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Urinary C-Type Natriuretic Peptide

A New Heart Failure Biomarker

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Objectives This study was conducted to determine whether urinary excretion of C-type natriuretic peptide (CNP) is elevated in acute decompensated heart failure (ADHF) and whether elevated levels predict adverse outcomes.

Background Urinary CNP has been detected in patients with heart failure, but its clinical significance and prognostic utility, compared to established kidney injury biomarkers, in ADHF is unknown.

Methods We measured 24-h urinary excretion and concurrent plasma concentrations of CNP22, CNP53, and NT-CNP53 in 58 ADHF patients and 20 healthy control subjects. Urinary kidney injury molecule (KIM)-1 and neutrophil gelatinase–associated lipocalin (NGAL) and plasma N-terminal pro-B type natriuretic peptide (NT-proBNP) were also measured. Mortality and all-cause rehospitalization/death were assessed over a follow-up of 1.5 ± 0.9 years.

Results ADHF patients had higher urinary excretion of all 3 CNP molecular forms than did controls. Plasma CNP22 and CNP53 were elevated in ADHF but showed limited correlation with urinary excretion, suggesting that mainly renal-derived CNP appears in urine. Plasma NT-proBNP and urinary KIM-1 were also elevated in ADHF ($p < 0.0001$); urinary NGAL was similar to that in controls. At 6 months, event-free survival values in ADHF patients were 86% for mortality and 59% for all-cause rehospitalization/death. On Cox regression analysis, urinary NT-CNP53 was the only predictor of mortality (hazard ratio: 1.7; 95% confidence interval: 1.1 to 2.4; $p = 0.01$) and all-cause rehospitalization/death (hazard ratio: 1.8; 95% confidence interval: 1.3 to 2.4; $p = 0.0004$), even after adjustment. Integrated discrimination analysis suggested that urinary NT-CNP53 combined with plasma NT-proBNP improved the prediction of adverse outcomes.

Conclusions The findings from this study support the clinical utility of urinary CNP molecular forms. In ADHF, urinary NT-CNP53 correlated with prognosis, better predicted outcomes than did urinary NGAL and KIM-1, and improved the prognostic value of plasma NT-proBNP. (J Am Coll Cardiol HF 2013;1:170–7) © 2013 by the American College of Cardiology Foundation

Acute decompensated heart failure (ADHF) is the leading cause of hospitalization in patients >65 years of age and continues to confer a disturbingly high mortality rate (1). Accurate risk stratification is important in the effort to improve outcomes in ADHF, as an aid to clinical decision making and appropriate patient selection for clinical trials. To this end, N-terminal pro-B-type natriuretic peptide (NT-proBNP), a circulating marker of ventricular stretch, remodeling, and neurohumoral activation, is strongly associated with prognosis in ADHF (2) and has been widely integrated into routine clinical evaluation. Nonetheless, ADHF pathophysiology is complex, and there is growing recognition that optimal risk assessment in ADHF patients may require a multimarker approach (3,4).

Renal dysfunction is a prevalent and independent predictor of adverse outcomes in patients with ADHF (5,6) and a candidate for inclusion in any multimarker strategy. However, conventional, creatinine-based estimates of glomerular function or urine albumin excretion fail to incorporate the potential prognostic impact of renal tubular injury, as was recently demonstrated in HF patients (7–9). Conversely, the novel urinary biomarker C-type natriuretic peptide (CNP) is produced in the kidney as well as in the endothelium, has been localized to renal tubules (10,11), and as part of the natriuretic peptide family may provide greater

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information concerning renal integrity and the cardio–renal interaction in ADHF.

CNP is synthesized as the precursor 103–amino acid (AA) protein proCNP (AA 1 to 103), which is then cleaved into NT-proCNP (AA 1 to 50) and CNP53 (AA 51 to 103) by the intracellular endoprotease furin (Fig. 1) (12). Additional downstream processing cleaves CNP53, giving rise to the biologically active mature form, CNP22 (AA 82 to 103), and an inactive form, NT-CNP53 (AA 51 to 81) (12,13). Although urinary CNP22 excretion has been shown to be elevated in stable heart failure (HF) (14), the clinical utility of other CNP molecular forms has not been studied. Furthermore, the prognostic significance of urinary CNP excretion in ADHF is unknown.

