Soluble ST2 protein in chronic heart failure is independent of traditional factors

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Abstract

Introduction: ST2 protein is the interleukin 33 (IL-33) receptor, whose serum level depends on the biomechanical strain of cardiac myocytes. The aim of this study was to analyse the relationship between soluble ST2 (sST2) level and traditional factors in patients with chronic heart failure.

Material and methods: Sixty-six patients (mean age 62 years, 75% males) in stable NYHA class I-III with left ventricular ejection fraction < 45% were included in the study. Clinical, biochemical, electrocardiographic, echocardiographic and angiographic data were analysed. Patients were divided into groups depending on sST2 median: > 0.28 ng/ml (n = 31) vs. ≤ 0.28 ng/ml (n = 35). sST2 was measured using a quantitative ELISA kit. In order to define factors associated with sST2 levels uni- and multivariate regression analysis was performed.

Results: There was no relationship between sST2 levels and age (p = 0.67), body mass index (p = 0.19), hsTnT (p = 0.7) or other analysed parameters (all p > 0.05), except for N-terminal prohormone B-type natriuretic peptide (NT-proBNP). A significant positive correlation between sST2 and NT-proBNP was found (p = 0.013, R = 0.395). Multivariate analysis revealed that the stage of coronary artery disease and NT-proBNP were independent factors associated with sST2 concentration (p = 0.04). Intriguing is the fact that the fewer the sclerotic changes present in arteries, the higher was the sST2 level (β = –0.381, p = 0.04).

Conclusions: sST2 protein is independent of traditional factors which usually affect levels of NT-proBNP. In chronic heart failure, sST2 protein may be of greater importance in idiopathic dilated cardiomyopathy than in ischaemic aetiology, which seems to be associated with the molecular mechanism (biomechanical strain) related to sST2.

Key words: ST2, chronic heart failure, biomarkers.

Introduction

The use of biomarkers is easy and helps to confirm the diagnosis, optimise the therapy and estimate the prognosis in heart failure (HF) [1]. However, serum concentrations of NT-proBNP, which are now widely used in clinical practice, depend on several factors such as age, sex, left ventricular hypertrophy, tachycardia, right ventricular overload, myocardial ischaemia, hypoxaemia, renal dysfunction, metabolic risk factors, liver cirrhosis, sepsis and infection [1, 2]. Therefore it is necessary to find an independent biomarker.

ST2 protein is an interleukin 33 (IL-33) receptor. The IL-33/ST2 pathway has recently been described as a novel, critical cardioprotective system.
[3, 4], which reduces fibrosis and hypertrophy of the myocardium and helps to preserve ventricular function. In chronic HF (CHF) mechanical stress/strain of the left ventricle (LV) wall is exerted on the individual cardiomyocyte, which results in upregulation of ST2 gene expression. Therefore serum concentrations of ST2 soluble form (sST2) depend on mechanical stress [4]. That phenomenon is called biomechanical strain. sST2 is therefore a biomarker of mechanical overload [4, 5]. Myocardial overproduction of ST2, as a result of mechanical strain in HF, leads to the inhibition of IL-33 protective effects. The researchers posed a hypothesis that sST2 is not only a marker of poor prognosis in the case of mechanical ventricular overload, but also a mediator of disease progression. High expectations are associated with this pathway as a target of new HF therapies [3].

sST2 protein is quite well known as an independent prognostic factor for acute HF [6-16]. However, there are only a few studies on sST2 in CHF. The aim of this study was to determine the relation between sST2 level and traditional clinical, electrocardiographic, biochemical, echocardiographic and angiographic factors assessed during comprehensive evaluation of patients with CHF. It was hypothesized that sST2 protein may be an independent biomarker of CHF, more useful in clinical practice.

