Original Article

Prognostic value of soluble ST2 biomarker in heart failure patients with reduced ejection fraction – A multicenter study

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ABSTRACT

Objective: To study the prognostic value of soluble Suppression of Tumorigenicity-2 (sST2) in heart failure patients with reduced ejection fraction (HFrEF).
Methods: In this prospective, observational, multicenter study, patients with heart failure (HF) and left ventricular ejection fraction (LVEF) <50% were included. Clinical evaluation and serum levels of sST2 were estimated at five time points during follow up. Study endpoint was the relationship of baseline and serial sST2 concentration in the blood to the composite endpoints of cardiac death and re-hospitalization for worsening of HF during one year follow up period.
Results: A total of 141 patients were enrolled. The mean age was 60 ± 10.4 years. At baseline evaluation, 49.6% patients were in New York Heart Association (NYHA) class III and 36.2% in class IV. Adverse events were observed in 57 patients (40.4%); 25 (17.7%) were re-hospitalized due to worsening of HF and 32 (22.7%) died due to cardiac causes. The median value of baseline sST2 was 46.36 ng/ml (IQR 31.30–78.38). sST2 concentration at baseline was significantly higher among patients with adverse events in comparison to patients without adverse events ($p = <0.001$). Receiver operating characteristic curve (ROC) for baseline sST2 concentration identified 49 ng/ml as optimal cut-off value to predict cardiac death and re-hospitalization, with a sensitivity and specificity of 72% and 75%, respectively.
Conclusion: In patients with HFrEF, sST2 concentration at baseline as well as on serial testing was significantly correlated with cardiac death and re-hospitalization for worsening of HF.
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1. Introduction

Heart failure (HF) is a growing health problem worldwide associated with high morbidity and mortality. The overall HF mortality rate remains high with an annual rate of 29.6% and five year rate of 50%.[1] Risk stratification of this multifactorial syndrome is crucial to identify patients who are likely to benefit from the best available and emerging therapies. Biomarkers play an important role in risk stratification of patients with HF.[2,3] Several biomarkers including Brain Natriuretic Peptide (BNP), N-Terminal-proBNP (NT-proBNP), Galectin-3, Soluble endothelin, Growth differentiation factor-15, Copeptin, Suppression of Tumorigenicity-2 (ST2) have been investigated in the diagnosis and prognosis of patients with HF.[4–9]

ST2 is a member of interleukin-1 (IL-1) receptor family which exists in membrane bound (ST2L) and soluble circulating forms (sST2). Binding of interleukin-33 (IL-33) to ST2L has been found to be cardioprotective reducing myocardial fibrosis, hypertrophy and apoptosis in experimental models.[10] sST2 acts as a decoy receptor of IL-33 and eliminates cardioprotective effects of IL-33/ST2L combination in a dose dependent manner. Increased concentration of sST2 in blood has been observed in conditions associated with cardiac fibrosis and remodeling. It has emerged as a strong predictor of cardiovascular outcomes in both acute and chronic HF and its estimation provided incremental value to BNP/NT-proBNP in the diagnosis and prognosis of patients with HF.[11–13] Serial measurement of sST2 has been found to be useful in predicting response to therapy in HF.[8] However, there is a paucity of data on

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Table 1
Demographic and baseline clinical characteristics of the enrolled patients.