We tested the hypotheses that excretion of NT-CNP53, which, like NT-proBNP, may have a longer half-life and be more resistant to degradation than its biologically active mature form, CNP22, would: 1) be associated with prognosis in patients with ADHF; 2) provide greater prognostic information than contemporary urinary biomarkers of tubular injury (kidney injury molecule [KIM]-1 and neutrophil gelatinase-associated lipocalin [NGAL]); and 3) be of incremental predictive value to plasma NT-proBNP in the risk stratification of patients with ADHF.

Methods

Patient population. Sixty ADHF patients and 20 healthy subjects were recruited. ADHF patients hospitalized at St Mary's Hospital, Mayo Clinic, Rochester, Minnesota, were prospectively identified and enrolled from an ongoing registry of consecutive admissions between August 2009 and August 2010. Patients with a clinical diagnosis of HF consistent with the Framingham criteria (15) were included. Patients with a urine collection incomplete or incorrect for adequate urinary biomarker analysis were excluded (n = 2), leaving a total of 58 consecutive ADHF patients providing consent. These patients underwent a baseline history assessment, physical examination, and transthoracic echocardiography as a part of routine clinical care. Collection of 24-h urine and plasma samples for the assessment of CNP molecular forms, urinary KIM-1 and NGAL, and plasma NT-proBNP were obtained on admission. Urine samples were collected on ice with acetic acid (30 ml of 1:1 acetic acid; 17.4 M). After the timed urine collection (mean, 22.9 ± 4 h), total volume was recorded and samples aliquoted and stored at −80°C until analysis. Results are expressed using the Modification of Diet in Renal Disease estimated glomerular filtration rate (eGFR) (16).

Control subjects were recruited from a population of volunteers from Rochester, Minnesota; all were nonsmokers and had no history of cardiovascular or systemic disease. The 24-h urine and plasma samples were collected on enrollment. All participants provided written informed consent for participation, and the study was approved by the institutional review board at the Mayo Clinic.

Urine biomarker assays. CNP22 (AA 82 TO 103). Urinary CNP22 was determined using a non-equilibrium radioimmunoassay (Phoenix Pharmaceuticals Inc., Burlingame, California), with an antibody that detects human CNP22, as previously described (11). The range of the standard curve was 0.5 to 128 pg, with a lower limit of quantification (LLOQ) of 0.5 pg. Interassay and intra-assay variability values were 11% and 5%, respectively. Recovery was 85%. Cross-reactivity values were 0% with atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), endothelin, and NT-CNP53 and 59% with CNP53.

Abbreviations and Acronyms

ADHF = acute decompensated heart failure
ANP = atrial natriuretic peptide
BNP = brain natriuretic peptide
CI = confidence interval
CNP = C-type natriuretic peptide
eGFR = estimated glomerular filtration rate
IQR = interquartile range
KIM = kidney injury molecule
LLOQ = lower limit of quantification
NGAL = neutrophil gelatinase–associated lipocalin
NT-proBNP = N-terminal pro-B type natriuretic peptide

CNP53 (AA 51 TO 103) AND NT-CNP53 (AA 51 TO 81). Urinary CNP53 and NT-CNP53 were determined using a similar nonequilibrium radioimmunoassay (Phoenix Pharmaceuticals Inc.), with antibodies that detect human CNP53 (CNP53 [AA 51 to 103]), and the first 29 amino acids of CNP53, starting from the amino-terminal only when separated from the ring structure (NT-CNP53 [AA 51 to 81]). For CNP53 and NT-CNP53, the range of the standard curve was 0.5 to 128 pg. Interassay and intra-assay variability values for CNP53 were 8% and 7%, respectively. Recovery was 81 ± 4%. Cross-reactivity values were 100% with CNP22 and 0% with NT-CNP53, ANP, and BNP. Interassay and intra-assay variability values for NT-CNP53 were 10% and 6%, respectively. Recovery was 82 ± 5.2%. Cross-reactivity values were 0% with ANP, BNP, CNP22, CNP53, and endothelin.

NGAL AND KIM-1. Urinary concentrations of NGAL and KIM-1 were measured using enzyme-linked immunoassay as per the manufacturer's instructions (Quantikine ELISA, R&D Systems, Inc., Minneapolis, Minnesota). The LLOQs were 0.012 and 0.009 ng/ml for NGAL and KIM-1, respectively. Intra-assay and interassay coefficients of variation were <5% and <8%, respectively. NGAL is recognized to form complexes with matrix metalloproteinase (MMP)-9; recombinant human MMP-9/NGAL complex demonstrated 0.3% cross-reactivity in the assay used. There was no significant cross-reactivity or interference in the KIM assay.