Material and methods

The study enrolled patients in stable NYHA class I-III with LV ejection fraction < 45%, 30% on average (range: 13-44%) and receiving optimized therapy. Exclusion criteria were as follows: NYHA class IV, acute coronary syndrome, acute heart failure, inflammatory states, and thyroid dysfunction. Medical history was collected and a 12-lead electrocardiogram was made at hospital entry. Echocardiography and coronary arteriography were performed according to the ASE/EAE and ESC recommendations. Using the M-mode, 2-dimensional and Doppler echocardiographic examinations, left (LA) and right atrial diameter (RA), left ventricular systolic (LVESD) and diastolic dimensions (LVEDD), left ventricular end-systolic (LVESV) and end-diastolic volume (LVEDV), and left ventricular ejection fraction (LVEF) were assessed. Conventional coronary arteriography was performed using a femoral or radial approach. To calculate the sclerotic alteration in coronary arteries we evaluated the degree of luminal obstruction in conventional visual quantification of coronary arteriography.

The following parameters were analysed: demographic and clinical (age, body mass index (BMI), smoking status), electrocardiographic from the standard 12-lead ECG (heart rate, heart rhythm – sinus rhythm or atrial fibrillation, LBBB, QRS duration, QTC), comorbidities (diabetes mellitus, arterial hypertension, stroke, coronary artery disease (CAD)), aetiology of heart failure (ischaemic or non-ischaemic – idiopathic dilated cardiomyopathy), basic laboratory tests such as morphology (haemoglobin, white blood cells, neutrophils), sodium levels, estimated glomerular filtration rate (eGFR), high-sensitivity C-reactive protein (hsCRP), high-sensitivity troponin T (hsTnT) and N-terminal prohormone B-type natriuretic peptide (NT-proBNP), echocardiographic variables (LVESD, LVEDD, LA, RA, LVEDV, LVESV, LVEF) and coronary arteriography (stenosis ≥ 50% of the left main coronary artery and ≥ 75% of other coronary arteries).

Blood for sST2 level determination was collected at hospital entry and stored at −76°C until analysed. ST2 serum levels were measured using a quantitative ELISA kit. Blood samples (5 ml) were collected into vacuum tubes containing clot activator and after the formation of a clot the samples were centrifuged for 5 min at 3000 rpm. The supernatant (serum) was immediately separated and frozen at (−20°C). The soluble ST2 level was measured using a sandwich ELISA kit (Medical and Biological Laboratories; Japan). The assay uses two monoclonal antibodies against two different epitopes of human sST2. Serum samples were incubated in microwells coated with the first anti-human sST2 monoclonal antibody. After the washing stage, the second incubation with the peroxidase-conjugated anti-human sST2 monoclonal antibody was conducted. After further washing, the peroxidase substrate was added into each well and the optical density was measured at 450 nm using a microplate reader.

Patients were divided into groups depending on sST2 median: > 0.28 ng/ml (n = 31) vs. ≤ 0.28 ng/ml (n = 35).

Statistical analysis

In order to define factors associated with sST2 levels, uni- and multivariate regression analysis was performed. Continuous variables are presented as mean values ± SD or median values with interquartile ranges (IQR) if not normally distributed. To compare continuous variables with non-normal distribution the Mann-Whitney U test was performed. Spearman correlation coefficients were used to assess correlations between sST2 and other continuous variables. Associations between nominal variables were examined using Pearson’s χ² test, Yates correction for χ² test in 2 × 2 contingency tables, and the exact Fisher test for larger than 2 × 2 contingency tables. Variables which were associated with sST2 level (p < 0.11) in the univariate tests were used in the multivariate model. Data were analysed with Statistica software version 8.0 (Pl). A p value < 0.05 was considered significant.
The investigation conforms to the principles outlined in the Declaration of Helsinki. The study was approved by the Bioethics Committee and all patients signed an informed consent form.

