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Original Paper

Prognostic Significance of Serum Cysteine-Rich Protein 61 in Patients with Acute Heart Failure

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Key Words
Acute heart failure • CCN1/CYR61 • Biomarker • Prognosis

Abstract
Background/Aims: Cyr61-cysteine-rich protein 61 (CCN1/CYR61) is a multifunctional matricellular protein involved in the regulation of fibrogenesis. Animal experiments have demonstrated that CCN1 can inhibit cardiac fibrosis in cardiac hypertrophy. However, no study has been conducted to assess the relation between serum CCN1 and prognosis of acute heart failure (AHF). ***Methods:*** We measured the serum CCN1 levels of 183 patients with AHF, and the patients were followed up for 6 months. The associations between CCN1 levels and some clinical covariates, especially left ventricular ejection fraction (LVEF), estimated glomerular filtration rate (eGFR), atrial fibrillation and age, were estimated. The AHF patients were followed up for 6 months. The endpoint was all-cause mortality. Kaplan-Meier curve analysis and multivariable Cox proportional hazards analysis were employed to evaluate the prognostic ability of CCN1. We used calibration, discrimination and reclassification to assess the mortality risk prediction of adding CCN1. ***Results:*** Serum CCN1 concentrations in AHF patients were significantly increased compared with those in individuals without AHF (237 pg/ml vs. 124.8 pg/ml, $p<0.001$). CCN1 level was associated with the level of NT-proBNP (r=0.349, $p<0.001$) and was not affected by LVEF, eGFR, age or atrial fibrillation in AHF patients. Importantly, Kaplan-Meier curve analysis illustrated that the AHF patients with serum CCN1 level >260 pg/ml had a lower survival rate ($p<0.001$). Multivariate Cox hazard analysis suggests that CCN1 functions as an independent predictor of mortality for AHF patients (LgCCN1, hazard ratio 5.825, 95% confidence interval: 1.828-18.566, $p=0.003$). In addition, the inclusion of CCN1 in

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the model with NT-proBNP significantly improved the C-statistic for predicting death (0.758, $p<0.001$). The integrated discrimination index was 0.019 ($p<0.001$), and the net reclassification index increased significantly after addition of CCN1 (23.9%, $p=0.0179$). ***Conclusions***: CCN1 is strongly predictive of 6-month mortality in patients with AHF, suggesting serum CCN1 as a promising candidate prognostic biomarker for AHF patients.

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Introduction

Acute heart failure (AHF) is characterized by an acute or sub-acute deterioration in cardiac function resulting from underlying heart disease and precipitating factors. AHF can lead to injuries of multiple organs, including heart, brain, kidney and liver, and such injury is associated with increased mortality [1]. According to data from the UK, AHF is the leading cause of hospital admission in individuals aged >65 years [2]. Approximately 30% of AHF patients in Europe die during 1-year follow-up [1]. Despite utilization of guideline-mandated therapy, little improvement has been made in AHF outcomes [3]. To tailor an appropriate therapeutic strategy for AHF patients, severity evaluation and risk stratification are important. Serum biomarkers such as B-type natriuretic peptide, amino terminal B-type natriuretic peptide (NT-proBNP), ST-2 and gelatin-3 have been shown to be effective markers for evaluating the prognosis of AHF [4-7]. However, these biomarkers have limitations for mortality prediction in patients with heart failure [8, 9]. Therefore, the discovery of novel biomarkers might improve risk stratification for patients with AHF.

CCN1 (also known as Cyr61-cysteine-rich protein 61, CYR61) belongs to the CCN family. The members of this family share a modular structure that includes an N-terminal secretory peptide followed by four conserved domains, including a region with sequence homology to insulin-like growth factor-binding protein, a von Willebrand factor type C repeat, a thrombospondin type I repeat and a carboxyl-terminal domain [10]. CCN1 was first identified as a serum-inducible immediate-early gene product in mouse fibroblasts [11]. Mounting evidence has demonstrated that CCN1 is a multifunctional matricellular protein that is involved in the regulation of wound healing, fibrogenesis and other inflammatory processes [10, 11]. CCN1 inhibits fibrosis by driving myofibroblasts into senescence and attenuating the fibrotic signaling of transforming growth factor-β [12]. Papetta et al. detected CCN1 immunopositivity in most cardiac tissue specimens obtained from young and middle-aged victims of sudden cardiac death and found that CCN1 may be associated with ischemic morphology and hypertrophy of myocardial fibers [13]. Another study showed that CCN1 levels are elevated in cardiomyocytes exposed to pressure overload or ischemic stress [14]. Nevertheless, the clinical evidence regarding serum CCN1 level and its clinical significance in patients with heart failure remains scarce. In the present study, we measured serum CCN1 levels in patients with AHF, obtained follow-up information on all-cause mortality after discharge, and used this information to evaluate the prognostic value of CCN1 for AHF patients.

