ELISA kit (Antibody Shop, Denmark). We chose urinary, but not plasma, NGAL as a reference because the urine level more likely reflects renal tubular injury in the ADHF setting.17 Urinary albumin was measured using an automatic analyzer (BNPro Spec; Siemens, Germany). All of the urinary biomarkers were normalized for urinary creatinine and expressed as micrograms per gram of creatinine.

Serum N-terminal prohormone of NT-proBNP was quantified with an immunoenzymo assay analyzer (Elecsys proBNP; Roche Diagnostics, IN). Creatinine levels were measured using an automatic biochemical analyzer (AU 480; Olympus, Japan). The eGFR was determined by the Chronic Kidney Disease Epidemiology Collaboration equation.42

Outcome Definitions
The primary outcome was the development of AKI defined as an increase in serum creatinine by 26.5 μmol/L (0.3 mg/dl) within 48 hours of admission or a 50% increase in serum creatinine from the preadmission level within 7 days of admission (mean of at least three
measurements over a 6-month period before admission) according to the Kidney Disease Improving Global Outcomes Clinical Practice Guidelines for Acute Kidney Injury. We did not use urine output criteria (<0.5 ml/kg per hour for >6 hours) for AKI diagnosis because of limited sensitivity when diuretics are administered, reduced specificity in the presence of dehydration, and lack of practicality in measurement when an indwelling urinary catheter is not present.

Secondary outcomes included the following: (1) all-cause mortality (number of deaths per 100 patients with ADHF) from admission to 1-year follow-up; (2) rehospitalization (number of patients who were discharged from the hospital and readmitted to any acute care hospital within 1 year divided by the total number of patients who were discharged alive from the hospital) after discharge during the 1-year follow-up; (3) failure in renal recovery defined as serum creatinine at the time of hospital discharge that did not return to the level on the day of admission; and (4) progression of AKI to CKD defined as an eGFR <60 ml/min per 1.73 m² for >3 months after AKI in those without preexisting CKD.

Statistical Analyses
SPSS 13.0 software was used for all analyses. To compare continuous variables, we used a two-sample t test or a Mann–Whitney rank sum test. To compare categorical variables, we used the Pearson chi-squared test. To measure the sensitivity and specificity of uAGT on admission at different cutoff values, a conventional ROC curve was generated and the AUC of uAGT was compared with those of other biomarkers and the clinical models. Optimal cutoffs were determined by selecting the data point that minimized the geometric distance from 100% sensitivity and 100% specificity on the ROC curve. To evaluate the utility of biomarkers on risk classification, we determined the NRI and the IDI, as previously described. We determined the adjusted odd ratios for AKI and prognosis with multiple logistic regression analysis. The selection of covariates was based on known risk factors of AKI in the ADHF setting, including age, clinical risk factors (hypertension, diabetes, serum creatinine, serum albumin, UACR, hemoglobin, and NT-proBNP), factors that may affect uAGT level (treatment with diuretics or RAS inhibitors), and study site. In these analyses, uAGT was modeled both as a categorical variable (categorized into quartiles) and a continuous variable (log-transformed).

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DISCLOSURES
None.

REFERENCES


