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Soluble ST2 reflects hemodynamic stress in non-ischemic heart failure

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ABSTRACT

Background: Elevated levels of soluble ST2 (sST2) are associated with adverse outcome in heart failure. A change in sST2 levels has also been shown to presage outcome. In vitro, ST2 expression is induced by myocardial stress and pro-inflammatory stimuli. The determinants of sST2 levels in vivo, and how they vary with clinical status over time, have not been well described. In a cohort of patients with non-ischemic heart failure, we aimed to assess the association between sST2-levels and hemodynamic parameters reflecting right and left ventricular pre- and afterload, and how these vary with time and clinical status.
Methods: We prospectively recruited 102 patients with a left ventricular ejection fraction of 26 ± 10% and a diagnosis of idiopathic dilated cardiomyopathy based on patient history, clinical examination, echocardiography and coronary angiography. Patients went through extensive baseline work-up and were re-examined after one year. Subsequently, heart transplantations and deaths were recorded. Determinants of sST2 were analyzed at baseline and after one year. Soluble ST2 was measured with a highly sensitive immunoassay.
Results: Soluble ST2 levels were associated with hemodynamic parameters, but these associations were attenuated with clinical improvement. Soluble ST2 was elevated in patients with severe symptoms, but did not vary with etiology, viral presence or the amount of myocardial fibrosis. Heart rate and right atrial pressure remained independent predictors of sST2 on multiple regression analysis.
Conclusions: Our results imply that in non-ischemic heart failure, sST2 reflects hemodynamic stress rather than pathogenic processes in the myocardium.

1. Introduction

Despite extensive research, the pathophysiology of heart failure remains incompletely understood. Different injurious stimuli, such as ischemia; altered loading conditions; toxins; mutations; infections; and autoimmune disease may all precipitate heart failure. The drivers of the subsequent pathophysiological process include hemodynamic stress and neuroendocrine excitation. More recently, it has been suggested that inflammation plays a role in the development of heart failure. As of today, only the inhibitors of the major neuroendocrine axes: beta-blockers, angiotensin converting enzyme inhibitors/angiotensin II receptor blockers and aldosterone antagonists, have been indisputably shown to improve outcome. However, as the effect of neurohormonal inhibition seems to have been exhausted [1–3], we need to explore other pathogenic pathways as potential new targets for therapy in heart failure.

The Interleukin-33 (IL-33)/ST2 (suppressor of tumorigenicity 2; interleukin 1 receptor-like 1) system, which is part of the IL-1 family, is fundamentally linked to inflammatory function and immune-mediated disorders, but recent data suggest that this pathway also plays an important role in cardiovascular disease [4]. ST2 is associated with cardiac fibrosis, and vitro experiments have shown that ST2 is expressed in cardiomyocytes subjected to mechanical stress [5]. Levels of the soluble form of ST2 (sST2) are elevated in patients with heart failure [6,7]. Moreover, circulating concentrations of sST2 are associated with outcome in patients with acute and chronic heart failure [7–10]. Whether the elevated levels of sST2 observed in heart failure stem from myocardial or extramyocardial sources, remains to be resolved [11].

Soon after the discovery of an association between ST2 and cardiac disease, large clinical studies were published on the association between sST2 and outcome. Moreover, several authors reported on the

Abbreviations: ST2, suppressor of tumorigenicity 2; sST2, soluble ST2; IL-33, Interleukin-33; ECG, electrocardiogram; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association.

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predictive value of a change in sST2 values over time [6,12–14]. However, the change in sST2 values has been evaluated largely without correcting for changes in clinical status or other potentially predictive variables. The factors that are associated with increased levels of sST2 in heart failure in vivo remain to be ascertained, and the development in sST2 levels with time has not been well elucidated. Finally, there are scarce data on the association between sST2 and pathogenic factors in patients with non-ischemic cardiomyopathy specifically.

