

ST2 Levels Before and After Treatment of NYHA III and IV Heart Failure

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ABSTRAK

Tujuan: untuk mengetahui apakah ST2 dapat berperan sebagai petanda perbaikan klinis pada pasien gagal jantung NYHA III dan IV. **Metode:** penelitian ini merupakan penelitian longitudinal, pre dan post-test tanpa kontrol. Subyek penelitian adalah 23 orang pasien gagal jantung klasifikasi NYHA III dan IV. Pemeriksaan ST2 dilakukan pada awal perawatan dan hari terakhir perawatan pasien di rumah sakit. **Hasil:** dari 23 orang pasien gagal jantung didapatkan klasifikasi NYHA III sebesar 70% dan IV sebesar 30%. Pasien laki-laki lebih banyak daripada perempuan (51.4% vs 48.6%). Median usia NYHA III 52 tahun dan rerata usia NYHA IV 58 tahun. Penyebab gagal jantung terbanyak adalah CAD 52% dan non CAD 48%. Kadar ST2 tidak berkorelasi dengan usia, lama perawatan, jenis kelamin dan faktor penyebab gagal jantung. Kadar ST2 pada NYHA IV $58,82 \pm 37,36$ ng/ml cenderung lebih tinggi dibandingkan NYHA III $30,75$ (14,4–84,5), tetapi perbedaan ini tidak bermakna ($p=0.89$). Kadar ST2 pada awal hari perawatan lebih tinggi bermakna dibandingkan pada akhir hari perawatan $31,4$ (14-129,2) ng/mL vs $18,4$ (7,6-77,8) ng/mL, dengan $p=0,001$. Hasil ini menunjukkan dengan adanya perbaikan klinis penderita, terjadi penurunan kadar ST2 yang bermakna. **Kesimpulan:** ST2 dapat digunakan sebagai petanda untuk menentukan perbaikan klinis gagal jantung NYHA III dan IV.

Kata kunci: ST2, Gagal jantung, NYHA III and IV.

ABSTRACT

Aim: to find whether ST2 can be used to determine clinical improvement in patients with NYHA III and IV heart failure. **Methods:** this is a longitudinal, pre and post-test study without a control group. Study subjects are 23 NYHA III and IV heart failure patients. ST2 was tested at the start and end of hospital treatment. **Results:** of 23 heart failure patients, 70% were classified as NYHA III while 30% were NYHA IV. There were more male subjects than females (51.4% vs. 48.6%). Median age for NYHA III heart failure patients was 52 years and mean age for NYHA IV heart failure patients was 58 years. Heart failure was mostly caused by coronary artery disease (52%). ST2 levels did not correlate with age, length of care, sex and cause of heart failure. ST2 levels in NYHA IV heart failure patients (58.82 ± 37.36 ng/mL) tended to be higher than the one in NYHA III group (30.75 [14.4-84.5] ng/mL), but the difference was statistically not insignificant ($p=0.89$). ST2 levels at the start of treatment was significantly higher than at the end (31.4 [14-129.2] ng/mL vs. 18.4 [7.6-77.8] ng/mL), $p=0.001$. This shows that clinical improvement is associated with significant reduction of ST2 levels. **Conclusion:** ST2 can be used as a marker to determine clinical improvement in NYHA III and IV heart failure.

Key words: ST2, heart failure, NYHA III and IV.

INTRODUCTION

Heart failure has become a growing problem in the world, causing a great number of cardiovascular morbidity and mortality. The disease is also a major source of expense due to its high rate of hospital stay.^{1,2} Several studies reported that the incidence of heart failure increases with age. In Northern America (United States and Canada) and Europe, the risk for a person to have heart failure is 1 in 5 with a mean age of more than 40 years.^{3,4} Life expectancy for heart failure patients is increasing because of improvements in health facilities and treatment.⁴ Those patients commonly have left ventricular dysfunction and will progress to a state of chronic heart failure, thus prompting them for in-hospitalization.⁵⁻¹⁰

The New York Heart Association (NYHA) classification is the most common classification to determine heart failure progressivity based on symptoms of functional limitation. Heart failure is classified into four functional classes. NYHA class I is asymptomatic, class II has mild symptoms, class III has moderate symptoms (dyspnea, fatigue, palpitation, or chest pain during light activities), and class IV has severe symptoms (dyspnea, fatigue, palpitation, or chest pain at rest).^{3,11} The NYHA classification is subjective so there is a need among clinicians for a more objective laboratory test to differentiate heart failure based on severity.

