学位論文

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Time Course Changes in Urinary Angiotensinogen and Circulating N-Terminal Pro–B-Type Natriuretic Peptide in Patients Hospitalized with Acute Heart Failure

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Abstract

Objective: Home care is important in patients with heart failure (HF) in order to maintain their quality of life. A biomarker that can be measured noninvasively is needed to optimize the home care of patients with HF. Urinary angiotensinogen (uAGT) is an indicator of the intrarenal renin-angiotensin system activity, which may be augmented in HF. We hypothesized that uAGT might be a urinary biomarker in HF.

Methods: We measured uAGT by an enzyme-linked immunosorbent assay and uAGT normalized by urinary creatinine (uCr)—designated uAGT/uCr—at admission and discharge in 45 patients hospitalized for HF.

Results: We found that both uAGT/uCr [median (interquartile range): 65.5 (17.1–127.7) μg/g Cr at admission; 12.1 (6.0–37.0) μg/g Cr at discharge; $P<0.01$] and N-terminal pro–B-type natriuretic peptide (NT-proBNP) levels [5422 (2280–9907) pg/mL at admission; 903 (510–1729) pg/mL at discharge; $P<0.01$] significantly decreased between admission and discharge along with an improvement in patient's clinical status [New York Heart Association scores: 3 (3–4) at admission; 1 (1–1) at discharge; $P<0.01$]. The generalized least squares model revealed that the time course changes in uAGT/uCr also correlated with those in NT-proBNP levels between admission and readmission in five patients readmitted for HF.

Conclusions: The results indicated that the time course changes in uAGT/uCr correlated with those in the NT-proBNP levels in patients with HF who showed a clinical improvement. Further investigation and development of a kit for the rapid measurement of uAGT are needed to evaluate the clinical utility of uAGT as a biomarker in HF.

Key words: Heart failure; Renin-angiotensin system; Urinary angiotensinogen; N-terminal pro–B-type natriuretic peptide

Introduction

Heart failure (HF) is a global health problem, and substantial healthcare resources are needed because of a remarkable increase in the number of such patients (1). Furthermore, repeated admissions for HF have been another major problem for these patients, in view of the rapid increase in the elderly population (2). Repeated episodes of HF affect the mortality rates and lead to a longer duration of hospitalization (3). Meanwhile, home care is important for patients with HF to help maintain their quality of life. As a result, home care must focus on the individual's changes in daily life and in providing early intervention for them. However, in home care, it is often difficult to measure N-terminal pro–B-type natriuretic peptide (NT-proBNP), an established HF marker that is useful in monitoring the time course changes in patients with HF(4) because blood must be collected. Therefore, developing a noninvasive biomarker for which measurement does not require blood samples and that can demonstrate the time course changes in patients with HF is an unmet clinical need in home care for such patients; with such a biomarker, the need for readmissions may be reduced or even prevented.

One of the main clinical characteristics of HF is the change in neurohumoral factors, which causes excessive fluid accumulation in the systemic circulation and leads to congestion in various tissues and organs, including the kidneys (5, 6). There is long-standing evidence that the sympathetic nervous system and the renin-angiotensin system (RAS) play an important role in the pathophysiological processes of HF and influence the degree of congestion in HF (6, 7). The intrarenal RAS, which is independently regulated from the systemic RAS, also plays a significant role in regulating the hemodynamics and cardiorenal interaction, which are often abnormal in HF (8, 9). Urinary angiotensinogen (uAGT) has been identified as an indicator of the intrarenal RAS activity, which may be augmented during renal congestion in patients with HF (9-11).

We therefore hypothesized that uAGT might be a urinary biomarker that can be useful to follow the time course changes in patients with HF. To test this hypothesis, we examined the correlation between the uAGT and NT-proBNP levels in patients with HF.

Materials and Methods

Study design and setting

This single-center, prospective exploratory study was performed at Kagawa University Hospital, Kagawa, Japan. The protocol of this study was approved by the Ethics Committee of Kagawa University Hospital (approval number: H29-071). This study was conducted in accordance with the ethical standards established in the 1964 Declaration of Helsinki and its later amendments. All subjects provided their written informed consent to participate.

Study participants and inclusion and exclusion criteria

We prospectively registered 45 patients who were hospitalized on an emergency basis for HF in Kagawa University Hospital from July 2017 to April 2018. HF was diagnosed according to Framingham criteria (satisfaction of two major criteria or one major and two minor criteria) (12). The inclusion criteria were an age of 18 years or older, measurements of uAGT and urinary creatinine (uCr) at admission and discharge, and survival and discharge from the hospital. Patients who were provided only comfort care, patients on dialysis, patients with cardiogenic shock, and patients after cardiac arrest were excluded.

General management of patients with heart failure

All patients with HF were given optimal medical therapy as judged by physicians when the patients were admitted: diuretics, beta-blockers, aldosterone antagonists, and angiotensin I–converting enzyme inhibitors (ACE-I) or angiotensin II receptor blockers (ARB). In this study, the use of RAS blockers was defined as the use of an ACE-I or ARB. All patients underwent routine blood testing, including tests of their renal function or serum electrolytes, such as creatinine (Cr) level, estimated glomerular filtration rate (eGFR), and serum sodium (Na) level. The eGFR was calculated using the Modification of Diet in Renal Disease equation modified for Japanese individuals (13). Cardiac function tests were also performed and included measurements of circulating NT-proBNP and echocardiography to measure the left ventricular ejection fraction (LVEF), early diastolic velocity (E/e′), inferior vena cava (IVC) diameter, and ventricular size. Echocardiography was performed by cardiologists who used Vivid S5 (GE Healthcare, Tokyo, Japan). LVEF at admission and discharge were measured by means of a modified Simpson's method with transthoracic echocardiography.

Measurement of uAGT and circulating NT-proBNP among patients with heart failure

Patients underwent urine sampling to measure uAGT and blood sampling to measure circulating NT-proBNP, which were performed at admission and discharge after achieving hemodynamic stabilization and a clinical improvement.