URINARY BIOMARKER EXCRETION. Mean urinary flow (milliliter per hour) was determined from total urine volume (milliliters) and urine collection time (hour). Urinary biomarker excretion was calculated as the product of urinary biomarker concentration (picogram per milliliter or nanograms per milliliter) and urinary flow rate (milliliter per hour) and adjusted for urinary creatinine excretion (nanograms per gram Cr).

JACC: Heart Failure Vol. 1, No. 2, 2013
April 2013:170–7

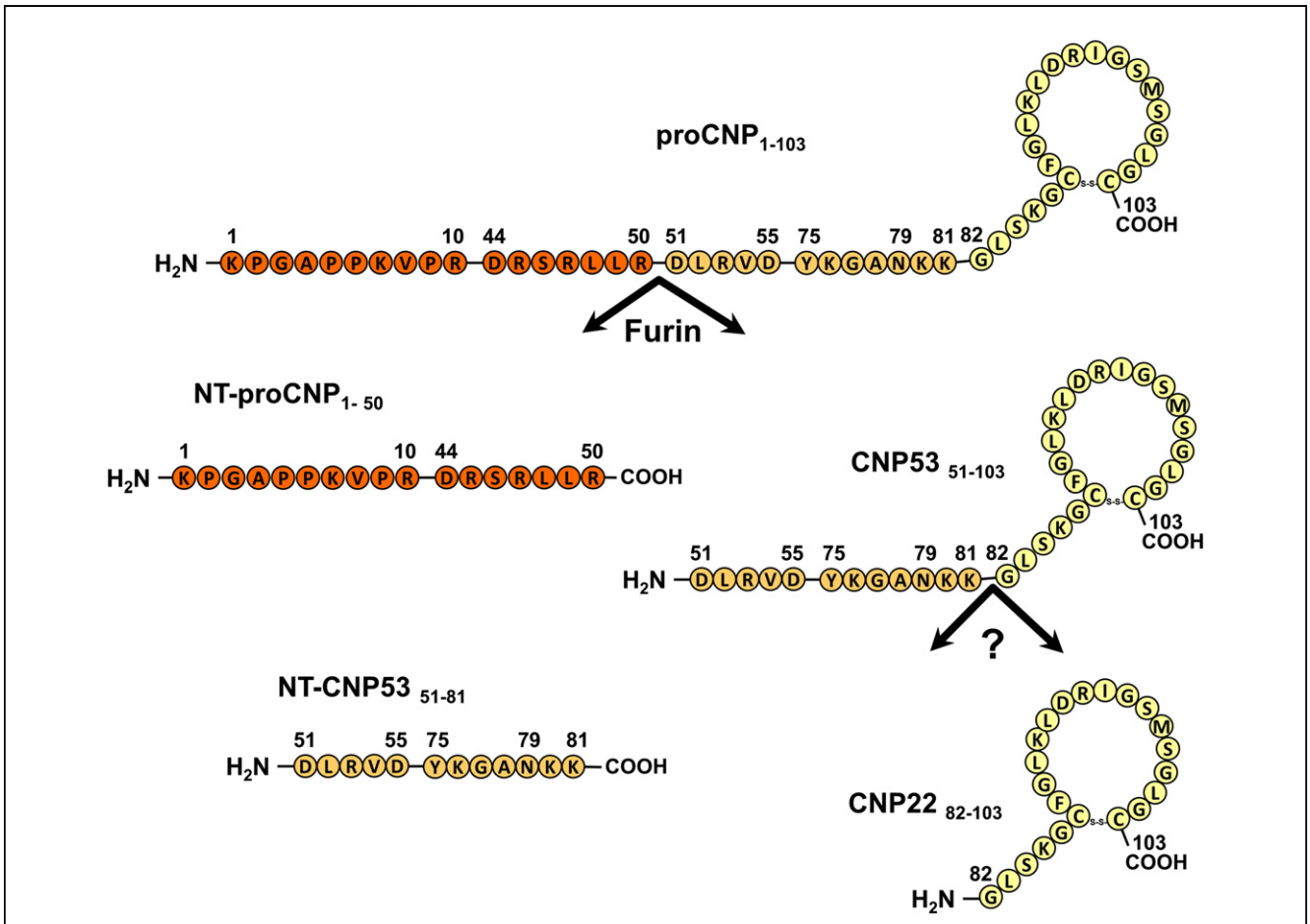

Figure 1 Biosynthesis and Processing of CNP

Precursor protein, pro C-type natriuretic peptide (CNP), is cleaved into N-terminal (NT)-proCNP and CNP53 by the intracellular endoprotease furin. Additional downstream processing cleaves CNP53 into the biologically active mature form CNP22, and an inactive form, NT-CNP53.