Materials and Methods

Subjects

We conducted a prospective and observational study of 183 hospitalized patients with AHF in the First Affiliated Hospital of Sun Yat-sen University from June 2014 to December 2015. The study was carried out according to the principles of Declaration of Helsinki and approved by the Medical Ethics Commission of First Affiliated Hospital of Sun Yat-sen University, China. Two cardiologists confirmed the diagnosis of AHF according to the current Chinese Society of Cardiology Heart Failure guideline. Patients older than 18 years old with symptoms and signs of AHF were consecutively included. Either new-onset AHF or acute decompensation of chronic heart failure was accepted. Informed consent was obtained from every patient. Coronary heart disease was confirmed by coronary angiography. Cardiomyopathy was diagnosed

by echocardiography and electrocardiogram according to the current Chinese Society of Cardiology Cardiomyopathy guideline. Patients with a severe infection, pulmonary embolism, severe liver dysfunction, malignant carcinoma or a history of recent cardiopulmonary resuscitation were excluded. The control group consisted of 20 individuals with matched age and gender that had no past history of heart failure or kidney dysfunction, as well as no symptoms or signs of AHF. They underwent laboratory examination (NT-proBNP＜300pg/ml) to further exclude AHF.

Clinical evaluation and follow-up

On admission, all the patients underwent an initial clinical assessment consisting of clinical history, physical examination, electrocardiogram, echocardiography, chest radiography and standard blood measurements including NT-proBNP, creatinine, hemoglobin and troponin T. Atrial fibrillation was identified by electrocardiogram before and during hospitalization. Left ventricular end diastolic dimension (LVEDD) and left ventricular ejection fraction (LVEF) were measured through M-mode and 2-dimensional Doppler-echocardiography.

The endpoint of the study was all-cause mortality. Fatal events were identified by checking medical records and contacting patients' family members. All patients were followed up for 6 months after discharge except 11 patients who lost contact during the follow-up.

Serum CCN1 measurement

Blood samples from patients with AHF were collected in coagulation promoting tubes, allowed to clot for 30 minutes, and centrifuged at 2000rpm for 10 minutes to separate the serum. After centrifugation, the serum samples were immediately aliquoted, properly coded, and frozen at -80°C until the CCN1 measurement. The serum levels of CCN1 were measured by an enzyme-linked immunosorbent assay (R&D Systems, Inc. Minneapolis, MN, USA) using monoclonal antibody against human CCN1 according to the manufacture's instruction. The serum used for this study only subjected to a single freeze-thaw cycle.

Statistical analysis

Normally distributed continuous variables were expressed as mean ± SD, while the non-normally distributed continuous variables (including CCN1 and NT-proBNP) were presented as medians and interquartile ranges. Categorized variables were expressed as number (%). Chi-square tests were used for comparisons of the categorical clinical characteristics between the patients with and without AHF. CCN1 and NT-proBNP were compared by the Wilcoxon's rank-sum test. Univariable Spearman correlation was used to evaluate the magnitude and significance of associations between serum CCN1 or NT-proBNP levels and other continuous covariates.

To evaluate the prognostic value of CCN1 or NT-proBNP at 6-month follow-up, we calculated the C-statistics that were identical to the area under the receiver operating characteristic (ROC) curves (AUC) after transforming log values of CCN1 and NT-proBNP. In a logistic model of endpoint including both LgCCN1 and Lg NT-proBNP, C-statistic was calculated to evaluate prognostic value of LgCCN1 plus LgNT-proBNP. Kaplan-Meier curves were constructed to investigate the prognostic value of levels of CCN1 or CCN1 plus NT-proBNP. Log transformed values of CCN1 and NT-proBNP were used in univariable Cox proportional hazards models. To identify the independent predictors of mortality for the cohort, multivariable Cox proportional hazards models were used. If variables' p values were <0.05 in univariable Cox proportional hazards model, they were entered into the multivariable model. And those parameters would be calculated with time-dependent covariates to check the proportional hazards assumption.