**Results**

This study included 66 patients (mean age: 62 years, 75% males). Characteristics of the studied population are shown in Table I. The most prevalent comorbidities in the study group were: arterial hypertension (44%), diabetes mellitus (20%) and stroke (10%). In this group, there were 53% former smokers and 14% current smokers. Coronary artery disease was demonstrated in 54% of patients examined. In the studied population the mean concentration of sST2 was 0.29 ng/ml, and the median value was 0.28 ng/ml, with 25th and 75th percentiles of 0.27 ng/ml and 0.29 ng/ml respectively. In this study, significant differences in NT-proBNP (p = 0.018) and QRS duration (p = 0.028) between groups with different sST2 levels were observed, with higher results in patients with sST2 above the median. Between the studied groups, there were no significant differences in LVEF, hsTnT or other analysed clinical, biochemical, echocardiographic and angiographic parameters, or in the frequency of current smokers (p = 0.5), prior myocardial infarction (MI) (p = 0.92), and comorbidities such as arterial hypertension (p = 0.88), diabetes mellitus (p = 0.11) or stroke (p = 0.24). Patients’ characteristics in both groups depending on sST2 concentration are presented in Table II. There was no relationship between sST2 levels and sex (p = 0.75), NYHA class (p = 0.12), smoking status (p = 0.50), presence of comorbidities such as arterial hypertension (p = 0.88), diabetes (p = 0.11),

| Variables | Mean | Median | Range | SD |
|-----------|------|--------|-------|----|
| Age [years] | 61.970 | 63 | 30-87 | 11.371 |
| Body mass index [kg/m²] | 26.769 | 26.4 | 16.6-37.1 | 3.736 |
| Systolic blood pressure [mm Hg] | 116.429 | 115 | 90-190 | 18.889 |
| Diastolic blood pressure [mm Hg] | 71.032 | 70 | 55-100 | 10.006 |
| Heart rate [bpm] | 81.048 | 78 | 53-170 | 22.778 |
| QRS duration [ms] | 106.267 | 100 | 60-180 | 27.977 |
| QTc [ms] | 385.433 | 380 | 260-509 | 54.8 |
| LVESD [cm] | 5.522 | 5.4 | 3.4-9.3 | 1.168 |
| LVEDD [cm] | 6.683 | 6.5 | 4.6-10.1 | 1.067 |
| LVESV [ml] | 138.732 | 118.5 | 46-418 | 73.376 |
| LVEDV [ml] | 195.696 | 180 | 83-490 | 84.008 |
| LA [cm] | 5.941 | 5.850 | 3.6-8 | 0.866 |
| RA [cm] | 5.191 | 5.1 | 3.5-8.1 | 0.897 |
| LVEF [%] | 29.477 | 30 | 13-44 | 7.209 |
| sST2 [ng/ml] | 0.29 | 0.28 | 0.26-0.59 | 0.02 |
| NT-proBNP [mg/dl] | 4043.533 | 2279 | 25.48-15600 | 4278.951 |
| hsTnT [mg/dl] | 0.063 | 0.026 | 0.004-0.36 | 0.085 |
| Creatinine [mg/dl] | 1.051 | 1 | 0.5-1.9 | 0.283 |
| hsCRP [mg/dl] | 14.115 | 5.9 | 0.4-157 | 28.370 |
| Sodium [mmol/l] | 137.088 | 138 | 127-144 | 3.622 |
| eGFR [ml/min/1.73 m²] | 83.369 | 80 | 27-185 | 31.032 |
| WBC [10³/µl] | 7.44 | 7.4 | 3.36-12.7 | 1.99 |
| Neutrophils [%] | 61.667 | 60.95 | 28.8-81.3 | 10.133 |
| Haemoglobin [mg/dl] | 14.334 | 14.3 | 9.2-18.8 | 1.887 |
stroke \( (p = 0.24) \) and other variables presented in Table III, except for NT-proBNP. A moderate positive correlation between sST2 and NT-proBNP \( (R = 0.34, \ p = 0.013) \) was found. Moreover, multivariate regression analysis revealed that the number of atherosclerotic coronary arteries and NT-proBNP were independent factors associated with sST2 concentration \( (p = 0.04) \). The fewer the sclerotic changes present in arteries and the higher the NT-proBNP level, the higher was the sST2 level \( (\beta = -0.381, \ p = 0.04) \). The above results are presented in Figure 1.