	Study population (n = 141)	Patients without adverse outcome (n = 84)	patients with adverse outcome (n = 57)	p-value
Age, years, mean ± SD	60.3 ± 10.4	59.5 ± 10.3	61.6 ± 10.7	0.244
Male, n(%)	108 (76.6)	67 (79.8)	41 (71.9)	0.281
BMI, Kg/m², mean ± SD	24.6 ± 3.8	25.1 ± 3.9	24.1 ± 3.8	0.133
Diabetes mellitus	102 (72.3)	53 (63.1)	49 (86)	0.002
Hypertension	84 (59.6)	46 (54.8)	38 (66.7)	0.157
Dyslipidemia	72 (51.1)	34 (40.5)	38 (66.7)	0.0023
Coronary artery disease	78 (55.3)	42 (50)	36 (63.2)	0.123
Cerebrovascular accident	13 (9.2)	7 (8.3)	6 (10.5)	0.658
NYHA class				
II	20 (14.2)	17 (20.2)	3 (5.3)	0.012
III	70 (49.6)	50 (59.5)	20 (35.1)	0.0044
IV	51 (36.2)	17 (20.2)	34 (59.6)	<0.001
IHD	78 (55.3)	42(50)	36(57)	0.123
Non-IHD	63 (44.7)	37 (44)	26 (45.6)	0.35
β-blockers	115 (81.6)	80 (95.2)	35 (61.4)	<0.001
ACEI/ARB	112 (79.4)	76 (90.5)	36 (63.2)	<0.001
Mineralocorticoid receptor Antagonists	81 (57.4)	54 (64.3)	27 (47.4)	0.046
sST2, ng/ml, mean± SD	71.7 ± 83.9	48 ± 36.8	106.6 ± 116.2	<0.001
LVEF%, mean ± SD	31.6 ± 7.1	32.4 ± 7.1	30.3 ± 7	0.087

Adverse events: cardiac-death and rehospitalisation for worsening of HF during one year follow-up.

the prognostic value of sST2 in patients with heart failure reduced ejection fraction (HFrEF) from the Indian subcontinent. This study examined the prognostic value of serum levels of sST2 at five time points during one year in predicting cardiac death and need for re-hospitalization in patients with HFrEF.

2. Methodology

This was a prospective, observational, multicenter study involving three tertiary care hospitals in Kerala, India, enrolling patients who were diagnosed to have HFrEF, between September 2014 and June 2015. The study was approved by the respective institutional ethics committees and informed consent was taken from patients prior to enrolment. Patients with clinical signs and symptoms of HF and left ventricular ejection fraction (LVEF) <50% were included in the study. Exclusion criteria were recent acute coronary syndrome or coronary revascularization in the preceding two months, myocarditis, cardiogenic shock, advanced liver or renal disease, malignancy or any medical condition substantially reducing life expectancy to less than one year.

Clinical examination of all patients was performed during enrolment, at discharge from hospital, one month, six month and one year. Functional status of patients was decided based on NYHA classification. Total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), glycated hemoglobin (HbA1c), serum creatinine, potassium, sodium, estimated glomerular filtration rate (eGFR), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) were estimated at baseline and during follow up visits. Left ventricular functional indices such as LVEF, left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), left ventricular end diastolic volume (LVEDV) and left ventricular end systolic volume (LVESV) were determined at baseline echocardiography.

2.1. Biomarker measurement

Blood sample for sST2 estimation was collected at the time of enrolment, at discharge from the hospital, one month, six month and one year visits and the plasma was stored at −70 °C until the time of assay. The sST2 was quantitatively measured using highly
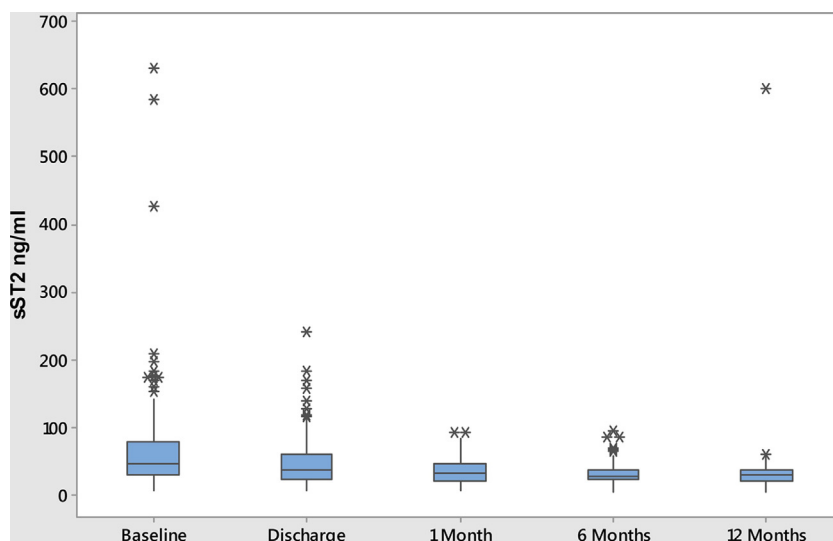

