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ORIGINAL RESEARCH ARTICLE

Circulating sST2 and catestatin levels in patients with acute worsening of heart failure: a report from the CATSTAT-HF study

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Abstract

Aims Soluble suppression of tumourigenicity 2 (sST2) and catestatin (CST) reflect myocardial fibrosis and sympathetic overactivity during the acute worsening of heart failure (AWHF). We aimed to determine serum levels and associations of sST2 and CST with in-hospital death as well as the association between sST2 and CST among AWHF patients.

Methods and results A total of 96 AWHF patients were consecutively enrolled, while levels of sST2 and CST were determined and compared between non-survivors and survivors. Predictive values of sST2 and CST for in-hospital death were determined by the penalized multivariable Firth logistic regression. The diagnostic ability of sST2 and CST for in-hospital death was assessed by the receiver operating characteristic analysis and examined with respect to the N-terminal pro-brain natriuretic peptide (NT-proBNP), high-sensitivity cardiac troponin I, and C-reactive protein. The in-hospital death rate was 6.25%. Serum sST2 and CST levels were significantly higher among non-survivors than survivors [146.6 (inter-quartile range, IQR 65.9–156.2) vs. 35.3 (IQR 20.6–64.4) ng/mL, $P < 0.001$, and 19.8 (IQR 9.9–28.0) vs. 5.6 (IQR 3.4–9.8) ng/mL, $P < 0.001$, respectively]. Both sST2 and CST were independent predictors of in-hospital death [Firth coefficient (FC) 6.00, 95% confidence interval (CI), 1.48–15.20, $P = 0.005$, and FC 6.58, 95% CI 1.66–21.78, $P = 0.003$, respectively], while NT-proBNP was not a significant predictor (FC 1.57, 95% CI 0.51–3.99, $P = 0.142$). In classifying non-survivors from survivors, sST2 provided area under the curve (AUC) of 0.917 (95% CI 0.819–1.000, $P < 0.001$) followed by CST (AUC 0.905, 95% CI 0.792–1.000, $P < 0.001$), while NT-proBNP yielded AUC of 0.735 (95% CI 0.516–0.954, $P = 0.036$). High-sensitivity cardiac troponin I and C-reactive protein were not found as significant classifiers of in-hospital death (AUC 0.719, 95% CI 0.509–0.930, $P = 0.075$, and AUC 0.682, 95% CI 0.541–0.822, $P = 0.164$, respectively). Among survivors, those with sST2 serum levels ≥35 ng/mL had significantly higher CST levels, compared with those with sST2 < 35 ng/mL (9.05 ± 5.17 vs. 5.06 ± 2.76 ng/mL, $P < 0.001$). Serum sST2 levels positively and independently correlated with CST levels in the whole patient cohort ($\beta = 0.437$, $P < 0.001$).

Conclusions Elevated sST2 and CST levels, reflecting two distinct pathophysiological pathways in heart failure, might indicate impending clinical deterioration among AWHF patients during hospitalization and facilitate prognosis beyond traditional biomarkers regarding the risk of in-hospital death (CATSTAT-HF ClinicalTrials.gov Number NCT03389386).

Keywords Acute decompensated heart failure; Catestatin; Heart failure; Hospital mortality; Risk stratification; Soluble suppression of tumourigenicity 2

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Introduction

The acute worsening of chronic heart failure (AWHF) or acute decompensated heart failure (ADHF) is the rapid or gradual deterioration in heart failure (HF) signs and symptoms among patients with established HF and accounts for more than two-thirds of all HF admissions.[1] Despite the guideline-directed optimal medical treatment, the in-hospital death rate of this condition remains high and ranges from 4% to 7% but can also well exceed 10% depending on the region of the world, co-morbidity burden, and syndrome severity of enrolled patients.[2–5] Factors independently associated with increased odds of in-hospital death are severe pulmonary oedema, acute kidney injury, sustained ventricular arrhythmias, and underlying respiratory process/pneumonia.[6,7] Likewise, cardiovascular (CV) death, especially in the form of sudden cardiac death (SCD), and HF progression to the terminal stage act as driving mechanisms behind in-hospital death among AWHF patients, while a substantial proportion of these patients die of complications caused by non-CV co-morbidities, especially those with preserved left ventricular ejection fraction (LVEF).[8–10]

Measurement of laboratory biomarker levels that reflect syndrome severity and have a downstream diagnostic, prognostic, and treatment implications is an integral part of the work-up of AWHF patients. Along with established CV biomarkers such as brain natriuretic peptide (BNP) or N-terminal proBNP (NT-proBNP), high-sensitivity cardiac troponin (hs-cTn), and C-reactive protein (CRP), novel and emerging biomarkers such as soluble suppression of tumourigenicity 2 (sST2) and catestatin (CST) might be utilized for prognostic purposes among AWHF patients. Of note, an sST2 is a soluble form of a decoy interleukin 33 (IL-33) receptor, and by reducing IL-33 bioavailability that exerts cardioprotective effects, it promotes cardiac hypertrophy, myocardial fibrosis, and ventricular dysfunction.[11] Increased levels of sST2 during hospitalization have been robustly associated with future adverse CV events, SCD, and overall death in both acute and chronic HF, independent of natriuretic peptide values.[12-14] On the other hand, CST is a potent adrenergic suppressor and catecholamine release inhibitor that likely reflects increased sympathetic nervous system (SNS) activity in HF and acts as a compensatory negative regulator of catecholamine metabolism, while higher CST levels independently predicted all-cause and cardiac death among chronic HF patients during the follow-up.[15] Previously, we demonstrated that higher CST levels were associated with ischaemic aetiology of HF and higher symptomatic burden among patients with ADHF.[16] However, no data exist on the mutual associations of sST2 and CST, as well as their potential diagnostic and prognostic utility regarding the short-term outcomes in HF such as in-hospital death. It is also unknown whether CST levels are increased among patients that are considered as a high risk of future adverse events following discharge, according to the elevated sST2 levels (≥35 ng/mL).