Plasma biomarker assays. Blood was drawn into ethylenediamine tetraacetic acid tubes and centrifuged at 4°C and 2,500 rpm for 10 min. One milliliter of plasma was aliquoted and stored at −80°C until assayed. Plasma concentrations of CNP molecular forms were determined using a nonequilibrium radioimmunoassay (Phoenix Pharmaceuticals Inc.), with anti–human CNP antibodies (17). Plasma NT-proBNP was measured using electrochemiluminescence immunoassay (18). The LLOQ for NT-proBNP was 5 pg/ml; interassay and intra-assay variability values were 3.1% and 2.5%, respectively. There was no cross-reactivity with CNP forms.

Statistical analysis. All urinary biomarkers demonstrated a non-Gaussian distribution; therefore, values are presented as medians (interquartile range [IQR]). For comparisons of data between the ADHF and control groups, nonparametric Wilcoxon rank-sum tests were used. Spearman rank correlation was used to ascertain relationships between continuous variables. Biomarker-excretion data were normalized using natural logarithmic transformation before Cox regression analysis to detect independent predictors of mortality and time to first nonelective all-cause rehospitalization/death. Mortality and rehospitalization were ascertained from institutional records, including local primary care data. Patients were otherwise censored at the time of last known follow-up. Survival rates were calculated using the Kaplan-Meier method. The discriminatory ability of biomarkers was compared using *c* statistics. Confidence intervals (CIs) were calculated for *c* statistics using an approximate jackknife method of calculating SEs. The integrated discrimination index (19) was utilized to evaluate the improvement in predictive accuracy using the combination of urinary NT-CNP53 and plasma NT-proBNP over the use of NT-proBNP alone, for study outcomes. Probability values are 2-sided; $p < 0.05$ was considered significant. Data were analyzed using JMP software version 9.0 (SAS Institute Inc., Cary, North Carolina) and SAS version 9.2 (SAS Institute Inc.).

Table 1 Clinical Characteristics

Characteristic	ADHF (n = 58)	Control (n = 20)	p Value
Age, yrs	70.1 ± 10.4	53.5 ± 6.1	<0.0001
Male	35 (59)	10 (50)	0.50
Ischemic etiology	19 (33)	0	–
Comorbidity			–
Hypertension	36 (62)	0	–
Diabetes	25 (43)	0	–
Thyroid disease	11 (19)	0	–
Atrial fibrillation	38 (66)	0	–
Previous CVA	7 (12)	0	–
CRT	14 (24)	0	–
Medications on admission			
ACEI or ARB	38 (66)	0	–
Beta-blocker	44 (76)	0	–
Loop diuretic	49 (84)	0	–
Aldosterone antagonist	12 (21)	0	–
Measurements			
LVEF, %	38.0 ± 18.9	–	–
Serum creatinine, mg/dl	1.2 ± 0.8	0.7 ± 0.18	<0.0001
eGFR, ml/min/1.73 m^2	60.5 ± 30.3	115.9 ± 21.1	<0.0001
Plasma biomarkers, pg/ml			
NT-proBNP	2,461 (1,222–6,994)	37.8 (21.9–67.3)	<0.0001
CNP22	11.7 (8.3–19.6)	6.4 (4.3–18.8)	0.005
CNP53	5.8 (5.0–7.6)	3.8 (3.6–4.3)	0.0001
NT-CNP53	6.1 (5.3–6.9)	6.5 (5.4–7.7)	0.56
Urine variables			
Volume, ml	1,824.8 ± 1,129.3	1,878.0 ± 653.7	0.80
Collection time, h	22.9 ± 4.0	24.0 ± 0	0.05
Creatinine, mg/dl	55.3 ± 37.8	75.5 ± 38.1	0.04
Protein/creatinine ratio, mg/mg	0.03 (0.02–0.08)	0.02 (0.01–0.02)	0.0007
Biomarker excretion, ng/g Cr			
KIM-1	1,354.0 (876.5–2,101.5)	475.0 (198.9–604.9)	<0.0001
NGAL	350.2 (137.2–1,405.7)	298.8 (225.2–458.3)	0.94
CNP22	14.0 (8.1–27.0)	7.2 (6.7–9.6)	0.0003
CNP53	115.2 (63.1–227.8)	64.7 (21.6–109.1)	0.02
NT-CNP53	35.8 (20.0–72.6)	19.4 (13.3–29.6)	0.002

Values are mean ± SD, n (%), or median (interquartile range).