We aimed to examine determinants of sST2 in a well characterized cohort of patients with non-ischemic, non-valvular heart failure. In particular, we assessed the associations between sST2 and potential pathogenic factors such as viral infection and fibrosis, as well as pre-defined hemodynamic parameters. Furthermore, we evaluated the development in sST2 plasma concentration with time and correlated clinical, echocardiographic and biochemical parameters with sST2 levels at inclusion and during follow-up. Finally, we looked at the ability of sST2 to predict heart transplantation and death from worsening heart failure.

2. Methods

The current results stem from a prospective cohort study performed at our tertiary care university hospital. The trial complies with the Declaration of Helsinki and was approved by the Regional Committee for Medical and Health Research Ethics South-East. All patients provided written, informed consent.

2.1. Patient population

Patients aged 18 years or above, admitted to our cardiology department with dilated cardiomyopathy, a left ventricular end diastolic internal diameter ≥ 6.5 cm (or an indexed value > 3.2 cm/m^2) and an ejection fraction ≤ 40% were included. Exclusion criteria were ischemic or hypertensive etiology; primary valvular heart disease; a known or suspected cause of cardiomyopathy, including acute or prior myocarditis; inotropic or mechanical support at admittance; an implantable cardiac device (pacemaker, cardioverter-defibrillator or biventricular pacemaker) and significant concomitant diseases. As we are the only center in Norway to perform heart transplantations, patients referred specifically for heart transplant evaluation were excluded to avoid outcome selection bias. Patients who were referred for diagnostic work-up, and were later found to be in need of heart transplantation, were not excluded. Atrial fibrillation was not an exclusion criterion, provided the ventricular rate had been well controlled.

2.2. Study procedures

At baseline, participants underwent physical examination; blood tests including screening for known monogenic causes of dilated cardiomyopathy; echocardiography; cardiac magnetic resonance imaging; ambulatory 24-hour electrocardiogram (ECG); exercise testing with measurement of peak oxygen uptake; and right-sided cardiac catheterization with endomyocardial biopsy. One year after inclusion, patients were invited for reassessment including echocardiography, exercise testing, cardiac magnetic resonance imaging and blood tests. Patients were later followed through the Norwegian National Population Register and our heart transplant database for mortality and heart transplantations, respectively.

2.3. Biochemical analysis

Peripheral blood samples were obtained in the non-fasting state at inclusion and at one year's follow-up. Blood was collected in glass tubes containing ethylenediamine tetraacetic acid as anti-coagulant, and immediately centrifuged. N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations were determined by an electrochemiluminescence immunoassay (Roche proBNP II, Roche Diagnostics, Basel, Switzerland). Levels of CRP were determined on a MODULAR Analytical platform, P800 module (Roche Diagnostics) using a particle-enhanced immunoturbidimetric assay (Tina-Quant CRP Gen.3, Roche Diagnostics).

Plasma for the measurement of sST2 was stored at −80 °C and thawed once for analysis. Soluble ST2 was measured with the Presage® ST2 Assay (Critical Diagnostics, San Diego, CA) as described by Dieplinger et al. [15]. The average intra-assay coefficient of variation for sST2 in the present study was less than 3%. The inter-assay coefficient of variation was 4.4% for a mean sST2 concentration of 22.6 ng/ml and 3.6% for a mean sST2 concentration of 71.2 ng/ml. The biochemical analyses were performed blinded to clinical data.

2.4. Echocardiography

Echocardiography was performed with Vivid 7 or E9 ultrasound scanners (GE Vingmed Ultrasound, Horten, Norway), using phased array transducers. Cine loops were digitally stored and later analyzed off line using Echo-Pac (GE Vingmed). 2D parameters and conventional Doppler measurements were obtained according to current recommendations [16,17]. Left ventricular ejection fraction was measured by Simpson's biplane method [16].