For these reasons, we aimed to compare circulating levels of sST2 and CST between AWHF patients who suffered in-hospital death and those that survived until discharge. Furthermore, we aimed to determine independent predictive values of sST2 and CST for the in-hospital death event, alongside traditional biomarkers such as NT-proBNP, high-sensitivity cardiac troponin I (hs-cTnI), and CRP. Secondarily, we aimed to determine CST levels, as a marker of SNS activation, among patients with sST2 levels <35 ng/mL and those with ≥35 ng/mL. Finally, examining the direct relationship between sST2 and CST in the whole patient sample and beyond natriuretic peptide levels has been of particular interest.

Methods

A study design, respective inclusion and exclusion criteria as well as baseline patient characteristics have been previously reported in the Serum Catestatin Expression and Cardiometabolic Parameters in Patients with Congestive Heart Failure (CATSTAT-HF) study.[16] In the mentioned study, a cohort of patients with acute worsening/decompensation of established chronic HF that survived to hospital discharge (N = 90) was enrolled between January 2018 and February 2019 at the Clinic for Cardiovascular Diseases of University Hospital of Split. An informed written consent was obtained from each participant enrolled in the study, while the study protocol was approved by the Ethics Committee of the University Hospital of Split (Approval No. 2181-147-01/06/M.S.-17-2) and University of Split School of Medicine Ethics Committee. The study was registered on 3 January 2018 at ClinicalTrials.gov registry before the recruitment of the first patient (CATSTAT-HF; ClinicalTrials.gov Number NCT03389386). The study adhered to the ethical guidelines laid out in the Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

In the present study, we included data of patients that met all required inclusion criteria and were enrolled in the study but died in hospital, and we compared data of these patients with the data of survivors comprising the aforementioned CATSTAT-HF cohort. None of the patients included in the analysis were enrolled because of *de novo* HF and had an infectious disease, active malignant disease or acute coronary syndrome, or stroke as the underlying aetiology of the index presentation. The acute coronary syndrome was ruled out by combining information based on symptoms, 12-lead electrocardiogram tracings, markers of myocardial injury, and/or results consistent with non-obstructive coronary artery disease

as assessed by diagnostic coronary angiography where performed. Patients with a severe valvular disease requiring surgical or percutaneous intervention were not included in the study. Furthermore, none of the patients were admitted in cardiogenic shock or received cardiopulmonary resuscitation before hospitalization.

All patients in the study underwent a detailed physical examination and had their medical history reviewed along with peripheral blood sampling and transthoracic echocardiography examination within 24 h of hospitalization by the same consultant cardiologist–investigator with high expertise in HF. A 12-lead electrocardiogram was recorded in all patients along with chest X-ray imaging, while arterial blood gas analysis was utilized in most patients. All patients were diagnosed and treated as per the European Society of Cardiology guidelines for the diagnosis and treatment of acute and chronic HF.[1]

The main goal of the current study was to compare serum sST2 and CST levels between patients that suffered in-hospital death and patients that survived to discharge. Furthermore, we sought to examine potential predictive and diagnostic values of sST2 and CST for the primary outcome of in-hospital death, along with traditional laboratory biomarkers reflecting ventricular haemodynamic overload (NT-proBNP), myocardial injury (hs-cTnI), and systemic inflammation (CRP). Finally, we aimed to assess and describe the relationship of sST2 with CST and to compare serum CST levels among patients with sST2 concentrations $<$35 ng/mL and those with $\geq$35 ng/mL.

Methods are described in detail in Supporting Information, *Appendix S1*.

Results

Baseline characteristics

A total of 96 patients with AWHF and in New York Heart Association Class II–IV were consecutively enrolled in the study. Of those, 90 patients survived until discharge comprising the CATSTAT-HF cohort, while six patients died during hospitalization making the in-hospital death rate of 6.25%. Adjudicated causes of death among these patients were SCD due to malignant ventricular arrhythmia in four cases, one patient suffered progressive pump failure resulting in cardiogenic shock while in hospital, and acute respiratory failure was the culprit in one case. The principal identified causes of HF worsening at admission among the whole patient cohort were uncontrolled arterial hypertension (*N* = 31, 32.3%), therapy non-adherence (*N* = 20, 20.8%), and arrhythmias [*N* = 19, 19.8%, of which atrial fibrillation with a fast ventricular response or atrial flutter were most common (10/19, 52.6%), followed by other supraventricular tachycardias (4/19, 21%), new-onset third-degree atrioventricular block (3/19, 15.7%), and ventricular tachycardia with haemodynamic stability (2/19, 10.5%)]. The underlying cause of decompensation was not identified in 26 patients (27.1%). Among patients that survived until discharge, mean catestatin concentration was 6.92 ± 4.56 ng/mL, while circulating levels of NT-proBNP, hs-cTnI, and CRP were 6254 ± 8112 pg/mL, 66.4 ± 53.6 ng/L, and 17.4 ± 16.26 mg/L, respectively. The patients' baseline characteristics have been described elsewhere[16] and are shown in Supporting Information, *Table S1*.