Furthermore, calibration was evaluated by the Hosmer-Lemeshow goodness of fit test. The clinical benefit in risk prediction of adding CCN1 to the clinical model was further assessed by reclassification analysis, including the integrated discrimination index (IDI) and the net reclassification index (NRI) [15, 16]. In the reclassification analysis, we used tertiles for the risk of death as a definition of meaningful risk categories: <20%, 20%-30%, and >30%. The NRI considered changes in the estimated mortality prediction probabilities that imply a change from one category to another, while the IDI considered the changes in the estimated mortality prediction probability as a continuous variable.

The reported p values were two-sided, and p<0.05 was considered statistically significant. All of the statistical analyses were performed using SPSS 24.0, with a graphical user interface of Graphpad Prism 6.0.

Results

Baseline clinical characteristics of the study population

The baseline characteristics of the AHF group and the control group are shown in Table 1. The mean age was 68 years in the AHF group and 69 years in the control group. There were 125 men (68%) in the AHF group and 12 men (60%) in the control group. Of the patients in the AHF group, 76% had prior heart failure, 56% had a history of hypertension and 45% had coronary heart disease, indicating that hypertension and coronary heart disease were the main causes of heart failure. Furthermore, 20% of the AHF group comprised patients with cardiomyopathy including dilated cardiomyopathy, hypertrophic cardiomyopathy, alcoholic cardiomyopathy and left ventricular noncompaction. Valvular heart disease was present in 8% of the AHF patients. In comparison with the patients in the control group, the AHF patients had a higher prevalence of atrial fibrillation. Serum creatinine, troponin T levels and LVEDD were significantly higher in the AHF group than in the control group, and AHF patients had lower estimated glomerular filtration rate (eGFR) and LVEF. Importantly, both serum NT-proBNP levels (median value: 5534 pg/ml, interquartile range: 2773-9954 pg/ml for the AHF group vs. median value: 73.5 pg/ml, interquartile range: 60.3-138.2 pg/ml for the control group; $p<0.001$) and serum CCN1 levels (median value: 237.0 pg/ml, interquartile range: 185.3-304.8 pg/ml for the AHF group vs. median value: 124.8 pg/ml, interquartile range: 91.2-161.0 pg/ml for the control group; $p<0.001$) were significantly higher in the AHF group than in the control group. These data demonstrate that serum CCN1 was significantly elevated in the patients with AHF.

Table 1. The baseline characteristics of patients in the AHF group and the control group. The data are expressed as an absolute number (percentage), the mean (standard deviation) or the median (25th-75th percentile). SBP, systolic blood pressure; DBP, diastolic blood pressure; ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro-B-type natriuretic peptide; CCN1, Cyr 61-cysteine rich protein 61; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end diastolic diameter

	AHF（n=183）	Control（n=20）	P-value
Age	68±15	69±11	0.833
Male	125(68%)	12（60%）	0.451
Prior heart failure	139（76%）	0（0%）	<0.001
Hypertension	103（56%）	10（50%）	0.591
Coronary heart disease	83 (45%)	9 (45%)	0.976
Cardiomyopathy	37（20%）	0（0%）	0.028
Valvular heart disease	15（8%）	1（5%）	0.614
Diabetes mellitus	55（30%）	4（20%）	0.347
Stroke	21（11%）	4（20%）	0.271
Smoking	63（34%）	3（15%）	0.078
Pacemaker	15 (8%)	0 (0%)	0.183
SBP	129±26	127±18	0.682
DBP	76±16	75±11	0.597
Heart rate	89±22	79±12	0.043
Atrial fibrillation	53 (29%)	0 (0%)	<0.001
Dyspnea	117（64%）	0（0%）	<0.001
Orthopnea	74（40%）	0（0%）	<0.001
Pulmonary moist rale	112（61%）	0（0%）	<0.001
Lower extremity edema	96（52%）	0（0%）	<0.001
Loop diuretics	161(88%)	1(5%)	<0.001
β-blockers	121(66%)	10 (50%)	0.153
ACEI / ARB	111(61%)	8 (40%)	0.075
Aldosterone antagonists	144 (79%)	1(5%)	<0.001
Creatinine (μmol/L)	108 (85-160)	74.0(60.8-101.3)	<0.001
eGFR (ml/min/1.73m^2)	57.7±30.6	93.2±27.7	<0.001
Hemoglobin (g/L)	123 (106-142)	129 (125-137)	0.127
Troponin T	0.04 (0.02-0.14)	0.008(0.003-0.58)	<0.001
CCN1 (pg/ml)	237.0(185.3-304.8)	124.8(91.2-161.0)	<0.001
NT-proBNP (pg/ml)	5534(2773-9954)	73.5(60.3-138.2)	<0.001
LVEDD (mm)	59（50-68）	48（45-49）	<0.001
LVEF (%)	46（33-61）	71（64-74）	<0.001