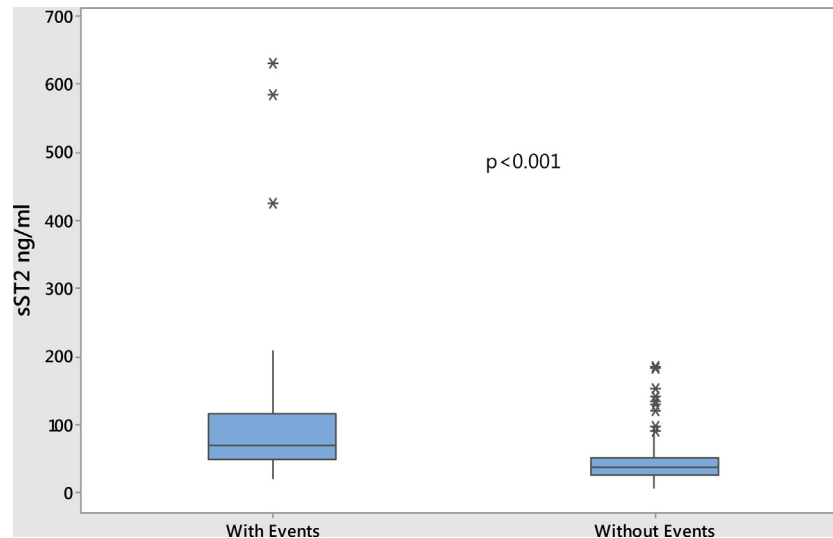**Fig. 1.** Distribution of sST2 over time.

Fig. 2. Distribution of sST2 concentration at baseline in patients with and without adverse events.

sensitive sandwich monoclonal immunoassay (Presage™ ST2 assay, Critical Diagnostics, San Diego, CA)[14] in a single laboratory.

2.2. Endpoint of the study

The study was intended to find out the relationship of basal and serial sST2 concentration to adverse outcomes like cardiac death and re-hospitalization for worsening of HF within one year follow up period. Cardiac death was defined as death due to an obvious cardiovascular cause or any death that was not clearly attributable to a non cardiovascular cause. Re-hospitalization was defined as admission to an in-patient unit or visit to emergency department that resulted in at least 24 h stay for heart failure symptoms. Worsening of HF was confirmed based on the presence of at least one of the following symptoms: dyspnea, orthopnea, paroxysmal nocturnal dyspnea, increasing fatigue or worsening exercise tolerance and presence of at least two of the following clinical signs: oedema, pulmonary crackles, jugular venous distension, rapid weight gain, tachypnea, S3 gallop, increasing abdominal distension/ascites, hepatojugular reflux and radiological evidence of HF.

2.3. Statistical analysis

Continuous variables were assessed for normality distribution using Shapiro-Wilk test. Variables associated to baseline sST2 levels were assessed using linear regression methods with the natural log-transformed form of sST2 as the dependent variable. The prognostic value of sST2 was assessed by multivariable Cox regression analysis. Covariates were retained if their p value was <0.1 and were entered into multivariable analysis. Receiver operating characteristic (ROC) curve analysis was used to determine the prognostic ability of sST2 for adverse outcomes at one year and to identify optimum cut-off points along with sensitivity, specificity, negative and positive predictive value. Kaplan-Meier curve analysis was performed to assess the predictive potential of this cut-off value for one year cardiac mortality and re-hospitalization.

Table 2
Univariable and multivariable Cox regression analysis for sST2 as predictor of 1-year cardiac mortality and need for re-hospitalization in patients with heart failure.