Patients that suffered in-hospital death did not significantly differ regarding the baseline characteristics compared with those that survived until discharge as shown in *Table 1*, except in the circulating levels of NT-proBNP and blood urea nitrogen that were significantly higher among those that died than survivors. Finally, patients that died had significantly higher Get With The Guidelines—Heart Failure score compared with patients that were alive to discharge (44.7 ± 5.1 vs. 36.7 ± 7.1 points, *P* = 0.008). None of the three shock indexes were able to significantly differentiate patients that suffered in-hospital death than those that survived to discharge (*p* = NS for all comparisons).

Circulating soluble suppression of tumourigenicity 2 levels among patients that survived until discharge

The average length of stay of patients that survived until discharge was 11 days (inter-quartile range, IQR 7–16 days). Among patients that survived until discharge (*N* = 90), the mean serum sST2 concentration was 43.96 ± 35.34 ng/mL with median of 35.33 ng/mL (IQR 20.57–64.41 ng/mL). Mean sST2 levels did not significantly differ between patients with ischaemic and non-ischaemic aetiology of HF (41.98 ± 32.16 vs. 46.16 ± 38.86 ng/mL, *P* = 0.596) and in respect to three clinical phenotypes stratified by the LVEF (47.89 ± 37.74 ng/mL for HF with reduced ejection fraction, 34.02 ± 35.89 ng/mL for HF with mid-range ejection fraction, and 45.01 ± 32.10 ng/mL for HF with preserved ejection fraction, *P* = 0.410). Furthermore, in this cohort, sST2 serum levels were not significantly associated with any of the measured echocardiographic parameters (Supporting Information, *Table S2*) and did not significantly correlate with any baseline anthropometric or clinical parameter, except New York Heart Association functional class (β = 0.442, *P* = 0.007) (Supporting Information, *Table S3*).

A majority of patients (*N* = 48, 53.3%) had circulating sST2 levels $<$35 ng/mL, while 42 patients (46.7%) had in-hospital sST2 levels $\geq$35 ng/mL and were classified as having a higher risk for adverse events following discharge.

Table 1 Baseline differences between patients that were alive to discharge and those that suffered in-hospital death

Variable	Alive to discharge	In-hospital death	*P*-value[a]
Age (years)	70.3 ± 10.2	70.8 ± 13.6	0.886
Female sex	47 (52.2)	3 (50.0)	0.951
BMI (kg/m^2)	30.2 ± 4.2	28.9 ± 3.4	0.464
Waist-to-hip ratio	0.98 ± 0.08	0.99 ± 0.11	0.588
SBP (mmHg)	137 ± 28	125 ± 16.43	0.278
MAP (mmHg)	100 ± 17	90 ± 12	0.126
HR at admission (b.p.m.)	95 ± 31	88 ± 10	0.580
NYHA functional class (mean)	3.0 ± 0.6	3.3 ± 0.5	0.200
Scores			
GWTG-HF risk score (points)	36.7 ± 7.1	44.7 ± 5.1	0.008
Modified shock index	1.14 ± 0.39	1.04 ± 0.22	0.494
Shock index	0.712 ± 0.267	0.717 ± 0.148	0.968
Age-adjusted shock index	52.8 ± 24.2	50.99 ± 15.6	0.861
Laboratory variables			
SaO_2 at admission (%)	89.7 ± 9.3	86.6 ± 9.6	0.484
pH at admission	7.43 ± 0.07	7.41 ± 0.06	0.663
Haemoglobin (g/L)	133 ± 19	137 ± 14	0.711
RDW (%)	15.1 ± 2.3	15.4 ± 2.0	0.757
Neutrophil-to-lymphocyte ratio	4.65 ± 2.99	6.14 ± 6.36	0.284
Sodium (mmol/L)	139 ± 3.7	137 ± 2.82	0.252
Potassium (mmol/L)	4.2 ± 0.4	4.3 ± 0.5	0.623
eGFR (CKD-EPI) (mL/min/1.73 m^2)	57.3 ± 24.9	48.2 ± 33.6	0.374
Creatinine (μmol/L)	118 ± 60	146 ± 67	0.267
BUN (mmol/L)	4.9 ± 2.6	7.3 ± 4.3	0.026
Albumin (g/L)	39 ± 4.1	37 ± 3.1	0.463
Fasting plasma glucose (mmol/L)	8.2 ± 3.0	8.6 ± 3.9	0.875
HbA1c (%)	6.6 ± 1.3	7.0 ± 1.2	0.633
Total cholesterol (mmol/L)	4.4 ± 1.3	3.7 ± 1.4	0.242
LDL cholesterol (mmol/L)	2.7 ± 1.1	2.2 ± 1.0	0.258
Triglycerides (mmol/L)	1.6 ± 0.6	1.5 ± 0.6	0.859
Echocardiography			
LVEF (%)	43.4 ± 16.4	43.6 ± 20.7	0.983
LVEDd (mm)	57.9 ± 9.4	57.3 ± 5.8	0.878
LVESd (mm)	42.6 ± 12.1	41.2 ± 10.2	0.764
Left atrium diameter (mm)	49.9 ± 8.9	46.3 ± 9.2	0.169
Electrocardiogram			
PR interval duration (ms)	188 ± 43	181 ± 23	0.783
QRS duration (ms)	121 ± 32	125 ± 18	0.761
QTc duration (ms)	440 ± 40	450 ± 25	0.565
Biomarkers of interest			
sST2 (ng/mL)	43.9 ± 35.3	122.6 ± 46.2	<0.001
CST (ng/mL)	6.9 ± 4.5	19.1 ± 8.9	<0.001
NT-proBNP (pg/mL)	6254 ± 8112	20 274 ± 16 210	<0.001
hs-cTnI (ng/L)	66.4 ± 53.6	94.2 ± 88.1	0.665
CRP (mg/L)	17.4 ± 16.26	22.8 ± 20.0	0.587