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; CNP22 = C-type natriuretic peptide-22; CNP53 = C-type natriuretic peptide-53; CRT = cardiac resynchronization therapy; CVA = cerebrovascular accident; eGFR = estimated glomerular filtration rate; IQR = interquartile range; KIM = kidney injury molecule; LVEF = left ventricular ejection fraction; NGAL = neutrophil gelatinase-associated lipocalin; NT-CNP53 = N-terminal fragment of C-type natriuretic peptide-53; NT-proBNP = N-terminal pro-B type natriuretic peptide.

Results

The baseline demographic and clinical characteristics of the study population are shown in Table 1. Patients with ADHF were older than controls, 40% were female, and the mean left ventricular ejection fraction was 38.4 ± 18.9%. Twenty-two ADHF patients (38%) presented with dyspnea alone, 4 (7%) presented with edema alone, and 24 (41%) presented with combined dyspnea and peripheral edema. Fifty-five percent presented in New York Heart Association class III. Plasma NT-proBNP was significantly elevated, and eGFR was reduced, in the ADHF group compared to control. Urinary creatinine concentration was lower in the ADHF group than in the control group, consistent with instigation or escalation of diuretic therapy during ADHF management.

Acute decompensated heart failure and urinary biomarker excretion. Median urinary concentrations of KIM-1 and all 3 CNP molecular forms were significantly higher in the ADHF group than in the control group, as was the urinary total protein/creatinine ratio (Table 1). Urinary NGAL excretion was similar between the 2 groups (p = 0.94). KIM-1 demonstrated a nonsignificant association with eGFR (Spearman ρ: −0.19; p = 0.1), and there was no significant relationship between any urinary biomarker and New York Heart Association class (III or IV) at presentation or left ventricular ejection fraction (off inotropes).

On assessment of correlations between excretion rates of urinary CNP and other measured biomarkers, moderate correlations were observed between urinary CNP molecular forms. However, only urinary CNP22 displayed any, albeit modest, correlation with its concentration in the plasma

JACC: Heart Failure Vol. 1, No. 2, 2013
April 2013:170–7

Table 2 Predictive Values of Urinary NT-CNP53 Excretion and Plasma NT-proBNP for Clinical Outcome in Patients With ADHF

	Outcome			
	Death		All-Cause Rehospitalization/Death	
Model	HR (95% CI)	p Value	HR (95% CI)	p Value
Urinary NT-CNP53				
Unadjusted	1.67 (1.14–2.37)	0.01	1.78 (1.30–2.39)	0.0004
Model 1	1.54 (1.05–2.22)	0.03	1.75 (1.28–2.36)	0.0007
Model 2	1.60 (1.06–2.38)	0.03	1.74 (1.26–2.36)	0.001
Model 3	1.67 (1.08–2.57)	0.02	1.79 (1.28–2.47)	0.0009
Plasma NT-proBNP				
Unadjusted	1.28 (0.85–1.93)	0.24	1.24 (0.94–1.65)	0.13
Model 1	1.35 (0.89–2.04)	0.16	1.26 (0.95–1.67)	0.11
Model 2	1.30 (0.85–1.98)	0.21	1.22 (0.91–1.63)	0.17

Univariate and adjusted Cox proportional hazard analysis, Ln-transformed data (hazard ratio are per 1 log unit increase). Model 1, adjusted for age; model 2, adjusted for age and urine protein/creatinine ratio; model 3, adjusted for age, urine protein/creatinine ratio, and plasma NT-proBNP.

CI = confidence interval; HR = hazard ratio; NT-CNP53 = N-terminal fragment of C-type natriuretic peptide-53; NT-proBNP = N-terminal pro-B-type natriuretic peptide.

(ρ: 0.28; p = 0.04) (Online Table 1). Urinary CNP22 (ρ: 0.45; p = 0.0003) and CNP53 (ρ: 0.33; p = 0.01) were weakly associated with plasma NT-proBNP; urinary NT-CNP53 was not. Urinary CNP22 (ρ: 0.68; p = 0.0001) and urinary KIM-1 (ρ: 0.78; p < 0.0001) demonstrated marked correlations with the urinary total protein/creatinine ratio; no correlation was evident with the other urinary biomarkers.