Variables	Univariable		Multivariable	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age (years)	1.032 (0.997–1.067)	0.070	1.028 (0.978–1.081)	0.283
Male gender	0.646 (0.323–1.292)	0.217		
Body mass index	0.920 (0.840–1.010)	0.076	0.937 (0.847–1.038)	0.212
Hypertension	0.613 (0.300–1.252)	0.179		
Diabetes	0.467 (0.194–1.122)	0.089	0.818 (0.318–2.100)	0.676
Dyslipidemia	0.349 (0.169–0.725)	0.005	0.905 (0.402–2.038)	0.810
History of CAD	0.742 (0.379–1.453)	0.384		
History of CVD	0.784 (0.277–2.220)	0.646		
Ischemic aetiology of HF	0.501 (0.065–3.837)	0.505		
Current smoking	0.549 (0.280–1.077)	0.081	0.596 (0.224–1.583)	0.299
Alcoholism	0.540 (0.274–1.065)	0.075	0.616 (0.214–1.776)	0.370
NYHA functional class	3.336 (1.828–6.08)	0.000	1.512 (0.717–3.189)	0.004
ACE-I/ARB	0.335 (0.170–0.661)	0.002	0.335 (.155−0.723)	0.005
β-blockers	0.180 (0.092–0.351)	0.000	0.325 (0.156–0.680)	0.003
MRA	1.228 (0.538–2.806)	0.626		
Creatinine clearance	0.993 (0.980–1.007)	0.338		
sST2, ng/ml	2.810 (1.997–3.996)	0.000	2.046 (1.246–3.358)	0.005

Variables were included in multivariable analysis (p < 0.1); The logarithm function of sST2 was used in the analysis. ACE-I – angiotensin converting enzyme inhibitor; ARB – Angiotensinogen receptor blocker; CAD- coronary artery disease; CVD- cerebrovascular disease CI – confidence interval; HR- hazard ratio; NYHA – New York Heart Association.

3. Results

A total of 141 patients were enrolled in the study. Baseline clinical and laboratory parameters of the entire cohort and that of patients with and without adverse outcomes are shown in Table 1. The mean age was 60 ± 10.4 years and majority were male patients (76.6%). Prevalence of diabetes mellitus and hypertension was 72.3% and 59.6% respectively, and dyslipidemia was present in 51.1%. History of ischemic heart disease was present in 55% and cerebrovascular accident in 9.2% patients. Among the enrolled patients, 31.5% patients had acute de novo HF, 46% acute on chronic HF and 22.5% chronic HF. At baseline evaluation, 49.6% patients were in NYHA class III and 36.2% in class IV. All patients were followed up for one year. Adverse outcomes were observed in 57 patients (40.4%); 25 (17.7%) were re-hospitalised due to worsening of HF and 32 (22.7%) died due to cardiac causes.

sST2 values at different time points of testing are shown in Fig. 1. The median value of baseline sST2 was 46.36 ng/ml (IQR 31.30–78.38). The concentration of sST2 at baseline was significantly higher among patients with adverse outcomes in comparison to patients without adverse outcomes ($p = <0.001$) (Fig. 2). Cox regression analysis demonstrated that baseline sST2 concentration was associated with adverse outcomes in univariable and multivariable model after adjusting for age, body mass index,