BMI, body mass index; BUN, blood urea nitrogen; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; GWTG-HF, Get With The Guidelines—Heart Failure; HbA1c, glycated haemoglobin; HR, heart rate; hs-cTnI, high-sensitivity cardiac troponin I; LVEDd, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESd, left ventricular end-systolic diameter; MAP, mean arterial pressure; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; SaO_2, peripheral arterial oxygen saturation; SBP, systolic blood pressure; sST2, soluble suppression of tumourigenicity 2.

Values are shown as mean ± standard deviation or *N* (%).

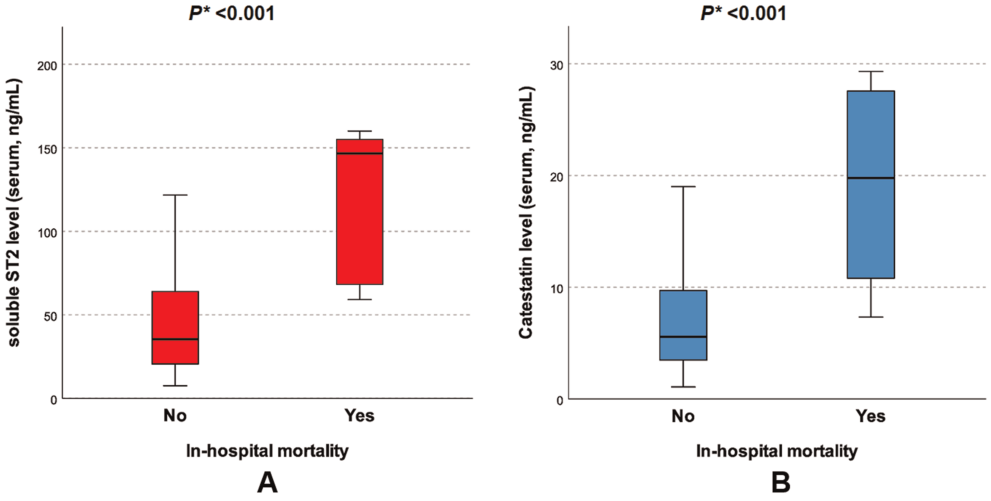
[a]An independent-samples *t*-test for continuous variables and Fisher's exact test for categorical variables were used for comparisons between two groups of interest, as appropriate.

Circulating levels of soluble suppression of tumourigenicity 2, catestatin, and traditional biomarkers in respect to in-hospital death

Patients that suffered in-hospital death had almost three-fold higher mean sST2 levels and significantly higher mean CST levels compared with survivors (*Table 1*). Likewise, mean levels of NT-proBNP were significantly higher among patients that suffered in-hospital death compared with survivors to discharge, while no significant difference was observed in mean hs-cTnI and CRP levels.

Because of non-normal distribution of these biomarkers in total patient sample, independent-samples Mann–Whitney *U* test was applied for all comparisons. Finally, this analysis showed that patients who died in hospital had significantly higher median sST2 levels compared with survivors [146.6

Figure 1 Comparison of subgroup of patients that survived to discharge and subgroup of patients that suffered in-hospital death in terms of (A) serum soluble suppression of tumourigenicity 2 (sST2) levels and (B) serum catestatin levels. *Mann–Whitney *U* test.



(IQR 65.9–156.2) vs. 35.3 (IQR 20.6–64.4) ng/mL, $P < 0.001$; *Figure 1A*], and similar finding was observed regarding the CST circulating levels [19.8 (IQR 9.9–28.0) vs. 5.6 (IQR 3.4–9.8) ng/mL, $P < 0.001$; *Figure 1B*]. Furthermore, patients who died in hospital did not have significantly higher medians of NT-proBNP [10 909 (IQR 3508–36 403) vs. 3586 (IQR 1361–7787) pg/mL, $P = 0.055$], hs-cTnI [60.9 (IQR 24.5–166.5) vs. 22.9 (11.6–49.0) ng/L, $P = 0.074$], and CRP [16.5 (IQR 9.5–33.0) vs. 8.4 (4.9–20.5) mg/L, $P = 0.138$], compared with patients that survived to discharge, respectively.