We measured uAGT using a method described by Nishijima et al. (14). Spot urine samples were frozen at −80°C after centrifugation. According to a previous study, one freeze-and-thaw cycle did not change the measured values of the uAGT concentrations (15). uAGT concentrations were measured with a human AGT enzyme-linked immunosorbent assay (ELISA) kit (IBL, Gunma, Japan). uCr was measured with assay kits (LabAssay Creatinine; Wako Co., Ltd., Wakayama, Japan) (9). uAGT was normalized by uCr (this measurement is designated "uAGT/uCr") and expressed in micrograms per gram of creatinine (16,17).

Data collection

The following data were collected: including age, sex, vital sign measurements (at admission and discharge), clinical status according to New York Heart Association (NYHA) functional class (at admission and discharge), clinical scenario, cause of HF, past medical history, medication details (medications taken before admission and medications added between admission and discharge), laboratory data, echocardiography data, duration of stay in the intensive care unit or coronary care unit, duration of hospital stay, and hospital mortality.

Statistical analysis

Demographic factors and baseline characteristics were summarized with descriptive statistics. Continuous variables were calculated as the means ± standard deviations or as medians with interquartile ranges (IQRs) according to their distribution. Categorical variables were calculated as numbers with percentages. Significant changes in the NYHA functional class, blood pressure, renal function, IVC diameter, NT-proBNP level, and uAGT/uCr between admission and discharge were evaluated in the paired *t* test or Wilcoxon signed-rank test as appropriate. The Spearman correlation coefficient was used to test the correlations between two continuous variables.

For the statistical analysis of the correlation of the time course changes in uAGT/uCr and NT-proBNP level, a generalized least squares (GLS) regression was employed with the use of the nlme package of R. In the gls regression, a subject factor was included as a random effect; time variable was modeled linearly; and the first order continuous auto-regressive variance-covariance structure was utilized because it provided the smallest value of Akaike Information Criteria. The natural logarithmic transformation was uAGT/uCr in order to achieve the normality of the residuals of the gls regression.

Statistical analyses were performed with the JMP® 14 software program (SAS Institute Inc., Cary, NC, USA) and R software program version 3.6.1 (https://www.r-project.org/). A two-sided P value of less than 0.05 was considered statistically significant for all analyses. Missing data were not replaced or estimated.

Results

Baseline characteristics of the study population

The study prospectively included 45 patients (Table 1). All patients were discharged after showing a clinical improvement (to NYHA functional class I or II). The mean hospital stay was 26.6 days. Most patients at admission were in NYHA functional class III or IV. All patients had a clinical profile of "wet and warm" perfusion. Study participants were typically elderly with various comorbidities, such as hypertension, diabetes, chronic kidney disease (CKD), and coronary artery disease.

In our study, the median baseline serum creatinine level was 0.98 mg/dL (IQR, 0.78–1.27 mg/dL) and that of baseline eGFR was 47.7 mL/min/1.73 m^2 (IQR, 38.9–58.5 mL/min/1.73 m^2). The median NT-proBNP level at admission was 5422 pg/mL (IQR, 2280–9907 pg/mL), and in 46.7% of patients, LVEF was less than 45% according to echocardiography at admission. Patients were treated with loop diuretics or RAS blockers (ACE-I and ARB); 9 patients who had not used RAS blockers before admission were prescribed RAS blockers for the first time between admission and discharge. A total of 15 patients who had not used aldosterone antagonists before admission were prescribed aldosterone antagonists for the first time between admission and discharge.

The median uAGT/uCr at admission was 65.5 μg/g Cr (IQR, 17.1–127.7 μg/g Cr), which did not significantly correlate with the serum creatinine levels at admission (rho=0.06, P=0.68; Supplemental Figure 1A), systolic blood pressure (rho=0.19, P=0.22; Supplemental Figure 1B), and LVEF (rho=0.10, P=0.52; Supplemental Figure 1C). At admission, uAGT/uCr did not significantly correlate with NT-proBNP level (rho=0.042, P=0.78; Supplemental Figure 2A).

Changes in the clinical characteristics and laboratory findings between admission and discharge in patients with heart failure

No significant difference in the blood pressure or renal function was observed between admission and discharge (Table 2). Along with the improvement in the clinical status (NYHA functional class: 3 [IQR, 3–4] at admission; 1 [IQR, 1–1] at discharge; P<0.01), IVC diameter significantly decreased between admission (18.2±3.9 mm) and discharge (10.0±1.5 mm; P<0.01), as did the NT-proBNP levels (from 5422 pg/mL [IQR, 2280–9907 pg/mL] at admission to 903 pg/mL [IQR, 510–1729 pg/mL] at discharge; P<0.01; Table 2). Of note, we found that uAGT/uCr also significantly decreased between

admission [65.5 μg/g Cr (IQR, 17.1–127.7 μg/g Cr)] and discharge [12.1 μg/g Cr (IQR, 6.0–37.0 μg/g Cr); P<0.01] in patients whose clinical status improved (Table 2). At discharge, uAGT/uCr significantly correlated with the NT-proBNP level (rho=0.40, P<0.01; Supplemental Figure 2B). On the other hand, uAGT/uCr did not significantly correlate with the serum creatinine levels at discharge (rho=0.21, P=0.16; Supplemental Figure 3A), systolic blood pressure (rho=0.08, P=0.59; Supplemental Figure 3B), and LVEF (rho=0.08, P=0.59; Supplemental Figure 3C). Further, uAGT/uCr did not significantly correlate with E/e′ at admission (rho=−0.24, P=0.11; Supplemental Figure 4A) and at discharge (rho=0.02, P=0.88; Supplemental Figure 4B).

Time course changes in the uAGT/uCr and NT-proBNP level in patients with heart failure

In our study, uAGT/uCr decreased in 43 patients with HF (Figure 1); in two patients, uAGT/uCr increased, although the NT-proBNP levels decreased between admission and discharge. Both of those two patients suffered a deterioration in the renal function between admission and discharge (for one patient, serum Cr was 2.20 mg/dL at admission and 2.64 mg/dL at discharge; for the other, Cr was 0.98 mg/dL at admission and 1.94 mg/dL at discharge).