B-type natriuretic peptide (BNP) is a member of the natriuretic peptides, of which secretion is stimulated by ventricular wall stretch and its plasma levels correlates with the severity of heart failure. Precursor of BNP is synthesized in ventricular myocytes and broken down by a protease to its active form (BNP) and amino terminal pro B-type natriuretic peptide (NT-proBNP).¹²⁻¹⁴ NT-proBNP levels in the blood equals BNP, but it has a longer half-life (60-120 minutes) than BNP (15-20 minutes).¹³⁻¹⁵ Patient characteristics influence NT-proBNP levels, which is higher in females and increases with age. NT-proBNP is also affected by kidney function because its clearance through kidneys.¹⁶⁻¹⁹

A new marker for heart failure that is currently gaining popularity is ST2. ST2 (growth Stimulation expressed gene 2) is a protein that

belongs in the interleukin-1 (IL-1 receptor) family and has two isoforms, transmembrane receptor form (ST2L) and soluble ST2 (ST2). In heart failure, ST2 will rise because of cardiomyocyte stretch.²⁰ Earlier reports state that high ST2 levels signifies an ongoing process of damage or remodeling in the heart. Heart remodeling cannot be monitored based on symptoms because it produces none. It also cannot be detected in physical examination. Radiographic examination has some value in detecting heart remodeling, but only if the damage is severe. In heart failure patients, ST2 levels strongly correlates with the worsening of disease and mortality.²¹ ST2 levels increases along with heart hypertrophy, fibrosis, and ventricle dysfunction. Expression of ST2 starts around one hour after myocyte stretch in a patient with myocardial infarct.²² Rehman et al.²³ in 2008 reported that patients with worse clinical symptoms in NYHA class IV group has higher ST2 levels than the ones in NYHA class III group. The advantage of ST2 over BNP/NT-proBNP is that it is not affected by conditions like sex, left ventricle hypertrophy, tachycardia, right ventricle overload, and factors external to the heart, such as hypoxemia, kidney dysfunction, metabolic risk factors, hepatic cirrhosis with ascites, and sepsis.²⁴

Muller showed that ST2 is superior than NT-proBNP as a prognostic marker.²⁵ Bhardwaj et al.²⁶ stated that not only did ST2 show heart muscle damage, but it also changed in response to the given treatment. As the heart improves, ST2 levels will drop. Hence, ST2 can be used for prognosis and treatment monitoring. Based on the results of those studies, we will conduct a research to discover the role of ST2 in determining clinical improvement in NYHA III and IV heart failure patients after treatment.

METHODS

This is a longitudinal, pre and post-test study without a control group. Study subjects were patients newly diagnosed with NYHA III and IV heart failure in Cipto Mangunkusumo hospital. Diagnosis of heart failure was established by cardiologist as noted in medical records. ST2 test was conducted upon entry to the hospital (before treatment) and upon discharge (after

treatment). Patients are discharged when clinical improvements are found. Clinical improvements include functional improvement of NYHA or fluid overload or electrolyte imbalance, are under control. Study subjects were NYHA III and IV heart failure patients that fulfilled the inclusion criteria.

The inclusion criteria are patients newly diagnosed with NYHA III and IV heart failure, 18-75 years of age, and willing to participate in the study by signing a written informed consent. Subject was considered a drop out if the patient withdrew, discharged him/herself against medical advice, or died. Sample size required to determine whether there was a significant difference between ST2 levels in the beginning and the end of hospital stay is 23. Sample size was calculated using the appropriate sample size formula with combined standard deviation of 0.6 ng/mL, d of 0.31, significance (2α) of 1.64 and power (β) of 0.89.²⁵

Six mL of vein blood was collected from the cubital vein in a tube without anticoagulant. Serum was separated under one hour after blood collection by centrifuging the sample at 3000 G for 10 minutes. Serum was then stored in Eppendorf tubes at -20°C . By the time a test is about to be performed, serum is thawed by removing it from the freezer and putting it in room temperature ($20-25^{\circ}\text{C}$). ST2 levels test was carried out using sandwich double monoclonal antibody ELISA and Presage®ST2 Assay reagent.²⁶

Before the ST2 test was performed, the instrument was calibrated. Afterward, within-run precision and accuracy analysis were carried out by measuring test control five consecutive times in the same day as sample testing.