Predictive value of soluble suppression of tumourigenicity 2 and catestatin levels for the in-hospital death event

In the univariable Firth logistic regression model and multivariable Firth logistic regression models that included all patients ($N = 96$) and that were adjusted for relevant confounders, log-transformed levels of sST2 and CST remained as independent and significant predictors of in-hospital death. Of note, Firth coefficients were 6.00

Table 2 Univariable and multivariable penalized likelihood Firth logistic regression models that examined selected biomarkers as the predictors of in-hospital death

	Univariable model			Multivariable model[a]		
Variable	Firth coefficient	95% CI	*P*-value	Firth coefficient	95% CI	*P*-value
sST2	8.43	3.55–16.26	<0.001	6.00	1.48–15.20	0.005
CST	7.34	3.12–13.82	<0.001	6.58	1.66–21.78	0.003
NT-proBNP	1.75	1.10–3.72	0.037	1.57	0.51–3.99	0.142
hs-cTnI	1.09	0.28–2.42	0.114			
CRP	1.14	0.53–2.96	0.185			
BUN	1.30	1.02–1.68	0.038	1.90	0.98–3.67	0.055
eGFR	0.98	0.95–1.02	0.375			
LVEF	0.91	0.88–1.04	0.344			
SBP	0.97	0.94–1.02	0.275			
BMI	0.92	0.74–1.15	0.459			
Haemoglobin	1.01	0.97–1.05	0.708			
Sodium	0.90	0.75–1.08	0.254			
NYHA class	2.51	0.61–10.30	0.203			
Age	1.01	0.93–1.09	0.885			
Sex	1.00	0.19–5.2	0.998			

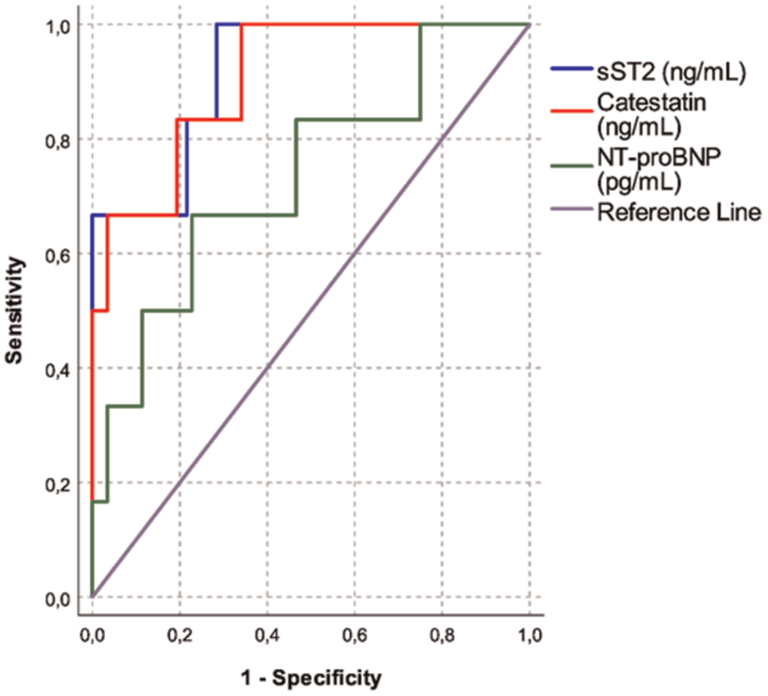
BMI, body mass index; BUN, blood urea nitrogen; CI, confidence interval; CRP, C-reactive protein; CST, catestatin; eGFR, estimated glomerular filtration rate; hs-cTnI, high-sensitivity cardiac troponin I; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; SBP, systolic blood pressure; sST2, soluble suppression of tumourigenicity 2.

[a]All multivariable logistic regression models were tested separately for each biomarker of interest (sST2, CST, NT-proBNP, hs-cTnI, and CRP) in respect to the binary outcome of in-hospital death and adjusted for covariates of age, sex, BMI, eGFR, BUN, haemoglobin, sodium, SBP, LVEF, and NYHA functional class. In addition, multivariable Firth regression models of sST2 and CST were also adjusted for log-transformed NT-proBNP levels.

[penalized 95% confidence interval (CI) 1.48–15.20, $P = 0.005$] for sST2 and 6.58 (penalized 95% CI 1.66–21.78, $P = 0.003$) for CST, as shown in *Table 2*. In contrast to this, of traditional biomarkers, hs-cTnI and CRP were not found as significant independent predictors of in-hospital death in the univariate Firth regression models, while NT-proBNP did not retain its significance in the multivariable Firth regression model (Firth coefficient 1.57, penalized 95% CI 0.51–3.99, $P = 0.142$). Sensitivity analyses with stepwise inclusion and exclusion of defined parameters defined in the multivariate models showed consistent associations of sST2 and CST with the outcome of in-hospital death. Respective likelihood plots derived from multivariable Firth regression models for sST2, CST, and NT-proBNP are available in Supporting Information, *Figure S1*.

Receiver operating characteristic (ROC) analysis performed in whole patient sample showed that of all five examined biomarkers, log-transformed levels of sST2 and catestatin provided the highest AUC values for the detection of in-hospital death event, and these models were significant (*Figure 2*). Of note, sST2 had AUC of 0.917 (95% CI 0.819–1.000, $P < 0.001$), CST provided AUC of 0.905 (95% CI 0.792–1.000, $P < 0.001$), while NT-proBNP yielded AUC of 0.735 (95% CI 0.516–0.954, $P = 0.036$). On the other hand, hs-cTnI and CRP were not

Figure 2 Areas under the curve for soluble suppression of tumourigenicity 2 (sST2), catestatin, and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) derived from the receiver operating characteristic analysis and overall model quality for each biomarker in detecting outcome of in-hospital death.