In addition, five patients (mean age 82.8±4.1 years) were readmitted for HF and similarly showed increased uAGT/uCr at readmission, as shown in Figure 1 (broken line). Of these five patients, three were diagnosed with HF with a preserved ejection fraction, and two were diagnosed with HF with a reduced ejection fraction at readmission. The median duration from discharge to readmission for these five patients was 85 (IQR, 20–181) days. No increase in serum creatinine of ≥0.3 mg/dL from discharge to readmission was observed, and there was no significant change in the serum creatinine level from discharge [1.10 mg/dL (IQR, 0.91–2.22 mg/dL)] to readmission [1.06 mg/dL (IQR, 0.85–2.24 mg/dL)] in the five patients (P=0.63). On the other hand, the uAGT/uCr and NT-proBNP levels tended to increase between discharge and readmission in all five patients (P=0.063, P=0.063, respectively). Therefore, we analyzed the correlation of the time course changes in uAGT/uCr and NT-proBNP levels between admission and readmission in those five patients in a GLS model. The time course changes in uAGT/uCr correlated significantly with those in NT-proBNP levels between admission and readmission (Regression coefficient between uAGT/uCr and NT-proBNP=0.59; P=0.0052; Figure 2).

Effect of initial prescription of RAS blockers on uAGT/uCr

In our study, there was no significant difference in uAGT/uCr at admission between 18 patients who had a prescription of RAS blockers before admission (95.8 μg/g Cr; IQR, 15.5–153.5 μg/g Cr) and 27 patients who had no prescription of RAS blockers at admission (62.7 μg/g Cr; IQR, 18.6–86.0 μg/g Cr; P=0.11).

Between admission and discharge, RAS blockers were prescribed for the first time to nine patients. We analyzed uAGT/uCr between admission and discharge for these patients separately. uAGT/uCr significantly decreased between admission and discharge not only in the nine patients who received new prescriptions of RAS blockers between admission and discharge (P=0.012), but also in the other 18 patients who did not use RAS blockers between admission and discharge (P<0.01).

Effect of the initial prescription of aldosterone antagonists on uAGT/uCr

There was no significant difference in uAGT/uCr at admission between 10 patients who had a prescription of aldosterone antagonists before admission (38.8 μg/g Cr; IQR, 8.7–349.9 μg/g Cr) and 35 who had no prescription of aldosterone antagonists at admission (70.9 μg/g Cr; IQR, 32.6–126.0 μg/g Cr; P=0.39).

Between admission and discharge, aldosterone antagonists were prescribed for the first time to 15 patients. We separately analyzed uAGT/uCr between admission and discharge for these patients. uAGT/uCr significantly decreased between admission and discharge in 20 patients who did not use aldosterone antagonists between admission and discharge (P<0.01) as well as in the other 15 who received new prescriptions of aldosterone antagonists between admission and discharge (P<0.01).

Discussion

Our prospective exploratory study demonstrated that the time course changes in uAGT/uCr correlated with those in the NT-proBNP levels in patients with HF who exhibited a clinical improvement. To the best of our knowledge, this is the first clinical study on the correlation between the time course changes in uAGT/uCr and NT-proBNP levels in patients with HF. Our study indicated that uAGT might be a urinary biomarker that can be monitored noninvasively for time course changes in patients with HF.

uAGT as a reliable marker for intrarenal RAS

Recently, uAGT has been suggested to be a reliable marker for intrarenal RAS and the angiotensin II levels, which are regulated independently of systemic RAS and not evaluated by plasma renin activity or plasma angiotensin II (18, 19). In several studies, the activation of the intrarenal RAS also occurs in conditions of tissue and organ congestion (20, 21). Schunkert et al. reported high levels of renal angiotensinogen, renin, and angiotensin II in rats with severe congestive HF (20). Quan and Baum found that acute changes in the extracellular fluid volume regulated the endogenous RAS in the proximal tubule in rats, independently of the systemic RAS (21). These findings support the significance of the relationship between the functional intrarenal RAS and fluid volume in HF and led us to examine the correlation between the uAGT and NT-proBNP levels in patients with HF.

Mechanism of increased uAGT in heart failure

HF is a pathophysiologic condition in which excessive fluid accumulation in the systemic circulation leads to congestion in various tissues and organs, including the kidneys. Patients with HF typically exhibit the signs and symptoms of congestion at admission. Neurohumoral factors, including the intrarenal RAS, as well as systemic RAS and the sympathetic nervous system, have been reported to show abnormalities with the congestion that accompanies HF (6, 9, 22). A previous study in our institution showed that the activation of the sympathetic nervous system as a result of HF increases the norepinephrine levels in plasma and the kidneys; in turn, the increased kidney norepinephrine stimulates uAGT expression and subsequently angiotensin II production in cortical tissues (9).

In our study, uAGT/uCr increased in two of the 45 patients between admission and discharge, although NT-proBNP levels decreased in all patients; both of those patients had CKD prior to admission

and developed acute kidney injury (AKI) defined according to the KDIGO Clinical Practice Guidelines between admission and discharge (23). As a result, they suffered a deterioration in the renal function between admission (in one, serum Cr was 2.20 mg/dL; in the other, 0.98 mg/dL) and discharge (in the first, 2.64 mg/dL; in the other, 1.94 mg/dL). These patients were presumed to be affected more by the deterioration of renal function than by the alleviation of HF. The relationship between CKD or AKI and increased uAGT/uCr has been reported in previous studies. At present, it may be necessary to carefully interpret the uAGT/uCr changes in such patients (11,24). As for LVEF and E/e′, there was no significant difference in uAGT/uCr between admission and discharge. It was assumed that uAGT/uCr reflected congestion rather than the degree of LVEF and cardiac function itself.

In some of our patients, various types and doses of RAS blockers or aldosterone antagonists were added between admission and discharge. We analyzed uAGT/uCr separately according to the new prescription of RAS blockers or aldosterone antagonists between admission and discharge because intrarenal RAS activity was attenuated with RAS blockers (7, 9, 25-27). The patients who received RAS blockers or aldosterone antagonists and those who did not receive showed similar decreases in uAGT/uCr. This implies that the decrease in uAGT/uCr was affected by the alleviation of HF as well as by the effect of RAS blockers or aldosterone antagonists.

Correlation between the uAGT and NT-proBNP level

NT-proBNP is an established marker of HF that is useful for monitoring the changes in patients with HF (4). Although the prerequisite for an ideal biomarker is that clinical course parallels biomarker variability, as with NT-proBNP levels in patients with HF (28), it has not been demonstrated for biomarkers other than NT-proBNP or BNP. As mentioned previously, we found the time course changes in uAGT/uCr to significantly correlate with those in the NT-proBNP levels in patients with HF who showed a clinical improvement.