Medications on admission for ADHF are shown in Table 1. On exploratory analysis, urinary NGAL was higher in the context of the use of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers (median [IQR]: 444.0 [219.2 to 2,144.1] ng/g Cr) versus no use of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers (177.3 [106.3 to 333.6] ng/g Cr) (p = 0.03), and urinary NT-CNP53 was lower in ADHF patients on loop diuretics compared to those not using them (34.0 [17.6 to 61.3] ng/g Cr vs. 60.4 [43.6 to 246.1] ng/g Cr; p = 0.01).

Plasma concentrations of C-type natriuretic peptide. Plasma CNP22 and CNP53 concentrations were elevated in the ADHF group compared to those in the control group, whereas plasma NT-CNP53 was similar (Table 1). Plasma CNP22 demonstrated limited association to its concurrent urinary excretion (ρ: 0.28; p = 0.04) and urinary NT-CNP53 excretion (ρ: 0.26; p = 0.05) but no significant correlation with urinary CNP53 (ρ: 0.24; p = 0.07) (Online Table 1). In contrast, neither plasma CNP53 nor plasma NT-CNP53 displayed any relationship to urinary excretion of any of the CNP molecular forms studied.

Clinical outcomes. Among the 58 ADHF patients studied, there were 18 deaths and 18 rehospitalizations (13 cardiovascular-related rehospitalizations) over a mean follow-up of 1.5 ± 0.9 years. Two patients were admitted for elective cardiac resynchronization therapy; these events were not included in the final analysis. At 6 months, the overall event-free survival rates (95% CI) were 85.8% (77.1 to 95.4) for mortality and 58.9% (47.2 to 73.4) for rehospitalization/death.

Univariate Cox regression analysis of baseline factors showed age to be the only variable associated with study outcomes (mortality: HR = 1.08 [95% CI, 1.02 to 1.15; p = 0.01]; rehospitalization/death: HR = 1.04 [95% CI, 1.00 to 1.09; p = 0.04]). Sex, New York Heart Association class, left ventricular ejection fraction, hypertension, and diabetes were not significantly associated with either outcome. Of the urinary and plasma biomarkers assessed, only urinary NT-CNP53 excretion was significantly predictive of mortality and all-cause rehospitalization/death on Cox regression analysis (Table 2). This association persisted after adjustments for age, urinary protein/creatinine ratio, and plasma NT-proBNP.

Six-month Kaplan-Meier event-free survival rates (95% CI) were lower in the subgroup with urinary NT-CNP53 excretion above the median compared to those in the subgroup with excretion rates below the median (mortality: 73.1% [57.9 to 92.3] vs. 96.7% [90.5 to 100.0]; rehospitalization/death: 46.3% [30.6 to 70.0] vs. 69.8% [55.1 to 88.5]) (Fig. 2). Moreover, on analysis of the *c* statistic for the occurrence of all-cause mortality, the addition of urinary NT-CNP53 to plasma NT-proBNP provided incremental predictive value compared to plasma NT-proBNP alone (Table 3). Examination of the integrated discrimination index provided further evidence that this combination improved prediction of adverse outcomes in this cohort. No other urinary or plasma biomarkers in this study demonstrated significant predictive value.

Discussion

This study investigated the prognostic value of urinary CNP in patients with ADHF. The major finding was that increased urinary excretion of NT-CNP53 was associated with increased mortality and also predicted the composite outcome of all-cause rehospitalization/death in patients with ADHF, independent of age, renal glomerular function, and