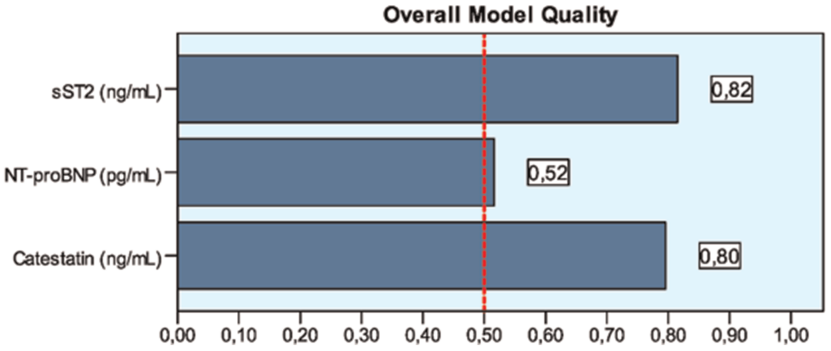


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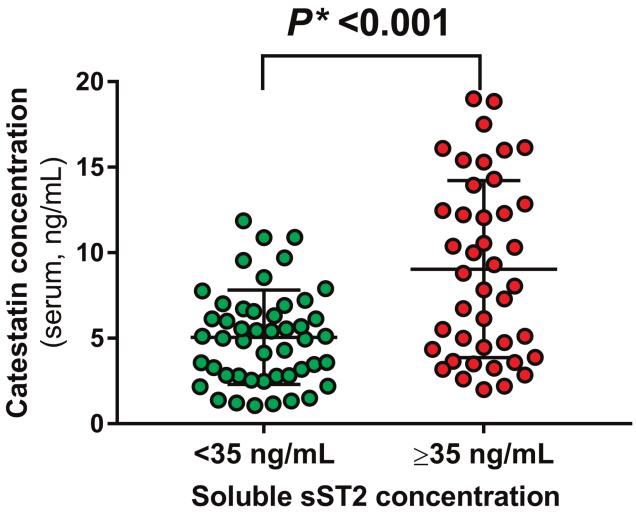
Figure 3 Circulating catestatin levels among patients that survived to discharge (N = 90), stratified into two groups based on serum soluble suppression of tumourigenicity 2 (sST2) levels <35 ng/mL (N = 48) and ≥35 ng/mL (N = 42), indicating a high risk of post-discharge adverse outcomes. *Student's t-test for independent samples.



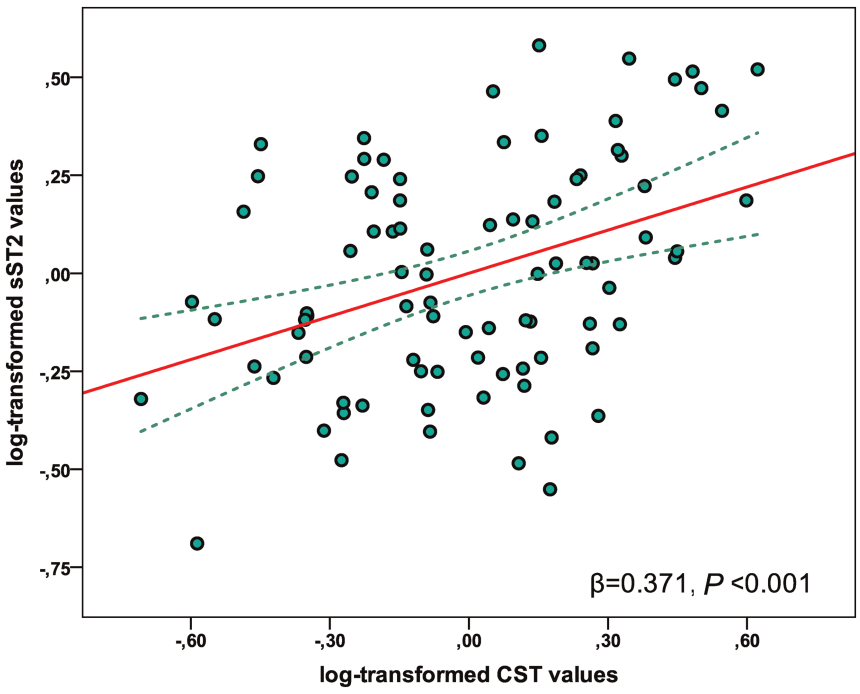
significant biomarkers in detecting in-hospital death event yielding AUC of 0.719 (95% CI 0.509–0.930, P = 0.075) and AUC of 0.682 (95% CI 0.541–0.822, P = 0.164), respectively. ROC analyses examining sST2, CST, and NT-proBNP for the outcome of in-hospital death revealed good model qualities (0.82 for sST2, 0.80 for CST, and 0.52 for NT-proBNP).

Association of soluble suppression of tumourigenicity 2 with catestatin in the context of acute worsening of chronic heart failure

Among patients that survived until discharge (N = 90), those with sST2 levels ≥35 ng/mL had significantly higher circulating levels of catestatin, compared with those with sST2 levels <35 ng/mL (9.05 ± 5.17 vs. 5.06 ± 2.76 ng/mL, $P < 0.001$, respectively) (*Figure 3*).