Association between the renal function and uAGT in heart failure

In previous clinical studies investigating uAGT in patients with HF, uAGT/uCr at admission or at time of AKI diagnosis was a strong predictor for AKI or progression of AKI (24, 29). In our study, eight patients developed AKI defined according to KDIGO Clinical Practice Guidelines between admission and

discharge (23). We found that patients with AKI tended to have an increased uAGT/uCr level at admission compared with those without AKI [219.3 μg/g Cr (IQR, 30.7–497.4 μg/g Cr) vs 62.4 μg/g Cr (IQR, 17.1–103.0 μg/g Cr); P=0.085].

As mentioned above, uAGT seems to be affected by changes in the renal function. In our study, uAGT/uCr increased in two patients who had CKD prior to admission and developed AKI between admission and discharge, although the NT-proBNP levels decreased. We separately analyzed the uAGT/uCr changes according to the development of AKI between admission and discharge. uAGT/uCr significantly decreased between admission and discharge in eight patients with AKI between admission [219.3 μg/g Cr (IQR, 30.7–497.4 μg/g Cr)] and discharge [46.2 μg/g Cr (IQR, 4.2–285.9 μg/g Cr), P=0.016] as well as in the other 37 patients without AKI between admission [62.4 μg/g Cr (IQR, 17.1–103.0 μg/g Cr)] and discharge [12.0 μg/g Cr (IQR, 6.0–28.7 μg/g Cr), P<0.01]. Furthermore, we confirmed the development of a worsening renal function (WRF) defined as increase in serum creatinine above 0.3 mg/dL (30) in 14 patients in our study. Similarly, we separately analyzed the uAGT/uCr changes according to the development of WRF between admission and discharge. uAGT/uCr significantly decreased between admission and discharge in 14 patients with WRF between admission [90.7 μg/g Cr (IQR, 26.1–349.9 μg/g Cr)] and discharge [22.5 μg/g Cr (IQR, 9.4–115.4 μg/g Cr), P<0.01] as well as in the other 31 patients without WRF between admission [54.7 μg/g Cr (IQR, 15.1–110.8 μg/g Cr)] and discharge [11.1 μg/g Cr (IQR, 5.7–28.6 μg/g Cr), P<0.01]. However, some patients seem to be affected more by the deterioration of the renal function than by the alleviation of HF such as two patients with CKD and AKI in this study. Thus, it may be necessary to carefully interpret the uAGT/uCr changes in patients with a deterioration of the renal function. In future studies, further examination of the effect of changes in the renal function on uAGT/uCr changes in HF are needed to evaluate the the clinical utility of uAGT as a biomarker.

Clinical implementation

Although the NT-proBNP level has been established as a biomarker for monitoring the clinical status of patients with HF, blood samples and blood sampling techniques are needed. Therefore, a biomarker for which measurement does not require blood samples is desirable for evaluating the clinical status of patients with HF quickly and simply, in order to prevent readmissions. Patient self-monitoring or

remote telemonitoring are also used as noninvasive methods to reduce hospitalizations for HF; however, the success of these methods depends on the patient's ability to perform them and to assess the results (31, 32). In previous studies, several devices have been tested as approaches for monitoring patients with HF (33-35). However, these methods also have some weaknesses, one of which is that they are relatively invasive. Although discussion and improvement in measuring uAGT are necessary before it can be put to practical use at clinical sites, uAGT might be a urinary biomarker for monitoring the time course changes in patients with HF not only at hospitals, but also at clinics or in home care, in which the measurement is objective and thus independent of the inspector's skill, as well as noninvasive. At present, uAGT measurement seems to be useful for revealing the relative time course changes in individual patients so as to reduce readmissions.

As we proceed in this work, we need to apply ELISA and immunochromatography to develop a kit for the rapid measurement of uAGT, taking the practical use in home care into consideration. The uAGT levels should be evaluated in more detailed studies with a large population of patients with HF to determine whether monitoring the uAGT level provides useful information and can thus potentially reduce readmissions.

Limitations

This study is associated with several limitations. First, there was some potential selection bias because this was an exploratory research conducted at a single center. Moreover, uncontrolled confounding factors may have been present. Second, the sample size in this study was relatively small. Therefore, we could not perform sufficient multivariate analyses and could not completely access the effects of severity of HF and renal function on uAGT/uCr. Third, the detailed dose or effect of medication used for HF management between admission and discharge, including the diuretic and antihypertensive, was not sufficiently considered. Fourth, we studied the NT-proBNP level, which can be measured in blood samples collected in normal storage. It is also necessary to consider studying the BNP level, which is less likely to be affected by the renal function.

Conclusions

This study indicated that the time course changes in uAGT/uCr correlated with those in the

NT-proBNP levels in patients with HF who showed a clinical improvement. uAGT might therefore be a urinary biomarker that is useful for monitoring the time course changes in patients with HF. Further investigation and the development of a kit for the rapid measurement of uAGT are needed to evaluate the clinical utility of uAGT as a biomarker in HF.