Table 2. Correlations between CCN1 and other clinical covariates in AHF patients. Abbreviations as in Table 1

Covariates.	CCN1		NT-proBNP	
	r	p Value	r	p Value
Age	0.106	0.154	0.199	0.007
SBP	-0.124	0.096	-0.016	0.828
DBP	0.039	0.597	-0.025	0.733
Heart rate	0.059	0.431	0.081	0.276
NT-proBNP	0.349	<0.001	-	-
CCN1	-	-	0.349	<0.001
Creatinine	0.077	0.301	0.520	<0.001
eGFR	-0.126	0.099	-0.545	<0.001
Troponin T	0.146	0.050	0.514	<0.001
Hemoglobin	-0.146	0.056	-0.103	0.177
LVEF	-0.155	0.048	-0.068	0.396
LVEDD	0.040	0.623	0.037	0.653

Correlation between CCN1 and other clinical covariates

To better understand the clinical characteristics of serum CCN1 in AHF, we examined the correlation between serum CCN1 levels and 11 clinical covariates related to HF. As shown in Table 2, serum CCN1 levels were associated with NT-proBNP levels (r=0.349，$p<0.001$) and had no association or an extremely weak association with LVEF levels (r=-0.155, $p=0.048$). In addition, CCN1 did not affect eGFR, troponin T or hemoglobin levels. To further evaluate the relationship between CCN1 and LVEF, we divided the patients into 3 groups according to their LVEF levels (≥50%, 40-49%, and <40%) [1]. However, there were no differences in CCN1 levels among these three groups ($p=0.731$; Fig. 1A).

To assess the impact of atrial fibrillation on CCN1, the patients were divided into 2 groups, a sinus rhythm group and an atrial fibrillation group. As shown in Fig. 1B, there was no difference in the CCN1 levels of these two subgroups, indicating that serum CCN1 levels are not affected by atrial fibrillation. In addition, in contrast to NT-proBNP, we found no significant correlation between serum CCN1 level and age (r=0.106, p=0.154; Table 2). Furthermore, when the AHF patients were divided by age into group 1 (less than 60 years of age), group 2 (60-75 years of age), and group 3 (more than 75 years of age), there were no statistically significant differences in serum CCN1 levels in the subgroups (p=0.354; Fig. 1C). Therefore, our data suggest that although serum CCN1 correlates with NT-proBNP, the level of serum CCN1 may not be affected by key covariates related to HF, including LVEF, atrial fibrillation and advanced age.

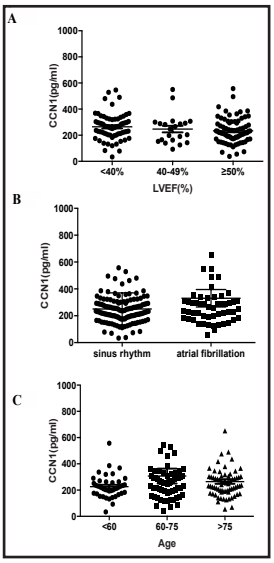

Fig. 1. CCN1 levels in AHF patients are not affected by LVEF, atrial fibrillation or age. Histogram showing the mean serum CCN1 levels in AHF patients with indicated LVEF(A), sinus rhythm and atrial fibrillation(B) or age (C).