Statistical Analysis

ST2 results were recorded and calculated for mean and standard deviation if they were normally distributed, or for median and range (minimum, maximum) if they were not.

All collected data were analyzed, with the exception of patients who died or were discharged from the hospital before observation was done. They were recorded as, respectively, lost cases or lost to follow up.

The study event was clinical improvement when a patient was discharged. Survival analysis was performed to evaluate ST2 as prognostic indicator of patients. ST2 levels at the start and end of treatment were analyzed using paired t-test for parametric data, and Wilcoxon signed rank for non-parametric data. ST2 levels in groups NYHA III and IV were analyzed using independent t-test for parametric data, and Mann-Whitney for non-parametric data. Logistic regression analysis was also performed to learn the effect of age, sex, causes of heart failure, and length of hospital stay to ST2 levels difference. Beforehand, bivariate analysis (Chi Square or Fisher's exact test) was carried out to determine variables that would be used in the logistic regression analysis. If none of the expected count values were <5 , the variable qualified for chi square. If at least one of the expected count values was <5 , Fisher's exact test was used for that variable. Variables that proceeded to the logistic regression analysis were the ones with $p < 0.25$. Strength of association between age, sex, causes of heart failure, length of hospital stay, and ST2 levels difference was described in the OR exponential β (EXP β).

RESULTS

Within-run precision and accuracy tests were performed using Presage®ST2 control kit BC 1066 for five consecutive times before sample testing. The range value for normal control is 18.8-30 ng/mL. Within-run CV was 3.63% and deviation was -6-2.4%.

During the study, there were 25 subjects. Twenty three finished the study and two dropped out. One died after 38 days of treatment, and one died at home on day 45 after discharging himself against medical advice. He was treated for 18 days. The subjects were NYHA III & IV heart failure patients who fulfilled inclusion criteria. There were 16 patients in the NYHA III group (69.6%) and seven in NYHA IV (30.4%).

Median age of study subjects was 54 years with a range of 29-67 years. In NYHA III group, the age range was 29-62 years with a median of 52 years, while in NYHA IV there were older subjects with an age range of 51-67 years and a median of 58 years.

There were more males than females. Thirteen subjects were males (56.5%) and 10 were females (43.5%). This was also found in both groups. NYHA III group had nine male subjects (39.1%), and NYHA IV group had four male subjects (17.4%) as seen in **Table 1**.

Table 1. Subject's characteristics

Study subjects	NYHA III group	NYHA IV group	Total
Total subjects, n (%)	16 (69.6)	7 (30.4)	23 (100.0)
Age (years), median (IQR)	52 (29-62)	58 (51-67)	54 (29-67)
Sex (males), n (%)	9 (39.1)	4 (17.4)	13 (56.5)
Causing factor			
- CAD	7 (30.5)	6 (26.1)	13 (56.6)
- Non CAD (HT, AF, VHD, DM)	4 (17.3)	6 (26.1)	10 (43.4)

CAD=coronary arterial disease, HT=hypertension, DM=diabetes mellitus, AF=atrial fibrillation, VHD=valvular heart disease

Median of ST2 levels at the start of treatment in NYHA III group was 30.75 (14.4-84.5 ng/mL), while the mean in NYHA IV group was 58.82±37.36 ng/mL. There was no significant difference between ST2 levels at the start of treatment in NYHA III and NYHA IV group with a p of 0.89. The boxplot in **Figure 1** shows that ST2 levels in NYHA IV group is higher than the one in NYHA III group, but the difference is insignificant.

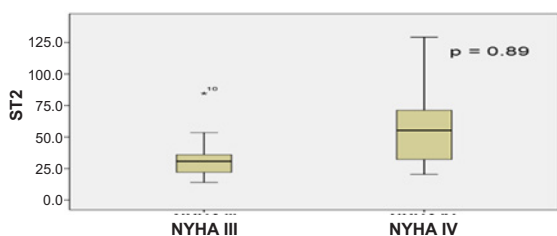


Figure 1. Boxplot of ST2 levels at the start of treatment in NYHA III and NYHA IV groups

Median of ST2 levels in groups III and IV at the start of treatment was 31.4 ng/mL with a range of 14.0-129.2 ng/mL, while median of ST2 levels in both groups at the end of treatment was 18.4 ng/mL, with a range of 7.6-77.8 ng/mL.