In the unadjusted and univariate linear regression model, log-transformed sST2 levels were in a significant and positive correlation with log-transformed CST levels (β = 0.437, t = 4.707, $P < 0.001$; model R^2 = 0.191, F = 22.159) among all patients (N = 96). Furthermore, multiple linear regression model adjusted for several covariates revealed that log-transformed sST2 levels remained in a significant and positive independent correlation with log-transformed CST levels as shown in the partial regression plot (β = 0.371, t = 3.706, $P < 0.001$; model R^2 = 0.308, F = 3.807) (*Figure 4*). Similarly, this relationship was attenuated but still confirmed in the multivariable-adjusted linear regression model among patients that survived to discharge (N = 90) with β = 0.283, t = 2.931, P = 0.014 (model R^2 = 0.194, F = 2.614).

Figure 4 A partial regression plot derived from multiple linear regression analysis showing multivariable-adjusted linear relationship of log-transformed serum soluble suppression of tumourigenicity 2 (sST2) and catestatin (CST) levels in the whole patient sample (N = 96).



Discussion

Our study showed that, among patients with AWHF, those that suffered in-hospital death had significantly higher circulating levels of sST2 and CST during hospitalization compared with those that were alive to discharge. Of note, both biomarkers were found to be independent predictors of in-hospital death, even when adjusted for NT-proBNP levels and other relevant baseline covariates. Furthermore, patients that survived to discharge and with sST2 levels ≥35 ng/mL, thereby designated as high risk of future adverse events, had nearly two-fold higher serum CST levels. Finally, a positive correlation of sST2 with CST, independently of NT-proBNP levels, was observed in a whole patient sample, while both biomarkers demonstrated superior diagnostic performance in the detection of in-hospital death, compared with traditional biomarkers such as NT-proBNP, hs-cTnI, and CRP, as demonstrated in our ROC analysis.

Prognostic impact of sST2 and CST on short-term outcomes such as in-hospital death has been poorly elucidated thus far. In contrast to this, a robust body of evidence shows that increased circulating levels of sST2, a biomarker reflecting myocardial fibrosis, inflammation, and adverse ventricular remodelling, have been consistently associated with an increased risk of all-cause and CV death as well as subsequent rehospitalizations in the longitudinal follow-up of discharged patients, in both the acute and chronic HF setting, and this prognostic value has been independent of traditional biomarkers such as NT-proBNP and high-sensitivity troponins.[17-19] On the other hand, increased CST levels during hospitalization were independently associated with an increased risk for all-cause and CV death among chronic HF patients during the follow-up period.[15] However, little or nothing is known about the potential utility and associations of sST2 and CST with short-term outcomes such as in-hospital death among patients admitted for the AWHF.

Hitherto, circulating CST levels have not been previously described in the literature regarding the short-term outcomes in patients with AWHF, and no previous studies described a relationship between CST and sST2, two CV biomarkers reflecting distinct pathophysiological mechanisms in HF.

Catestatin is a polyvalent peptide that is proteolytically cleaved from precursor chromogranin A molecule that is co-stored and co-released with catecholamines from the storage vesicles in adrenal chromaffin cells and adrenergic neurons.[20] It acts as a potent catecholamine release inhibitor by binding to the neuronal nicotinic acetylcholine receptor thereby blunting the exocytosis of neurohormones including catecholamines and also exerts direct vasodilatative and hypotensive effects through stimulation of histamine release from mast cells via heterotrimeric G-protein signalling.[21] Even more, CST directly and locally modulates adrenergic signalling by abolishing the adverse effects of norepinephrine at the level of β_1 and β_2 adrenergic receptors in cardiac myoblasts.[22] Our previous study showed that levels of CST were significantly higher among ADHF patients with ischaemic aetiology of the syndrome than those with non-ischaemic aetiologies and among patients that had more severe symptoms.[16] These increased CST levels were most likely observed as the compensatory mechanism to increased sympathetic activation that, in the decompensated HF stage and especially among those with established ischaemic disease and advanced symptoms, significantly overpowers neurohormonal pathways that exert cardioprotective effects. Therefore, a complex interplay of chromogranin A-derived peptides such as CST and natriuretic peptides is crucial in cardioprotection against catecholamine-evoked stress.[23]

A present study showed that CST levels were significantly higher among AWHF patients that died than survivors. This is a novel finding in this setting, and we hypothesize that this might be due to an increased propensity for the onset of ventricular tachyarrhythmias and SCD among patients with increased CST levels during hospitalization for the acute worsening of HF. Importantly, almost all patients that suffered in-hospital death in our study died because of sustained malignant arrhythmias and HF progression to a terminal stage. Previously, Pei *et al*. demonstrated that among patients with acute myocardial infarction, CST was an independent predictor of malignant arrhythmias during hospitalization and the incidence of malignant arrhythmias closely paralleled increasing CST levels.[24] This study is of relevance because it was focused on in-hospital outcomes and it also showed that levels of NT-proBNP, high-sensitivity CRP, and, most importantly, norepinephrine increased in a CST concentration-dependent manner, thus clearly demonstrating that CST levels are a 'mirror image' of excessive catecholamine spillover into circulation further perpetuating detrimental effects on the myocardium. Finally, two to-date available experiments, in animal acute myocardial infarction and hypertension models, showed that lack of CST was associated with significantly higher rates of ventricular arrhythmia induction, prolonged ventricular repolarization, increased heart rate, and increased QT interval variability, while exogenous CST administration diminished cardiac sympathetic drive.[25,26] These data altogether suggest that high circulating CST levels likely reflect profound SNS disturbance and adverse electrophysiological perturbations that might predispose myocardium to electrical instability, and these levels, although high, are likely insufficient to compensate for sympathetic and catecholamine toxicity.