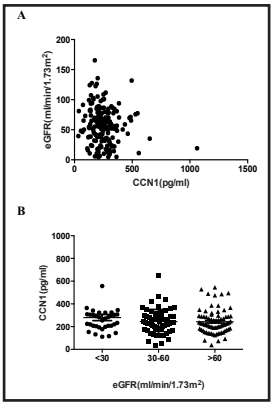

Fig. 2. There is no significant association between CCN1 levels and eGFR in AHF patients. (A) Scatter plot of serum CCN1 levels according to various eGFR. (B) Box Plot of serum CCN1 levels according to eGFR-grouped values in AHF patients.

Relationship between CCN1 and renal function

It is known that NT-proBNP levels can be affected by renal dysfunction; this would affect the diagnostic and prognostic utility of NT-proBNP [17, 18]. To understand the relationship between serum CCN1 and renal function, a Spearman correlation was performed. Interestingly, serum CCN1 levels were not significantly correlated with eGFR (r=-0.126, p=0.099; Fig. 2A). To further address whether CCN1 is affected by renal dysfunction, we divided the patients into 3 subgroups according to their eGFR values (>60 mL/min/1.73 m^2, 30-60 mL/min/1.73 m^2 and <30 mL/min/1.73 m^2) and analyzed their serum CCN1 levels. As shown in Fig. 2B, serum CCN1 levels were similar in these three subgroups (p=0.324). Therefore, our findings suggest that serum CCN1 may not be affected by renal insufficiency in AHF patients.

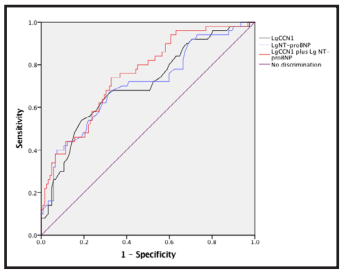

Fig. 3. CCN1 is as valuable as NT-proBNP in predicting mortality for AHF patients. The ROC of LgCCN1, Lg NT-proBNP and combination of Lg CCN1 and Lg NT-proBNP evaluated death rate at 6 months.

Serum CCN1 level as a predictor of mortality in AHF patients

During the 6-month follow-up period, 50 patients died. The serum CCN1 levels of AHF patients who died during the follow-up period were significantly higher than those of the patients who did not (375.32±90.76 pg/ml vs. 227.38±92.66 pg/ml, p=0.039). ROC analysis demonstrated that LgCCN1 was a significant predictor of 6-month mortality as evidenced by the C-statistic of 0.704 (the 95% confidence interval [CI]: 0.616-0.792, p<0.001). The optimal cutoff value is 2.415, and the prediction had a sensitivity of 66% and a specificity

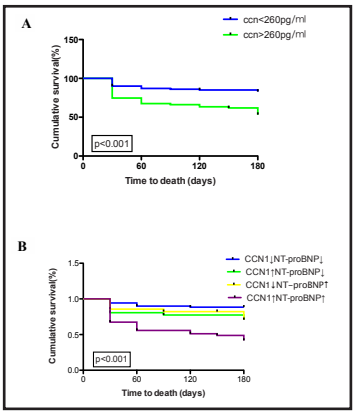

Fig. 4. CCN1 levels are significantly associated with the cumulative survival rate of AHF patients. Kaplan-Meier survival curves for AHF patients (A) according to CCN1 cutoff value (blue line means that CCN1 concentrations were lower than 260pg/ml, and, green line means that CCN1 concentrations were higher than 260pg/ml); (B) the combination levels of CCN1 plus NT-proBNP, using a cutoff value of 260pg/ml for CCN1 and 6456.5 pg/ml for NT-proBNP. ↓means below cutoff value, ↑means above cutoff value. Blue line: CCN1↓NT-proBNP↓, green line: CCN1↑NT-proBNP↓, yellow line: CCN1↓NT-proBNP↑ and purple line: CCN1↑NT-proBNP↑.