diabetes, dyslipidemia, creatinine clearance, smoking, NYHA functional class, use of angiotensin converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB), β-blockers (BB), with adjusted hazard ratio of 2.046; 95% CI 1.246-3.358; $p = 0.005$ (Table 2). sST2 at baseline and discharge were significantly correlated to death or hospitalization with a p value of <0.001 each. The baseline concentration of sST2 was significantly higher in patients with worse symptoms defined by NYHA class ($p < 0.001$) as shown in Fig. 3A. The relationship between serial sST2 concentration and percentage of patients in NYHA class IV is shown in Fig. 3B. Kruskal-Wallis test showed that a prompt and sharp fall in sST2 concentration from baseline to discharge was significantly related to rate of survival and re-hospitalization at one year (p value = 0.010). Receiver operating characteristic curve (ROC) for baseline sST2 concentration identified 49 ng/ml as optimal cut-off value to predict cardiac death and re-hospitalization, with a sensitivity and specificity of 72% and 75% respectively (Fig. 4). An area under ROC curve (AUC) was 0.784 (95% CI 0.707-0.860) which indicates the discriminative potential of this value of sST2 between high and low risk patients. Positive predictive value of this cut-off value was 66.12% and negative predictive value was 78%. Kaplan-Meir curve analysis (Fig. 5) demonstrated that patients with sST2 > 49 ng/ml had higher occurrence of adverse outcomes (HR 3.265; $p < 0.001$)
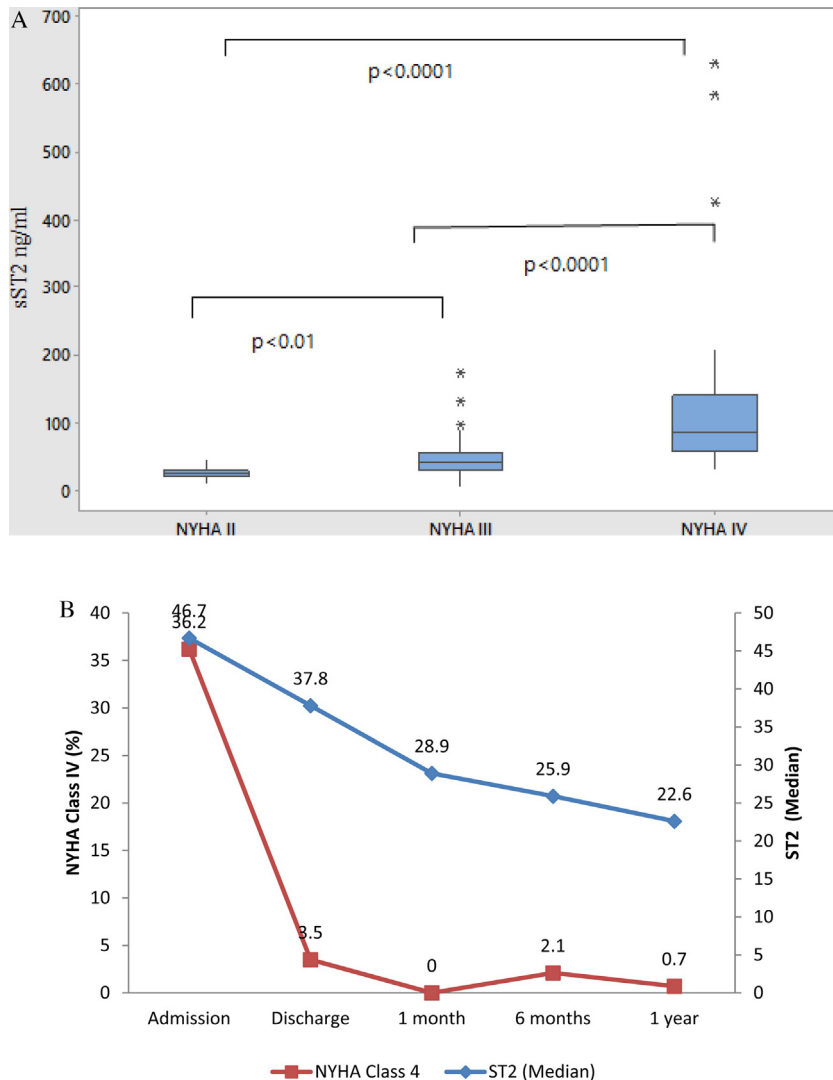

Fig. 3. A: sST2 distribution according to NYHA classification at baseline. B: ST2 (median) values and NYHA (Class IV) at different time points.