Of note, ~30–50% of patients with chronic HF and reduced LVEF die of SCD, while failing fibrotic and scarred myocardium presents a highly vulnerable substrate prone to acute mechanical failure and initiation of asystole, bradyarrhythmias, electromechanical dissociation, and incessant ventricular tachyarrhythmias.[27,28] Likewise, elevated circulating levels of sST2 reflect active pathophysiological processes of adverse

myocardial remodelling, fibrosis, and systemic inflammatory response, which all contribute to worsening of the myocardial substrate and might negatively impact on short-term in-hospital outcomes.[29] Previously, sST2 was found as a significant predictor of SCD among patients with coronary artery disease and HF with preserved systolic function and was associated with an increased risk of death, HF, and ventricular arrhythmias in patients with mildly symptomatic HF (MADIT-CRT trial).[30,31] Finally, a study by Pascual-Figal *et al*. showed that elevated sST2 concentrations were predictive of SCD in patients with chronic HF and reduced LVEF.[14] However, all these studies examined long-term post-discharge endpoints and did not focus on any of the in-hospital outcomes.

Based on our novel finding that serum sST2 levels significantly and positively correlate with CST levels, it could be hypothesized that excessive adrenergic activity and a high degree of pathologic myocardial remodelling and inflammation, as reflected in high circulating CST and sST2 levels, synergistically potentiate likelihood of adverse events such as in-hospital death. Even more, we showed that, among patients that survived to discharge, those with sST2 levels ≥35 ng/mL and who are conventionally considered at higher risk for adverse post-discharge outcomes had almost two-fold higher CST levels, which might implicate insidious sympathetic overactivity in these patients and could make them more prone in suffering cardiac and all-cause death but perhaps fatal arrhythmogenic assault as well. Importantly, in a penalized logistic regression adjusted for relevant covariates, we showed that both sST2 and CST had independent and similar predictive value for the event of in-hospital death, beyond the baseline NT-proBNP levels as a marker of myocardial stretch, while other two biomarkers measuring myonecrosis (hs-cTnI) and systemic inflammation (CRP) were not significantly associated with the in-hospital mortality. Finally, our ROC analysis demonstrated that serum levels of sST2 and CST were comparable in their diagnostic performance and provided significantly higher AUC values in discriminating patients who died than survivors, compared with traditional biomarkers of NT-proBNP, hs-cTnI, and CRP.

Our study has notable limitations as it was a single-centre study that enrolled a relatively low number of patients and registered a low number of events of interest (in-hospital death), thus limiting the interpretation of results concerning the mortality outcomes. Furthermore, it is possible that some of the traditional risk factors that might be associated with in-hospital mortality did not reach statistical significance in the univariate model due to low number of cases, thus limiting our multivariate regression model and its results despite penalized likelihood. Likewise, we did not capture changes in LVEF during hospitalization (from admission to discharge) or from last known LVEF to admission LVEF, which would likely provide more insight about the magnitude of HF worsening. Furthermore, a direct pathophysiological link of sST2 with CST could not be established because of a lack of mechanistic studies in this setting. However, all our analyses were performed with rigorous methodology and were adjusted for many important confounders, while sensitivity analyses showed consistent associations of sST2 and CST with reported outcomes. Future mechanistic and clinical studies with larger patient enrolment and a representative number of in-hospital events are required to confirm and validate these findings in order to facilitate risk stratification in clinical practice.

In conclusion, our results show that sST2 and CST might serve as useful biomarkers for identifying individuals at high risk of in-hospital death among patients admitted with the acute worsening of HF. Soluble ST2 and CST reflect distinct pathophysiological pathways in HF; thus, high circulating levels of both biomarkers measured during hospitalization, beyond traditional biomarkers, might be an indicator of impending clinical deterioration and increased risk of death while in hospital. Finally, patients traditionally considered as high risk according to sST2 cut-offs had nearly double the concentration of CST, and this might also contribute to future adverse events. Altogether, these findings may implicate more stringent monitoring and aggressive treatment of these patients in clinical practice and further support the concept of multimarker strategy use in HF for the purposes of risk stratification.

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Conflict of interest

None declared.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Supporting information

Table S1. *Baseline characteristics of patients that survived until hospital discharge (N = 90) comprising CATSTAT-HF cohort*

Table S2. *Results of the multiple linear regression that show univariate associations of serum sST2 levels with transthoracic echocardiographic*

parameters measured among all patients that survived until hospital discharge (N = 90)

Table S3. *Results of the multiple linear regression that show univariate and multivariate associations of serum sST2 levels with anthropometric, clinical and laboratory parameters among all patients that survived until hospital discharge (N = 90)*

Figure S1. *Estimated predictive effect sizes of sST2, CST and NT-proBNP for the dependent outcome of in-hospital death, adjusted for covariates in the multivariable Firth logistic regression model.*

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