of 68.9%. In addition, the C-statistic of LgNT-proBNP was 0.705 (95% CI: 0.616-0.794, p<0.001), and the cutoff point is 3.81. The predictive sensitivity of events was 68%, and the specificity was 67.2%. When we combined CCN1 and NT-proBNP, the C-statistic increased to 0.754 (95% CI: 0.675-0.832, p<0.001; Fig. 3). We converted the cutoff value of LgCCN1 2.415 to the serum CCN1 value of 260 pg/ml and transformed the cutoff value of LgNT-proBNP (3.81) to the NT-proBNP value of 6456.5 pg/ml. To explore whether serum CCN1 can serve as a predictor of poor outcome in AHF, Kaplan-Meier survival curves for death in the AHF patients were analyzed according to the CCN1 cutoff values. As shown in Fig. 4A, patients with serum CCN1 levels higher than 260 pg/ml had higher risk of mortality than those with lower serum CCN1 levels (log-rank test, p<0.001). Furthermore, we found that the combination of high serum CCN1 level (higher than the cutoff value of 260 pg/ml) and high serum NT-proBNP level (higher than the cutoff value of 6456.5 pg/ml) identified the AHF patients with the lowest survival rate. In contrast, the AHF patients with the highest survival rate had both serum CCN1 levels and NT-proBNP levels that were lower than the respective cutoff values (log-rank test, p<0.001; Fig. 4B). Collectively, these data suggest that serum CCN1 level can indeed be used as a predictor of mortality in AHF patients.

Table 3. Univariate and multivariate Cox proportional hazard analysis for CCN1 and other variables with all cause death. HR: hazard ratio; CI: confidence interval; abbreviations as in Table 1

	Univaraible			Multivariable		
Variable	HR	CI	p Value	HR	CI	p Value
Age	1.026	1.004-1.048	0.019	1.014	0.990-1.038	0.247
Gender	0.767	0.433-1.357	0.362			
Prior heart failure	1.053	0.539-2.056	0.880			
Hypertension	1.035	0.844-1.269	0.743			
Coronary heart disease	0.805	0.428-1.515	0.502			
Diabete mellitus	0.805	0.428-1.515	0.502			
Valvular heart disease	1.960	0.881-4.358	0.099			
Cardiomyopathy	0.490	0.209-1.151	0.102			
Stroke	1.292	0.581-2.873	0.529			
Smoking	0.979	0.546-1.758	0.945			
SBP	0.996	0.985-1.007	0.453			
DBP	0.981	0.963-1.000	0.049	0.984	0.965-1.004	0.110
Heart rate	0.991	0.977-1.005	0.200			
Atrial fibrillation	0.708	0.363-1.383	0.313			
LVEF	1.005	0.986-1.023	0.628			
LVEDD	0.996	0.971-1.022	0.765			
eGFR	0.980	0.970-0.990	<0.001	0.992	0.979-1.005	0.221
Troponin T	1.123	0.942-1.338	0.195			
Hemoglobin	0.997	0.987-1.008	0.560			
Lg NT-proBNP	3.916	2.089-7.341	<0.001	1.888	0.879-4.054	0.103
Lg CCN1	9.557	3.612-25.285	<0.001	5.825	1.828-18.666	0.003

Univariate Cox proportional hazard analysis was then performed to evaluate the variables in predicting mortality of AHF. We discovered that serum CCN1 (LgCCN1; hazard ratio [HR]: 9.557, 95% CI: 3.612-25.285, p<0.001), NT-proBNP (LgNT-proBNP; HR: 3.916, 95% CI: 2.089-7.341, p<0.001), age (HR: 1.026, 95% CI: 1.004-1.048, p=0.019), diastolic blood pressure (DBP, HR: 0.981, 95% CI: 0.963-1.000, p=0.049)， and eGFR (HR: 0.980, 95% CI: 0.970-0.990, p<0.001) were risk factors in AHF. These five independent variables were checked by the assumption of proportional hazards and further subjected to multivariate Cox proportional hazard analysis. We found that serum CCN1 (LgCCN1 HR: 5.825, 95% CI: 1.828-18.566, p=0.003) still presented independent prognostic utility (Table 3). Interestingly, NT-proBNP was no longer an independent predictor of AHF in our cohort of patients (Table 3).

The prognostic significance of serum CCN1 was further explored. In the ROC analysis, the C-statistic increased to 0.754 (p<0.001) after adding LgCNN1 to LgNT-proBNP. The Hosmer-Lemeshow test showed good calibration for the models with and without CCN1 (p=0.123 for all comparisons). The integrated discrimination improvement (IDI) was estimated at 0.019 (p<0.001). The reclassification of patients with and without mortality is summarized in Table 4. The net reclassification index (NRI) was significant with the individual inclusion of CCN1 (index: 10% for decedents, 13.9% for survival patients, and 23.9% overall; p=0.0179). Therefore, the NRI and IDI proved that the addition of CCN1 improved the risk stratification of AHF. In summary, these data further indicate that measurement of serum CCN1 levels might provide important valuable prognostic information for AHF patients.