Figure 2 shows that ST2 levels at the start of treatment was significantly higher than the end.

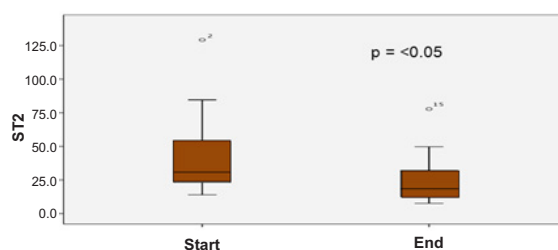


Figure 2. Boxplot of ST2 levels in NYHA III and IV groups at the start and end of treatment

The test yielded a p value of 0.001, indicating a significant difference. The median of ST2 levels at the start of treatment was significantly different than at the end of treatment.

The effect of age, sex, causes of heart failure, and length of hospital stay to ST2 levels difference was analyzed with logistic regression analysis. Beforehand, the variables were tested against ST2 levels difference using Fisher's exact test or Chi Square. ST2 levels difference is the difference between ST2 levels median at the start and the end of treatment. Its value is classified into two, ≤13 ng/mL and >13 ng/mL.

Table 2. Bivariate analysis of age, sex, causes of heart failure, and length of hospital stay against ST2 level difference

Variables	Δ ST2		p
	n ≤ 13	n > 13	
Age, n (%)			
- ≤50 years	4 (33.3)	1 (0.1)	0.317*
- >50 years	8 (66.7)	10 (91.0)	
Sex, n (%)			
- Male	7 (58.3)	6 (54.5)	1.00*
- Female	5 (41.7)	5 (45.4)	
Causes of heart failure, n (%)			
- CAD	8 (66.7)	5 (45.4)	0.414*
- NonCAD	4 (33.3)	6 (54.5)	
Length of hospital stay, n (%)			
- ≤20 days	8 (66.7)	4 (36.4)	0.146**
- >20 days	4 (33.3)	7 (63.6)	

* = Chi Square test, ** = Fisher's exact test

Logistic regression analysis was done to the length of hospital stay variable (p<0.25).

Sex, age, and causes of heart failure were not included. The odd ratio (OR) value (Exp β) of 0.286 and $p=0.153$. Length of hospital stay did not have a significant effect to ST2 levels difference.

The curve in **Figure 3** shows a weak positive correlation between length of hospital stay and ST2 levels difference ($r=0.346$; $p=0.106$).

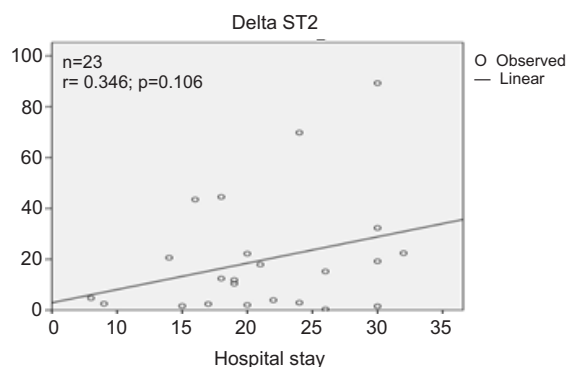


Figure 3. A curve depicting correlation between length of hospital stay and ST2 level difference

DISCUSSION

Within-run precision test for ST2 assay yielded a CV of 3.63%, suggesting an acceptable analytical performance. The manufacturer's CV is 3.4% for normal control and 2.7% for the abnormal. Within-run CV in this study does not differ much from the manufacturer's, and fulfill the manufacturer's regulation which was $<6.5\%$.²⁹ As a comparison, Maisel acquired a CV of 5.4% and Khan obtained 6.5% in their respective studies.²⁷

The study accuracy test also gave a good result because ST2 levels that was acquired for normal control was still in manufacturer's required range. The sample testing was done in one day.