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References

1. Schocken DD, Benjamin EJ, Fonarow GC, et al. Prevention of heart failure: a scientific statement from the American Heart Association Councils on Epidemiology and Prevention, Clinical Cardiology, Cardiovascular Nursing, and High Blood Pressure Research; Quality of Care and Outcomes Research Interdisciplinary Working Group; and Functional Genomics and Translational Biology Interdisciplinary Working Group. Circulation 117:2544-2565, 2008.
2. Hamaguchi S, Kinugawa S, Tsuchihashi-Makaya M, Goto D, Tsutsui H. Weekend versus weekday hospital admission and outcomes during hospitalization for patients due to worsening heart failure: a report from Japanese Cardiac Registry of Heart Failure in Cardiology (JCARE-CARD). Heart Vessels 29:328-335, 2014.
3. Solomon SD, Dobson J, Pocock S, et al. Influence of nonfatal hospitalization for heart failure on subsequent mortality in patients with chronic heart failure. Circulation 116:1482-1487, 2007.
4. Masson S, Latini R. Amino-terminal pro-B-type natriuretic peptides and prognosis in chronic heart failure. Am J Cardiol 101:56-60, 2008.
5. Fonarow GC, Heywood JT, Heidenreich PA, Lopatin M, Yancy CW. Temporal trends in clinical characteristics, treatments, and outcomes for heart failure hospitalizations, 2002 to 2004: findings from Acute Decompensated Heart Failure National Registry (ADHERE). Am Heart J 153:1021-1028, 2007.
6. Ross EA. Congestive renal failure: the pathophysiology and treatment of renal venous hypertension. J Card Fail 18:930-938, 2012.
7. Kobori H, Nangaku M, Navar LG, Nishiyama A. The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. Pharmacol Rev 59:251-287, 2007.
8. Velez JC. The importance of the intrarenal renin-angiotensin system. Nat Clin Pract Nephrol 5:89-100, 2009.
9. Rafiq K, Noma T, Fujisawa Y, et al. Renal sympathetic denervation suppresses de novo podocyte injury and albuminuria in rats with aortic regurgitation. Circulation 125:1402-1413, 2012.
10. Kobori H, Harrison-Bernard LM, Navar LG. Urinary excretion of angiotensinogen reflects intrarenal angiotensinogen production. Kidney Int 61:579-585, 2002.
11. Yamamoto T, Nakagawa T, Suzuki H, et al. Urinary angiotensinogen as a marker of intrarenal angiotensin II activity associated with deterioration of renal function in patients with chronic kidney disease. J Am Soc Nephrol 18:1558-1565, 2007.
12. Nieminen MS, Harjola VP. Definition and epidemiology of acute heart failure syndromes. Am J Cardiol 96:5g-10g, 2005.
13. Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 53:982-992, 2009.
14. Nishijima Y, Kobori H, Kaifu K, et al. Circadian rhythm of plasma and urinary angiotensinogen in healthy volunteers and in patients with chronic kidney disease. J Renin Angiotensin Aldosterone Syst 15:505-508, 2014.
15. Nishijima Y, Kobori H, Sofue T, et al. Important aspects of urine sampling for angiotensinogen measurement: time and preservation conditions in healthy individuals. Tohoku J Exp Med 228:333-339, 2012.
16. Katsurada A, Hagiwara Y, Miyashita K, et al. Novel sandwich ELISA for human angiotensinogen. Am J Physiol Renal Physiol 293:F956-960, 2007.
17. Suzaki Y, Ozawa Y, Kobori H. Quantification of human angiotensinogen by a novel sandwich ELISA. Peptides 27:3000-3002, 2006.
18. Kobori H, Alper AB, Jr., Shenava R, et al. Urinary angiotensinogen as a novel biomarker of the intrarenal renin-angiotensin

system status in hypertensive patients. Hypertension 53:344-350, 2009.

19. Kobori H, Nishiyama A, Harrison-Bernard LM, Navar LG. Urinary angiotensinogen as an indicator of intrarenal Angiotensin status in hypertension. Hypertension 41:42-49, 2003.

20. Schunkert H, Tang SS, Litwin SE, et al. Regulation of intrarenal and circulating renin-angiotensin systems in severe heart failure in the rat. Cardiovasc Res 27:731-735, 1993.

21. Quan A, Baum M. Endogenous angiotensin II modulates rat proximal tubule transport with acute changes in extracellular volume. Am J Physiol 275(1 Pt 2):F74-78, 1998.

22. Ronco C, McCullough PA, Anker SD, et al. Cardiorenal syndromes: an executive summary from the consensus conference of the Acute Dialysis Quality Initiative (ADQI). Contrib Nephrol 165:54-67, 2010.

23. Koeze J, Keus F, Dieperink W, van der Horst IC, Zijlstra JG, van Meurs M. Incidence, timing and outcome of AKI in critically ill patients varies with the definition used and the addition of urine output criteria. BMC Nephrol 18:70, 2017.

24. Yang X, Chen C, Tian J, et al. Urinary angiotensinogen level predicts AKI in acute decompensated heart failure: a prospective, two-stage study. J Am Soc Nephrol 26:2032-2041, 2015.

25. Mogensen CE. Early glomerular hyperfiltration in insulin-dependent diabetics and late nephropathy. Scand J Clin Lab Invest 46:201-206, 1986.

26. Komine N, Khang S, Wead LM, Blantz RC, Gabbai FB. Effect of combining an ACE inhibitor and an angiotensin II receptor blocker on plasma and kidney tissue angiotensin II levels. Am J Kidney Dis 39:159-164, 2002.

27. Nishiyama A, Yoshizumi M, Rahman M, et al. Effects of AT1 receptor blockade on renal injury and mitogen-activated protein activity in Dahl salt-sensitive rats. Kidney Int 65:972-981, 2004.

28. Anand IS, Florea VG, Fisher L. Surrogate end points in heart failure. J Am Coll Cardiol 39:1414-1421, 2002.

29. Chen C, Yang X, Lei Y, et al. Urinary biomarkers at the time of AKI diagnosis as predictors of progression of AKI among patients with acute cardiorenal syndrome. Clin J Am Soc Nephrol 11:1536-1544, 2016.

30. Damman K, Navis G, Voors AA, et al. Worsening renal function and prognosis in heart failure: systematic review and meta-analysis. J Card Fail 13:599-608, 2007.

31. Goldberg LR, Piette JD, Walsh MN, et al. Randomized trial of a daily electronic home monitoring system in patients with advanced heart failure: the Weight Monitoring in Heart Failure (WHARF) trial. Am Heart J 146:705-712, 2003.

32. Fonarow GC, Corday E. Overview of acutely decompensated congestive heart failure (ADHF): a report from the ADHERE registry. Heart Fail Rev 9:179-185, 2004.

33. Packer M, Abraham WT, Mehra MR, et al. Utility of impedance cardiography for the identification of short-term risk of clinical decompensation in stable patients with chronic heart failure. J Am Coll Cardiol 47:2245-2252, 2006.

34. Yu CM, Wang L, Chau E, et al. Intrathoracic impedance monitoring in patients with heart failure: correlation with fluid status and feasibility of early warning preceding hospitalization. Circulation 112:841-848, 2005.