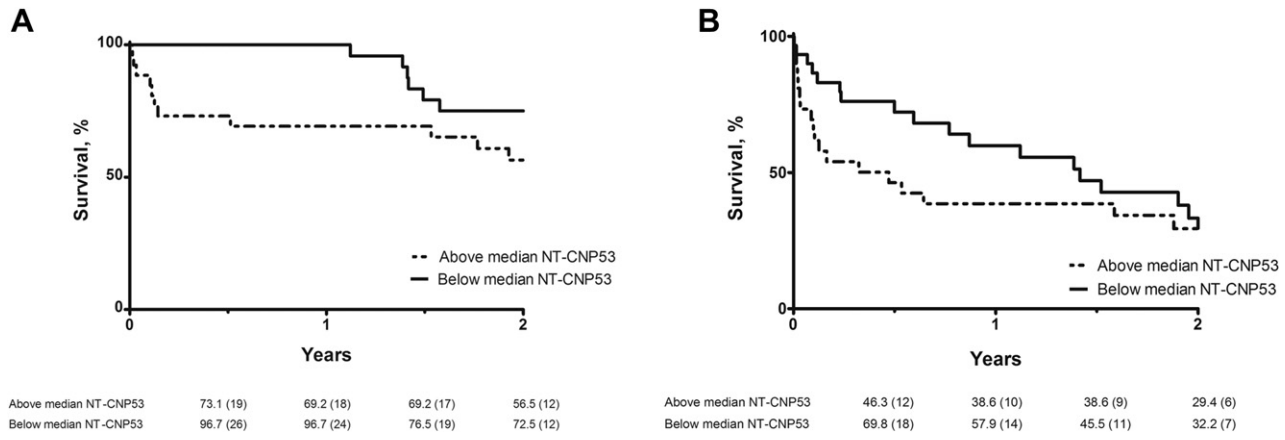

Figure 2 **Mortality and All-Cause Rehospitalization/Death**

Kaplan-Meier survival analysis for **(A)** mortality and **(B)** all-cause rehospitalization/death. Event-free survival curves for patients with urinary NT-CNP53 excretion above and including **(dashed lines)** and below **(solid lines)** the median value. Values are survival rates (%) and number of patients at risk **(in parentheses)** at each 6-month interval. Abbreviations as in Figure 1.

plasma NT-proBNP. Furthermore, elevated NT-CNP53 excretion offered incremental predictive value to plasma NT-proBNP and was the only urinary biomarker among those investigated to demonstrate prognostic significance. These findings support accumulating evidence for the involvement of CNP in HF pathophysiology and, more specifically, that its detection in urine may contribute to risk stratification in ADHF patients.

Urinary C-type natriuretic peptide in heart failure. CNP is predominantly produced in the kidney (11,14,20) and the endothelium (17), but has also been detected in other tissues (21–23). Despite limited diuretic or natriuretic activity relative to that of ANP and BNP, CNP acts as a venodilator (24), and modestly elevated plasma levels have been detected in HF patients, although markedly less than elevations of ANP and BNP (25). Urinary CNP most likely derives from a combination of glomerular filtration, transtubular reuptake, and secretion from post-glomerular blood, as well as tubular secretion of locally produced CNP. On account of its low circulating levels, susceptibility to rapid removal by the clearance receptor (NPR-C), and degradation by neutral endopeptidase, CNP detected in urine is predominantly thought to reflect local renal production (14). The lack of significant correlations between urinary excretion rates and plasma concentrations of CNP peptides in the present study supports this hypothesis. In addition, the detection of 3 distinct CNP molecular forms in the urine of both ADHF patients and control subjects suggests local renal processing of CNP occurs too.

Few studies have described urinary CNP excretion in HF patients, and these initial studies, confined to the biologically active mature form CNP22, have yielded conflicting results (14,26–28). Compared with healthy controls, increased urinary CNP excretion was observed by Mattingly et al. (14) (n = 6) and Ng et al. (26) (n = 34) in stable HF and ADHF patients, respectively; however, this finding was not reported by Bentzen et al. (27) (n = 11) or Kalra et al. (28) (n = 16). This disparity may be due to differentially timed collections of CNP22, which is especially prone to rapid degradation. Urinary excretion rates for other molecular forms of CNP in HF have not previously been published. Although the understanding of CNP biology continues to evolve, it is currently thought to serve as a renoprotective peptide, activated by humoral mechanisms and/or hypoxia in the setting of renal injury, to preserve glomerular function and suppress profibrotic processes (11).

Urinary C-type natriuretic peptide and prognosis in heart failure. Renal dysfunction portends a poor prognosis in ADHF (5,6). In this study, >50% of ADHF patients exhibited an eGFR <60 ml/min (chronic kidney disease stage ≥2), consistent with previous reports (5,6). Although reduced renal perfusion is a major determinant, recent studies have highlighted an independent association between

Table 3 **Measures of Predictive Accuracy**

Model	C-Index (95% CI)	Integrated Discrimination Improvement, % ± SE	p Value
Death			
Plasma NT-proBNP	0.57 (0.43–0.71)	–	–
Urinary NT-CNP53	0.66 (0.53–0.78)	–	–
Both	0.69 (0.56–0.82)	30 (11)*	0.004*
Death/rehospitalization			
Plasma NT-proBNP	0.56 (0.46–0.66)	–	–
Urinary NT-CNP53	0.67 (0.59–0.76)	–	–
Both	0.69 (0.61–0.78)	17 (5.0)*	0.001*

*Versus plasma NT-proBNP alone.
Abbreviations as in Table 2.