### Discussion

The main finding of this study is the observed association between sST2 and the number of atherosclerotic coronary arteries. To the best of our knowledge, this study is the first to reveal such a relationship. Our result is consistent with the role of the IL-33/ST2 pathway in inflammatory diseases, including atherosclerosis. ST2 protein inhibits the proinflammatory effect of IL-33 [17, 18]. We hypothesise that higher concentrations of sST2 in primary
Soluble ST2 protein in chronic heart failure is independent of traditional factors

Table III. Spearman rank order correlations between sST2 and other variables

| Variables          | Value of p | R  |
|--------------------|------------|----|
| **Demographic and clinical** |            |    |
| Age                | 0.665      | 0.06 |
| Body mass index    | 0.189      | 0.18 |
| Systolic blood pressure | 0.853      | 0.02 |
| Diastolic blood pressure | 0.386      | 0.02 |
| **ECG**            |            |    |
| Sinus rhythm       | 0.164      | −0.18 |
| Heart rate         | 0.609      | 0.07 |
| QRS duration       | 0.174      | 0.18 |
| QTc                | 0.574      | 0.08 |
| **Echocardiographic** |            |    |
| LVESD              | 0.584      | −0.08 |
| LVEDD              | 0.503      | −0.09 |
| LVESV              | 0.540      | −0.06 |
| LVEDV              | 0.650      | −0.06 |
| LA                 | 0.420      | 0.11 |
| RA                 | 0.878      | 0.02 |
| LVEF               | 0.889      | 0.02 |
| **Biochemical**    |            |    |
| NT-proBNP          | 0.012      | 0.39 |
| hsTnT              | 0.703      | −0.1 |
| Creatinine         | 0.552      | −0.07 |
| hsCRP              | 0.645      | 0.07 |
| Sodium             | 0.065      | −0.25 |
| eGFR               | 0.670      | 0.06 |
| WBC                | 0.864      | 0.02 |
| Neutrophils        | 0.339      | 0.13 |
| Haemoglobin        | 0.361      | 0.12 |

Figure 1. Diagram of relationship between sST2 level, NT-proBNP level and the number of atherosclerotic coronary arteries
death, in acute HF and acute coronary syndromes [6-16]. Recently, some studies concerning the role of sST2 in the assessment of CHF prognosis have been performed [20-23].

Our study has limitations. The main limitation is the small number of patients. There is a need for further research conducted on a larger population. Quantification and detection of coronary artery stenoses were done under conventional visual evaluation without an algorithm standardized methodology such as the Rotterdam Coronary Artery Algorithm Evaluation Framework.

In conclusion, sST2 protein is independent of traditional factors, such as age and BMI, which usually affect levels of NT-proBNP. In chronic heart failure, sST2 protein may be of greater importance in idiopathic dilated cardiomyopathy than in ischaemic ure, sST2 protein may be of greater importance in aetiology of HF, which seem to be associated with the molecular mechanism (biomechanical strain) related to sST2.

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References

1. Dickstein K, Cohen-Solal A, Filippatos G, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008. Eur Heart J 2008; 29: 2388-442.
2. Bao Y, Shang X, Zhou L, et al. Relationship between N-terminal pro-B-type natriuretic peptide levels and metabolic syndrome. Arch Med Sci 2011; 7: 247-56.
3. Kakkar R, Lee RT. The IL-33/ST2 pathway: therapeutic target and novel biomarker. Nat Rev Drug Discov 2008; 7: 827-40.
4. Sanada S, Hakuno D, Higgins LJ, Schreiter ER, McKen zie AN, Lee RT. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. Circulation 2010; 107: 721-6.
5. Weinberg EO, Shimpo M, Hurwitz S, Tominaga S, Rolueau J, Lee RT. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. Circulation 2003; 107: 721-6.
6. Boisot S, Beede J, Isackson S, et al. Serial sampling of ST2 predicts 90-day mortality following destabilized heart failure. J Card Fail 2008; 14: 732-38.
7. Sabatine MS, Morrow DA, Higgins LJ, et al. Complementary roles for biomarkers of biomechanical strain ST2 and N-terminal prohormone B-type natriuretic peptide in patients with ST-elevation myocardial infarction. Circulation 2008; 117: 1936-44.
8. Bayes-Genis A, Pascual-Figal D, Januzzi JL, et al. Soluble ST2 monitoring provides additional risk stratification for outpatients with decompensated heart failure. Rev Esp Cardiol 2010; 63: 1171-8.