35. Bourge RC, Abraham WT, Adamson PB, et al. Randomized controlled trial of an implantable continuous hemodynamic monitor in patients with advanced heart failure: the COMPASS-HF study. J Am Coll Cardiol 51:1073-1079, 2008.

Figure legends

Figure 1. Changes in uAGT/uCr (urinary angiotensinogen [uAGT] normalized by urinary creatinine [uCr]) between admission and discharge in patients with heart failure. The graph shows uAGT/uCr at admission and discharge in patients with heart failure. Five patients (broken line) were readmitted because of heart failure; they exhibited increased uAGT/uCr in comparison with those at discharge.

Figure 2. Correlation between the time course changes in uAGT/uCr (urinary angiotensinogen [uAGT] normalized by urinary creatinine [uCr]) and those in N-terminal pro–B-type natriuretic peptide (NT-proBNP) levels in five patients who were readmitted. The mean values are expressed as closed circles, and standard errors are represented by error bars. The generalized least squares model was utilized to examine the correlation of time course changes in the uAGT/uCr and NT-proBNP level.

Supplemental Figure 1. Correlations among the serum creatinine level (A), systolic blood pressure (SBP) (B), left ventricular ejection fraction (LVEF) (C), and uAGT/uCr (urinary angiotensinogen [uAGT] normalized by urinary creatinine [uCr]) at admission.

Supplemental Figure 2. Correlations between uAGT/uCr [urinary angiotensinogen (uAGT) normalized by urinary creatinine (uCr)] and levels of N-terminal pro–B-type natriuretic peptide (NT-proBNP) at admission (A) and discharge (B).

Supplemental Figure 3. Correlations among the serum creatinine level (A), systolic blood pressure (SBP) (B), left ventricular ejection fraction (LVEF) (C), and uAGT/uCr [urinary angiotensinogen (uAGT) normalized by urinary creatinine (uCr)] at discharge.

Supplemental Figure 4. Correlations between uAGT/uCr [urinary angiotensinogen (uAGT) normalized by urinary creatinine (uCr)] and E/e′ at admission (A) and discharge (B).

Table 1. Baseline clinical and laboratory characteristics of the study population

Variables	All patients (N=45)
Mean age (years)	76.9±11.7
No. of men	23 (51.1%)
Mean body mass index (kg/m^2)	21.8±3.9
No. of patients with NYHA functional class III or IV	41 (91.1%)
Mean systolic blood pressure (mmHg)	122.3±18.0
Mean heart rate (beats/min)	86.5±18.0
Medical history	
No. of patients with hypertension	28 (62.2%)
No. of patients with diabetes mellitus	12 (26.7%)
No. of patients with atrial fibrillation	18 (40.0%)
No. of patients with chronic kidney disease	18 (40.0%)
No. of patients with stroke/transient ischemic attack	5 (11.1%)
No. of patients with coronary artery disease	18 (40.0%)
Cardiac diseases	
No. of patients with ischemic cardiomyopathy/coronary artery disease	10 (22.2%)
No. of patients with nonischemic cardiomyopathy	12 (26.7%)
No. of patients with valvular disease	7 (15.6%)
No. of patients with arrhythmia	10 (22.2%)
No. of patients with hypertensive heart disease	3 (6.7%)
No. of patients with other conditions	3 (6.7%)
Laboratory data	
Median hemoglobin (g/dL)	11.3 [10.1–12.7]
Median serum creatinine level (mg/dL)	0.98 [0.78–1.27]
Median eGFR (mL/min/1.73 m^2)	47.7 [38.9–58.5]
Median serum albumin level (mg/dL)	3.7 [3.3–4.0]
Median serum sodium level (mEq/L)	139 [137–141]
Median NT-proBNP level (pg/mL)	5422 [2280–9907]
Echocardiography parameters	
Mean LVEF (%)	48.8±17.9
No. of patients with LVEF < 45%	21 (46.7%)
Mean LV diastolic diameters (mm)	48.3±10.3
Mean inferior vena caval diameter (mm)	18.2±3.9
Medication at admission	
No. of patients taking beta-blockers	18 (40.0%)

No. of patients taking ACE-I/ARB	18 (40.0%)
No. of patients taking aldosterone antagonists	10 (22.2%)
No. of patients taking loop diuretics	23 (51.1%)
No. of patients taking sodium–glucose cotransporter-2 inhibitors	2 (4.4%)
No. of patients taking ACE-I/ARB for the first time during hospitalization	9 (20.0%)
Mean duration of ICU or CCU stay (days)	6.9±8.2
Mean hospital stay (days)	26.6±18.1

Data are expressed as mean ± standard deviation, number (percentage), or median [interquartile range]. NYHA, New York Heart Association; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro–B-type natriuretic peptide; LVEF, left ventricular ejection fraction; LV, left ventricle; ACE-I, angiotensin I–converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ICU, intensive care unit; CCU, coronary care unit.

Table 2. Changes in NYHA functional class, blood pressure, renal function, IVC diameter, NT-proBNP level, and uAGT/uCr between admission and discharge in patients with HF

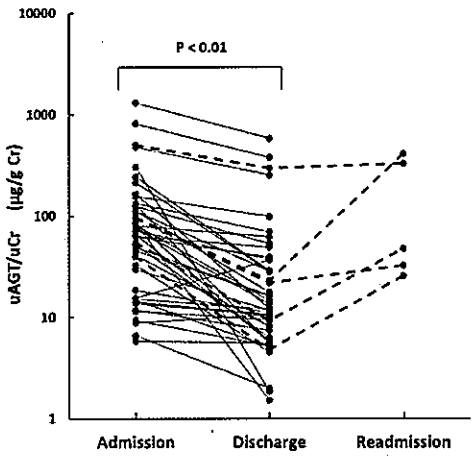
Results	Admission	Discharge	*p*-value
NYHA functional class	3 [3–4]	1 [1–1]	<0.01
Mean systolic blood pressure (mmHg)	122.3±18.0	119.2±16.1	0.27
Median serum creatinine level (mg/dL)	0.98 [0.78–1.27]	1.01 [0.73–1.29]	0.43
Median eGFR (mL/min/1.73 m^2)	47.7 [38.9–58.5]	46.8 [35.8–73.6]	0.43
Mean IVC diameter (mm)	18.2±3.9	10.0±1.5	<0.01
Median NT-proBNP level (pg/mL)	5422 [2280–9907]	903 [510–1729]	<0.01
Median uAGT/uCr (μg/g Cr)	65.5 [17.1–127.7]	12.1 [6.0–37.0]	<0.01

Data are expressed as mean ± standard deviation or median [interquartile range].