There were 23 study subjects, comprised of 16 subjects (69.6%) in NYHA III group and 7 subjects (30.4%) in NYHA IV group. Out of all study subjects, there were more males (56.5%) than females (43.5%). This finding is similar to a study conducted by Djuliana in Jakarta in 40 heart failure and chronic kidney disease patients which had 70% males and 30% females.²⁸ The Framingham off spring study in 2012 obtained 55% females and 45% males out of its 3450

study subjects.²⁹ The Rotterdam study in 2004 found a higher incidence of heart failure in males (17.6/1000 persons/year) than females (12.5/1000 persons/year).³⁰ Mosterd reported that males experience heart failure more frequently than females (15 vs. 12/1000 persons per year).³¹ This is caused by sex related life style. Alcohol consumption, smoking, and lack of motivation to seek for care are found more in males than females.³²

This study is limited to adults aging 18-75 years old because heart failure in pediatric patients has different causes. Most of the study subjects in this study were 51-60 years old (12 subjects). There were two subjects who were less than 40 years old, three between 40-50 years old, and six who were more than 60 years old. The median for age in NYHA III group was 52 years and the mean in NYHA IV group was 58 years. There was no significant difference between the age of both groups ($p=0.076$). NYHA IV group had relatively older subjects. The Framingham heart study reported that heart failure incidence increased with age, specifically from 20 to >80 years old.³³ In the MONICA (Multinational Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg study, there were 1274 subjects, 2.8% were 25-35 years old, and 15.8% were above 65 years old.³¹ The Hillington study reported a heart failure incidence of 0.2/1000 persons per year in the age of 45-55 years and 12.4/1000 persons per year in the age of >85 years.³¹ Heart failure in older age is caused by aging where there are increasing tissue rigidity, decreasing tissue elasticity, increasing fibrosis, and calcification, generating intimal thickness, vascular rigidity, hypertension, left ventricular hypertrophy, arrhythmia, and the heart's losing ability to increase cardiac output as a response to stress.^{33,34}

In this study, the causative factors were CAD (56.6%), hypertension (26.1%), DM (8.7%), AF (4.3%), and VHD (4.3%). In another perspective, CAD was 56.6% and non CAD was 43.4%. In a study conducted by Khan, the causative factors of heart failure were CAD (35%), hypertension (27%), and rheumatic heart disease (13%).²⁷ Lee reported that in his study, the causing factors were CAD (52%), hypertension (26%),

and rheumatic heart disease (8%).³⁵ In a study conducted by Klatsky to 2594 subjects, 60% of heart failures were caused by CAD, and 40% by non CAD causes.³⁶ Miller found that CAD caused more heart failures (68%) compared to non CAD (32%).²² This is also the case with Frazier's study (59% of cases were caused by CAD).³⁷ According to American College of Cardiologist/American Heart Association, persons with high risk of developing heart failure are patients with hypertension, CAD, rheumatic fever, cardiomyopathy, and DM.² In this study, we also found VHD and AF as causes of heart failure in two subjects aging less than 40 years old.

CAD and hypertension are caused by atherosclerosis, which is the most frequent cause of heart failure. Hypertension increases progression of CAD and triggers acute myocardial infarct by altering blood flow velocity in the vasculars.^{2,32,37,38}

Median of ST2 levels at the start of treatment in NYHA III group was 30.75 (14.4-84.5 ng/mL), while the mean in NYHA IV group was 58.82±37.36 ng/mL. There was no significant difference between ST2 levels at the start of treatment in NYHA III and NYHA IV group ($p = 0.89$). This was probably caused by the small sample size. The higher ST2 levels in NYHA IV group compared to NYHA III group shows that ST2 levels increase corresponds with heart structure damage severity. A study by Rehman Januzzi in 2008 found that ST2 levels in patients with class IV heart failure was higher than in patients with class III and II heart failures.²⁰

In this study, the ST2 levels in NYHA III and IV groups at the start of treatment was significantly higher than the end (31.4 [14-129.2] ng/mL vs. 18.4 [7.6-77.8] ng/mL, $p=0.001$). This result shows that ST2 levels decreases significantly as the patient experiences clinical improvement. This is in line with what Bhardwaj concluded in 2010, that ST2 levels was not just an indicator of heart muscle damage, but it also changed as the body improves with treatment. The better the heart gets, the lesser the ST2 levels.²⁶

Bivariate and logistic regression analysis showed that age, sex, causes of heart failure, and

length of hospital stay had no effect to ST2 levels difference. Even though length of hospital stay yielded an OR of 0.286 but it was statistically insignificant. This is in accordance with a study from Weinberg that reported that race, age, and factors causing heart failure had no effect to ST2 levels.

CONCLUSION

This study found that ST2 levels could be used as a marker of clinical improvement as indicated by a significant decrease between pre and post treatment.

The change of ST2 levels between the start and end of treatment is not affected by age, sex, causes of heart failure, and length of hospital stay.

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