Table 4. Reclassification among patients with and without levels of CCN1 who were dead and those who were survival during follow-up. Abbreviations as in Table 1

Model with NT-proBNP	Model with NT-proBNP +CCN1			
Patients who died	Low tertile (<20%)	Medium tertile (20%-30%)	High tertile (>30%)	Total no.
Low tertile (<20%)	5	2	3	10
Medium tertile (20%-30%)	5	7	6	18
High tertile (>30%)	0	1	21	22
Total no.	10	10	30	50
Patients who did not die				
Low tertile (<20%)	34	5	2	41
Medium tertile (20%-30%)	32	22	10	64
High tertile (>30%)	1	1	15	17
Total no.	67	28	27	122

Discussion

The present study found that elevation of serum CCN1 level is significantly associated with the severity of AHF in a cohort of 183 patients. Unlike NT-proBNP, serum CCN1 levels are not likely to be affected by LVEF, kidney dysfunction, age or atrial fibrillation. Kaplan-Meier analysis showed that a high level of serum CCN1 is most likely to indicate deteriorated outcome for AHF patients. Moreover, multivariate Cox proportional hazard analysis demonstrated that serum CCN1 represents an independent risk factor for AHF mortality. Furthermore, CCN1 improved the risk stratification for death in AHF patients as estimated by model discrimination and reclassification. Therefore, the results of our current study suggest that serum CCN1 has great potential to serve as a novel biomarker for evaluating the prognosis of AHF patients.

CCN1, which was originally identified as a growth factor-inducible immediate early gene in fibroblasts [11], plays essential roles in cardiovascular development, embryogenesis, inflammation regulation, wound healing and fibrogenesis [10]. It has been reported that CCN1 is rapidly activated by serum or purified platelet-derived growth factor stimulation in fibroblasts and that it is secreted and accumulates in the extracellular matrix. Once associated with the extracellular matrix, its half-life increases to more than 24 hours, in contrast to the short half-life (approximately 30 min) of intracellular and cell surface-associated CCN1. CCN1 plays an essential role in regulating matrix structural integrity [19, 20]. It has also been reported that CCN1 expression can be induced by angiotensin II and phenylephrine or ischemic stress in cardiomyocytes. Expression of CCN1 is nearly absent in the non-failing heart, whereas increased CCN1 levels have been detected in the atrial myocardium in an animal model of chronic heart failure [21]. Interestingly, Yoshida et al. found that CCN1 increased the survival of myocytes by activating the integrin β1-Akt pathway under conditions of oxidative stress, whereas knockdown of CCN1 decreased the number of surviving cells under these conditions, indicating that CCN1 is a protector of ischemic myocytes [22]. Moreover, CCN1 inhibited cardiac fibrosis in cardiac hypertrophy induced by transverse aortic constriction [23], suggesting that inhibition of fibrosis might be the core mechanism through which CCN1 protects against cardiac remodeling. Moreover, it was shown in several animal experiments that the expression of CCN1 protein increased in cardiac tissue in a model of heart failure [21, 24, 25]. Consistent with these reports, our study showed that serum CCN1 concentrations were significantly higher in AHF patients than in individuals without HF. In addition, NT-proBNP, which is closely related to cardiac function, was correlated with serum CCN1 in clinical AHF patients. Thus, our data suggest a close

association of CCN1 with AHF. It is well known that deteriorated cardiac remodeling leads to a transition from compensated cardiac hypertrophy to heart failure, and extracellular matrix materials such as procollagen, which is related to cardiac remodeling, have shown prognostic significance in heart failure [26]. Therefore, serum CCN1 could be a valuable potential biomarker in patients with AHF.