Significant changes in NYHA functional class, blood pressure, renal function, IVC diameter, NT-proBNP level, and uAGT/uCr between admission and discharge were evaluated in the paired t-test or Wilcoxon signed-rank test as appropriate.

NYHA, New York Heart Association; eGFR, estimated glomerular filtration rate; IVC, inferior vena cava; NT-proBNP, N-terminal pro–B-type natriuretic peptide; uAGT, urinary angiotensinogen; uCr, urinary creatinine; uAGT/uCR, uAGT normalized by uCr; HF, heart failure.

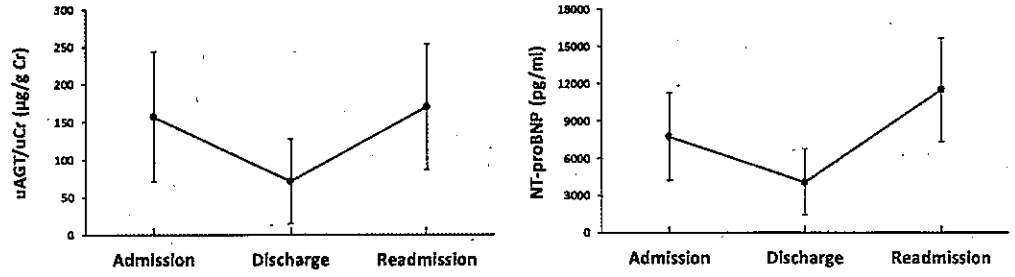
Figure 1



1

Figure 2

Regression coefficient between uAGT/uCr and NT-proBNP=0.59
P=0.0052



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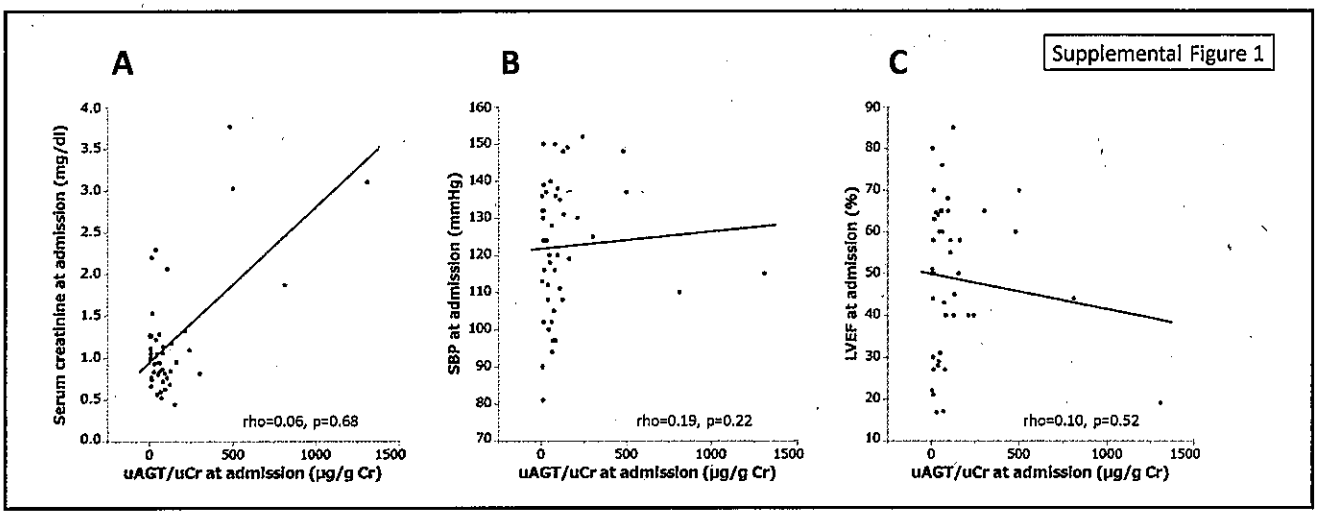
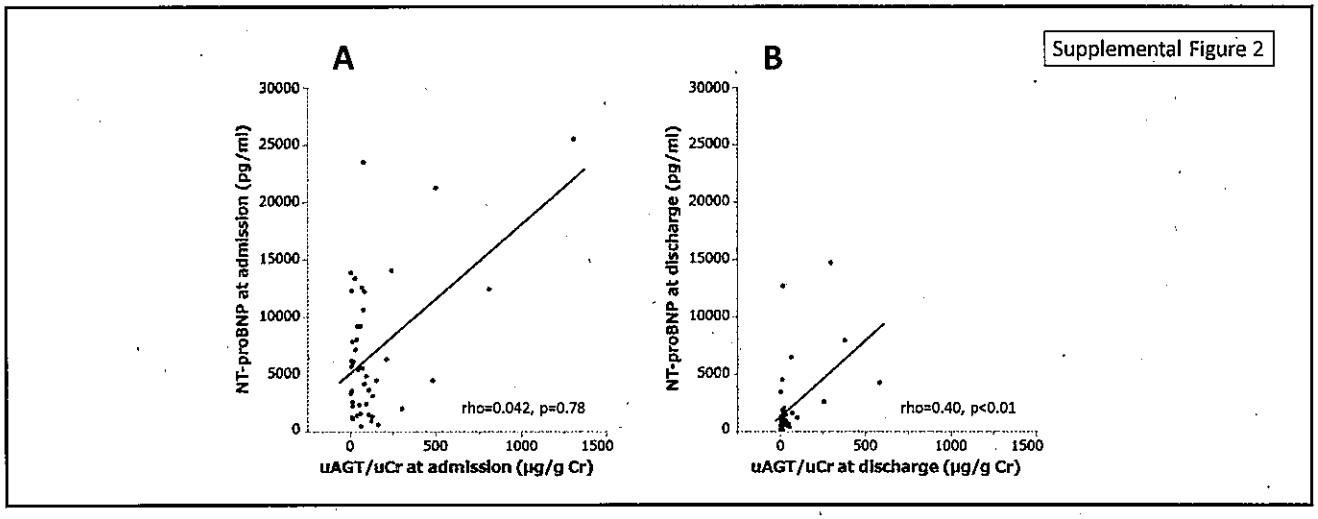
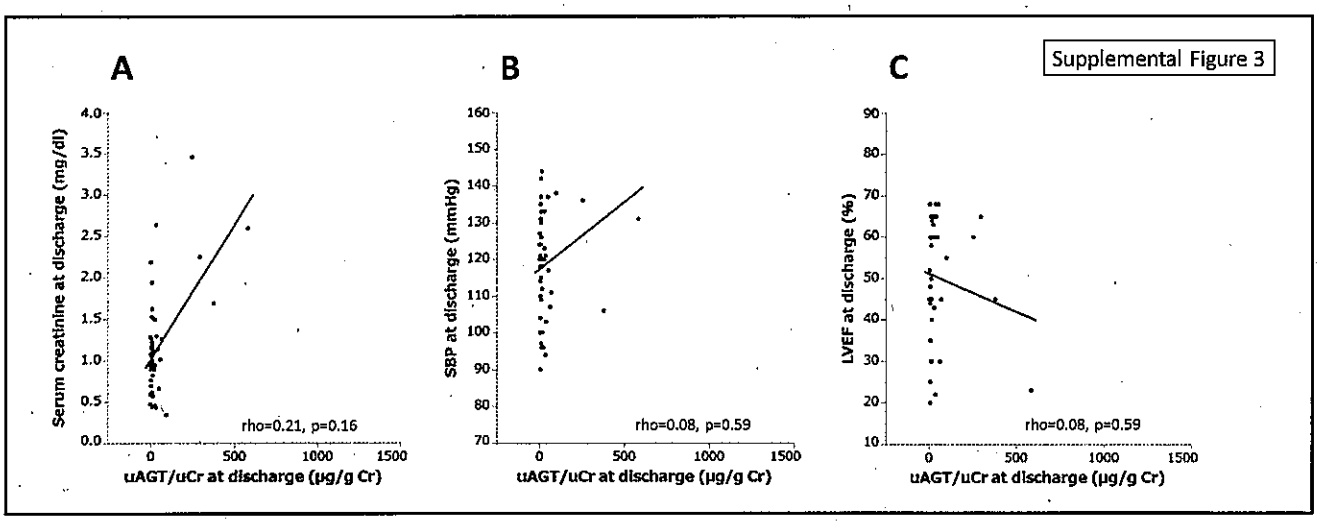