JACC: Heart Failure Vol. 1, No. 2, 2013
April 2013:170–7

elevated urinary excretion of tubulospecific proteins such as KIM-1 and NGAL, and adverse outcomes in HF patients (7–9). These biomarkers of structural tubular damage are postulated to reflect a decline in renal function or adverse remodeling preceding a reduction in GFR and/or highlight an additional insult such as renal hypoxia. In the human kidney, CNP has been localized to the renal tubules (10), and thus its excretion in urine is proposed to be an additional marker of tubular integrity. However, only patients with clinically stable HF have been investigated, thus providing a rationale for the present examination in ADHF.

Herein we observed a >2-fold increase in KIM-1 excretion among ADHF patients compared to that in controls. However, in contrast to the findings from a prior report (9), urinary NGAL excretion was unchanged and neither KIM-1 nor NGAL were significantly associated with clinical outcomes. Notably, all 3 forms of urinary CNP were elevated in ADHF, and the excretion of NT-CNP53, in particular, outperformed both KIM-1 and NGAL as an indicator of mortality and the combined endpoint of all-cause rehospitalization/death.

Currently, the mechanism of NT-CNP53 generation and its relationship to HF prognosis remains unclear. Conceivably, tubular dysfunction sufficient to elevate NT-CNP53 may exceed the capacity of renal homeostatic mechanisms, already maximally employed in ADHF, thereby conferring a poorer prognosis. Because natriuretic peptides are counterregulatory in HF, CNP-induced vasorelaxation may counterregulate increases in peripheral vascular resistance. A net local excess of NT-CNP53, relative to the biologically active form CNP22, could portend a maladaptive neurohumoral response, corroborating the lack of prognostic significance of urinary CNP22 and plasma CNP forms observed in this study. Clearly, further studies are needed to address these key questions and to delineate mechanisms of CNP activation in ADHF.

Urinary C-type natriuretic peptide in a multimarker risk prediction strategy for heart failure. There is increasing interest in the use of multimodal biomarkers for risk prediction in HF. The findings from this study suggest that additional utilization of urinary NT-CNP53 may offer a more precise estimation of renal dysfunction in ADHF, including the detection of tubular dysfunction and/or renal parenchymal injury or remodeling. Furthermore, urinary NT-CNP53 provided incremental predictive value to established prognostic markers in ADHF—age, renal glomerular function (eGFR, urine protein/creatinine ratio), and plasma NT-proBNP—supporting its use as a complementary index of disease severity within a multimarker approach to ADHF assessment. Specifically, the combination of plasma NT-proBNP and urinary NT-CNP53 provided the optimal prognostic information in this ADHF cohort. Although both are biologically inactive, these 2 differentially localized natriuretic peptides are thought to reflect distinct pathological insults, NT-proBNP resulting from myocardial pressure-volume overload, and NT-CNP53, a marker of renal injury and/or dysfunction. The absence of a statistical correlation between them supports this notion and highlights their combined value for an integrated cardiorenal risk-prediction strategy in ADHF.

Study limitations. This was a small study, but follow-up was 100%. A larger study including stratification by HF etiology and new versus recurrent presentation is now warranted to confirm the present findings. Heterogeneous ADHF-management strategies and polypharmacy may have masked associations pertaining to the urinary biomarkers. On exploratory analysis, no marked correlations were observed between medications on presentation and urinary biomarker levels, but further investigation is needed to accurately define pharmacodynamic effects on CNP excretion. Ascertainment of clinical outcomes was restricted to our institution and local services because the majority of patients admitted to our HF service exclusively utilize institutional or locally provided clinical care. Inclusion criteria were deliberately broad to reflect the spectrum and high risk of HF patients presenting for hospital admission.

Conclusions

In this proof-of-concept study, elevated urinary excretion of NT-CNP53 was significantly associated with adverse outcomes in ADHF patients, independent of eGFR and with incremental prognostic value to plasma NT-proBNP. These findings highlight the importance of the cardio–renal interaction in determining clinical outcomes in ADHF and support a potential role for a dual natriuretic peptide multimarker approach, which combines plasma NT-proBNP and urinary NT-CNP53, for risk stratification in this population.

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Key Words: acute decompensated heart failure ■ biomarkers ■ C-type natriuretic peptide ■ outcomes.

APPENDIX

For more details on the results of this study, please see the online version of this article.