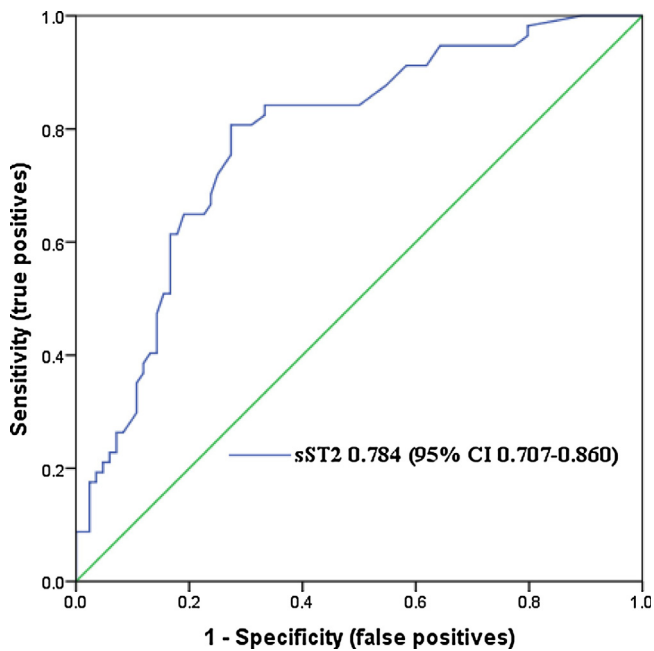**Fig. 4.** Receiver operating characteristic curve analysis of sST2 for predicting 1-year cardiac mortality and re-hospitalization.
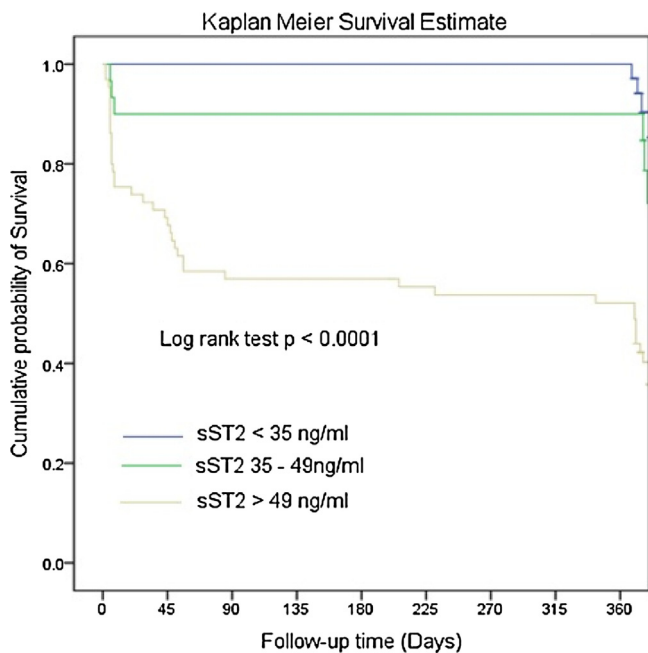

Fig. 5. Kaplan Meier curves for 1-year cardiac mortality and re-hospitalization for different levels of sST2.

Among 141 patients, 81.6% received BB, 79.4% ACE-I or ARB and 57.4% mineralocorticoid receptor antagonist (MRA). BB use was 61.4% in patients with events and 95.2% in those who had no events (p = <0.001). Similarly, ACE-I or ARB use was 63.5% Vs 90.5% (p = <0.001), MRA 47.4% Vs 64.3% (p = 0.046) in patients with and without adverse outcomes. Multivariate analysis showed that usage of BB and ACE-I or ARB was independently associated with decreased occurrence of death or re-hospitalization with HR of 0.333 (p = 0.005) and 0.325 (p = 0.003), respectively. Kaplan Meier survival analysis was conducted to compare patients on a single

drug, two drugs or all three drugs. Based on log rank test, the survival distributions for the three groups were significantly different (p < 0.0001). Death rate was significantly high (p = 0.0075) in patients who did not receive any of the drugs compared to those who received all the three drugs. Event reduction by the use of three drugs was more evident in patients with baseline concentration of sST2 >35 ng/ml and serial testing showed that this benefit was associated with a decline in sST2 concentration. Mean LVEF at baseline was $31.6 \pm 7.1\%$, and no significant difference was noticed among patients with and without adverse outcomes.