Supplemental Figure 1
A
B
C
Serum creatinine at admission (mg/dl)
SBP at admission (mmHg)
LVEF at admission (%)
rho=0.06, p=0.68
rho=0.19, p=0.22
rho=0.10, p=0.52
uAGT/uCr at admission (μg/g Cr)
uAGT/uCr at admission (μg/g Cr)
uAGT/uCr at admission (μg/g Cr)

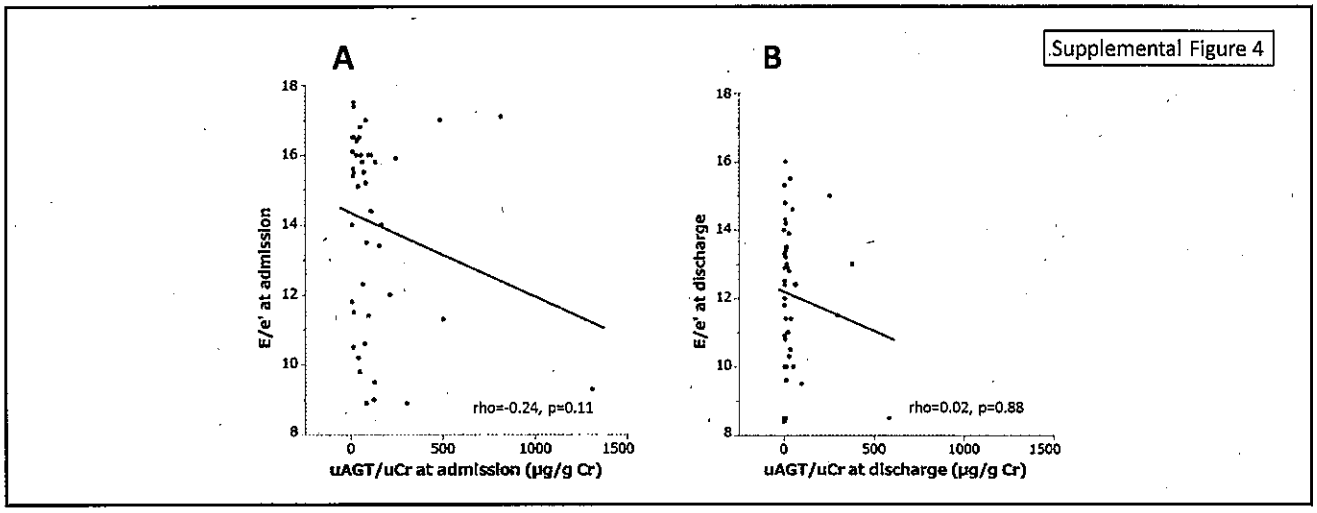
3


Supplemental Figure 2
A
B
NT-proBNP at admission (pg/ml)
NT-proBNP at discharge (pg/ml)
rho=0.042, p=0.78
rho=0.40, p<0.01
uAGT/uCr at admission (μg/g Cr)
uAGT/uCr at discharge (μg/g Cr)

4


Supplemental Figure 3
A
B
C
Serum creatinine at discharge (mg/dl)
SBP at discharge (mmHg)
LVEF at discharge (%)
rho=0.21, p=0.16
rho=0.08, p=0.59
rho=0.08, p=0.59
uAGT/uCr at discharge (μg/g Cr)
uAGT/uCr at discharge (μg/g Cr)
uAGT/uCr at discharge (μg/g Cr)

5


Supplemental Figure 4
A
B
E/e' at admission
E/e' at discharge
rho=-0.24, p=0.11
rho=0.02, p=0.88
uAGT/uCr at admission (μg/g Cr)
uAGT/uCr at discharge (μg/g Cr)

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