Our study revealed that serum CCN1 levels were elevated in AHF patients who died during the follow-up period. This suggested that CCN1 is associated with the risk of mortality in AHF. Kaplan-Meier analysis showed that AHF patients with high levels of CCN1 had a higher probability of mortality. At the same time, multivariate Cox proportional hazard models showed that CCN1 represents an independent risk factor for all-cause death. ROC analysis of 6-month death further showed that CCN1 could predict events in AHF patients as effectively as NT-proBNP. NT-proBNP that is released in response to volume expansion and pressure overload is a well-established biomarker in predicting heart failure [27, 28], and CCN1 has been shown to be involved in the regulation of fibrogenesis associated with cardiac remodeling. The use of a combination of biomarkers associated with different pathophysiological pathways could produce additive prognostic information. Our study showed that combining NT-proBNP with CCN1 increased the C-statistic from 0.705 (C-statistic of NT-proBNP) to 0.754, suggesting that inclusion of CCN1 as well as NT-proBNP could make it possible to better predict adverse outcomes in patients with AHF. Moreover, use of the statistical tools NRI and IDI to assess and quantify the improvement in risk prediction further proved that the inclusion of CCN1 increased prognostic discrimination in AHF patients. Thus, the results of our study imply that the use of CCN1 as a biomarker improves risk stratification in AHF.

Renal impairment is closely associated with heart failure [29]. Notably, renal dysfunction not only decreases the specificity and sensitivity of NT-proBNP for diagnosing AHF [30] but also affects the prognostic ability of NT-proBNP [18]. One of the reasons for this might be that natriuretic peptides are partially excreted by the kidney [17]. Our study showed that CCN1 levels were not affected by eGFR. Although CCN1 is localized in the thick ascending limb of Henle's loop, distal and proximal tubules and collecting ducts and has been reported to be downregulated in diseased kidneys, the results of animal experiments have shown that heart failure slightly attenuates the expression of CCN1 protein in the kidney [25]. Our study provides important clinical evidence that renal insufficiency does not affect the serum concentration of CCN1 in HF patients. After adjusting for age, DBP and eGFR, the multivariate Cox proportional hazard model including both CCN1 and NT-proBNP showed that CCN1 was still an independent predictor of poor outcome, whereas NT-proBNP was no longer a mortality predictor in AHF. This implies that CCN1 might offer an advantage over NT-proBNP for evaluating the prognosis of AHF patients with kidney dysfunction. Therefore, our study highlights the prognostic significance of serum CCN1 in AHF patients, especially those with renal failure.

B-type natriuretic peptide and NT-proBNP are the most useful biomarkers of heart failure, but their expression is greatly affected by age and atrial fibrillation. Although age is an important factor influencing the effectiveness of NT-proBNP [31], some studies show that aging sarcopenia is associated with increased expression of CCN1 [32]. Interestingly, our study showed that serum CCN1 levels are not affected by age. In addition, atrial fibrillation, which is the most common arrhythmia in patients with heart failure, can elevate the level of NT-proBNP [33] and impair the diagnostic accuracy of B-type natriuretic peptide in heart failure patients [34]. Although CCN1 protein was reported to be upregulated in the atria in an animal model of chronic heart failure, there was no significant change in serum CCN1 levels in AHF patients with atrial fibrillation in our cohort, suggesting that atrial fibrillation might not affect serum CCN1 in AHF patients. In addition to age and atrial fibrillation, CCN1 is also not affected by LVEF, despite the role of CCN1 in regulating cardiac remodeling. It has been found that biomarkers for cardiac remodeling show no significant changes in HFpEF or HFrEF [35]. The reason for this might be that cardiac fibrosis contributes to the process of both HFpEF [36] and HFrEF [37]. Because CCN1 is quite important in cardiac remodeling, it is

reasonable that the level of CCN1 increases both in HFpEF and HFrEF. Furthermore, in human right atrial appendage specimens, CCN1 protein levels showed no statistically significant differences in different left ventricular ejection fractions [21]. This might be explained by our observation that CCN1 is not correlated with LVEF. Collectively, our study demonstrates the possible advantages of CCN1 over NT-proBNP in the evaluation of the prognosis of HF.

The present study has some limitations. First, it is a single-center study with limited sample size. A larger cohort and extended clinical data are needed to strengthen the prognostic significance of serum CCN1 in AHF patients. Second, although CCN1 was shown to provide prognostic information in AHF patients, we did not validate its prognostic power in patients with chronic heart failure. To further explore the potential value of CCN1 as a prognostic marker, we will include chronic heart failure patients in a future study.

Our study proved for the first time that CCN1 can be used as an independent risk factor for improved mortality risk prediction in AHF, suggesting that it is an effective predictor for the prognosis of AHF. These data may provide clinical validation and an additional method for the prognostic evaluation of patients with AHF.

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Disclosure Statement

The authors declare there are no conflict of interests.

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