2.5. Magnetic resonance imaging

Magnetic resonance imaging was performed with Siemens 1.5 Tesla scanners (Siemens Avanto and Siemens Sonata; Siemens Medical Systems, Erlangen, Germany). Seven millimeter thick short axis slices covering the entire left and right ventricles were acquired, and endocardial borders were traced manually at a PACS work station (Sectra Medical Systems AB, Linköping, Sweden). Ventricular volumes and ejection fractions were calculated by short axis slice summation. Ten to 20 min after the intravenous injection of 0.2 mmol/kg of gadoterate meglumine (Guerbet, Villepinte, France), images with late gadolinium enhancement were acquired. The total volume of late myocardial enhancement, presumably reflecting myocardial inflammation and/or fibrosis, was quantified by visual analysis of short axis slices covering both ventricles.

2.6. Right-sided heart catheterization

Right-sided heart catheterization was performed using a Swan–Ganz pulmonary artery thermodilution catheter (Baxter Health Care Corp, Santa Ana, CA). Intracardiac pressures were recorded, and cardiac output was measured by the thermodilution technique. Endomyocardial biopsies were obtained from the right ventricular side of the myocardial septum for viral genome detection, and conventional and electron microscopy.

2.7. Viral genome detection

Total nucleic acid was extracted from endomyocardial biopsies. Real-time polymerase chain reaction assays for the detection of enteroviruses (including coxsackievirus and echovirus), adenovirus, human Parvovirus B19, Epstein–Barr virus, cytomegalovirus, and human herpes virus 6 were performed as described elsewhere [18].

2.8. Conventional and electron microscopy

Endomyocardial specimens obtained for light microscopy were fixed with formalin, embedded in paraffin, sliced into 5-μm sections, and stained with hematoxylin and eosin as well as hematoxylin phloxine saffron and Congo stains for light microscopic examination.

Biopsy specimens scheduled for ultrastructural examination were fixed with glutaraldehyde and examined by electron microscopy.

2.9. Exercise testing and measurement of peak oxygen consumption

Symptom-limited exercise testing was performed using an electrically braked bicycle ergo meter. The test employed an individualized, stepwise protocol. Simultaneous hemodynamic and gas exchange monitoring was performed (Cardiovit CS-200, Schiller, Baar, Switzerland and Ganshorn PowerCube, Ganshorn, Niederlauer, Germany). Peak oxygen uptake is reported in ml/kg/min and as a percentage of age and gender adjusted reference values [19].

2.10. Ambulatory twenty four-hour electrocardiogram

Ambulatory 24-hour ECGs were recorded with Medilog AR4 digital Holter monitors (Schiller AG, Baar, Switzerland) and edited in Medilog Excel 3 by experienced nurses. The edited recordings were then reviewed by a physician. Ventricular tachyarrhythmia was defined as three or more consecutive ventricular beats.

2.11. Genetic testing

Genetic analyses were performed in all participants. DNA sequencing included the translated exons with flanking intron sequences of the genes MYH7, MYBPC3, MYL2, MYL3, TNNI3, TTNNT2, LMNA and ACTC. The pathogenicity of identified missense mutations was assessed with the bioinformatics software programs PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/).

2.12. Statistics

Values are presented as mean ± standard deviation or median (interquartile range) depending on distribution. Baseline characteristics were analyzed according to sST2 tertiles, using one way ANOVA for symmetric, continuous variables, Kruskal–Wallis test for asymmetric continuous variables, and χ^2 test for categorical variables. Determinants of sST2 levels were analyzed by bivariate and multiple linear regression, with log transformed sST2 values as the dependent variable. Associations between categorical variables and log-transformed sST2 levels were analyzed by Student's t-tests or one-way ANOVA. For the latter analyses, post-hoc testing was performed.

The predictive value of baseline sST2 for heart transplantation or death due to heart failure was assessed by Cox proportional hazard analyses. The number of events was quite low, and did not allow for multivariate analysis. All statistical analyses were performed with the Statistical Package for Social Sciences version 18 software (SPSS Inc. Chicago, IL). Two-sided probability values were considered significant at $p < 0.05$.