4. Discussion

The sST2 is considered as a marker of myocardial fibrosis and remodeling. Recent meta-analysis on studies on sST2 found that it is a strong predictor of cardiovascular outcomes in both acute and chronic HF.[15,16] It has independent as well as additive prognostic value along with natriuretic peptides. We investigated the prognostic utility of sST2 in 141 patients diagnosed to have HFrEF. This cohort represented patients with HF who seek medical care in daily practice, comprising a heterogeneous group of acute heart failure, decompensated and stable chronic HF.

4.1. Baseline sST2 value

Baseline sST2 value of >35 ng/ml has been accepted by US Food and Drug Administration[17] as a predictor of worse prognosis. In the HF-ACTION study the median sST2 level was 23.7 ng/ml and only 19.6% of patients had sST2 levels above the prognostic cut point of 35 ng/ml.[18] In our study, the median value was 46.36 ng/ml and 65.2% had sST2 value >35 ng/ml, which could be due to the inclusion of high proportion of patients with NYHA class IV, diabetes mellitus, and ischemic heart disease. Baseline sST2 value was found to be correlating with death and hospitalization during the study period. Ky et al in Penn Heart Failure Study (PHFS) found an independent association with a baseline value of sST2 and adverse outcomes.[19] In this study, the ROC curve revealed a baseline sST2 of 49 ng/ml as optimal cut-off value to predict cardiac death and re-hospitalization. This relative high value could be due to inclusion of 77.5% patients with acute de novo as well as de compensated chronic HF. Across the entire cohort, baseline sST2 value strongly correlated with HF symptoms defined by NYHA class. The relationship between functional class and sST2 values have been documented earlier by Wojtezak et al. [20]

4.2. Serial sST2 value

Serial measurement of cardiac markers could be useful for HF evaluation and management.[4–6] Weinberg et al., in a sub-study of Prospective Randomized Amlodipine Survival Evaluation (PRAISE-2) found that serial changes but not baseline ST2 values were associated with increased risk of death or transplantation.[21] Although repeated measurement of sST2 was helpful in the evaluation of patients with decompensated HF,[6] very little is known about the usefulness of serial measurements of sST2 at multiple time points in HF patients. Gaggin et al reported that among various cardiac biomarkers only sST2 appeared to provide incremental prognostic data beyond the initial measurement.[8] In this study, it was observed that apart from the baseline concentration of sST2, value at discharge was correlated with adverse outcomes and functional status, although no such relationship was seen with sST2 concentrations at 1 and 6 month. Moreover, serial testing revealed that 81% of cardiac death and 16% of re-hospitalization occurred in those patients who continued to have sST2 value more than 35 ng/ml during the entire study period.

Serial testing also revealed that the decline in baseline sST2 value at the time of discharge was associated with reduction in one year mortality and improvement in NYHA functional class.

Patients who received all three groups of drugs- BB, ACE-I or ARB and MRA exhibited a significant decline in sST2 concentration among patients with baseline value of >35 ng/ml and was associated with better outcome than those who were on either one or two drugs. Serial measurements revealed that reduction in sST2 concentration by the use of three drugs resulted in better reduction of adverse outcomes compared to the use of one or two drugs. The relationship between sST2 concentration and the drug response observed in this study possibly supports the potential use of sST2 estimation in guiding HF therapy.

5. Conclusion

In this study of patients with HFrEF, estimation of sST2 at baseline and at the time of discharge from hospital was significantly correlated with cardiac death and re-hospitalization for worsening of HF. A higher baseline value was associated with worse outcome. Moreover, serial testing at multiple points during treatment revealed that lower sST2 concentration was associated with reduced mortality and improved functional status. Standard drug treatment for HF has been found to decrease the sST2 level which has got an impact on the survival rate. Further studies will be required to establish the role of sST2 estimation in guiding HF therapy.

5.1. Study limitation

NT-proBNP, the most widely used biomarker for risk prediction in patients with HF was not analyzed in our study and hence additive use of these biomarkers in this group of patients with acute and chronic HF could not be commented.

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