DOI:
10.1016/j.amjcard.2016.04.017

Document Version
Peer reviewed version

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PII: S0002-9149(16)30498-2

DOI: 10.1016/j.amjcard.2016.04.017

Reference: AJC 21812

To appear in: *The American Journal of Cardiology*

Received Date: 24 December 2015

Revised Date: 4 April 2016

Accepted Date: 6 April 2016

Biological Variability of Soluble ST2 in Patients with Stable Chronic Heart Failure and Implications for Monitoring

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Short Title: Biological Variability of ST2 in CHF

Abstract

Soluble ST2 (sST2) is a novel biomarker implicated in myocardial remodelling and fibrosis. Recent studies in normal subjects have suggested the biological variability (BV) of sST2 is significantly lower than that of the B-type natriuretic peptides, BNP & NTproBNP. It may, consequently, be a better biomarker for monitoring patients with chronic heart failure (CHF). To date, no published studies have examined the BV of sST2 in a heart failure population. Blood samples from 50 outpatients with pharmacologically optimised stable CHF and persistent left ventricular dysfunction (ejection fraction (EF) <40%). were collected at baseline, 1 hour, 1 month, 3 months and 6 months. Using log-transformed data, mean intra-individual coefficients of variation (CV_I) and subsequent reference change values (RCV) were calculated for both NTproBNP and sST2. Results demonstrate significantly lower CV_I and RCV for sST2 compared with NTproBNP at 1 month (12.02 (36%) vs. 36.75 (103%)) p<0.001, 3 months (12.23 (36%) vs. 40.98 (114%)) p<0.001 and 6 months (16.41 (47%) vs. 46.02 (128%)) p<0.001. In conclusion**,** the BV of sST2 is significantly lower than that of NTproBNP in patients with CHF. These results support previous indications that sST2 may be a better biomarker for monitoring such patients.

Keywords: Heart Failure, Monitoring, Biomarkers

Introduction

sST2 is a member of the interleukin 1 (IL-1) receptor family that has recently been identified as a novel biomarker for cardiac remodelling and fibrosis. Raised concentrations are known to be predictive of mortality in patients with acutely decompensated heart failure (ADHF)[1-3]. Moreover, several studies have provided evidence for the prognostic role of serial measures of sST2 in patients with ADHF[4-6]. The role of sST2 in CHF is, however, less well defined[7,8]. Recently, Wu et al[9],, have demonstrated that the RCV of sST2 in healthy volunteers to be much lower than that of BNP or NTproBNP, indicating it may be a better marker for serial monitoring. Despite such promising results, it is likely that variation amongst patients with disease will be greater than that in healthy individuals. To date, no studies analysing the BV or RCV of sST2 in CHF have been reported.

Methods

Fifty patients with CHF, New York Heart Association (NYHA) Class I-III and left ventricular EF ≤40% were recruited from the heart failure clinics at Kings College Hospital, London. A subset of this cohort has been previously described[10]. All were on optimum tolerated heart failure medications. Target dose levels were defined according to current guidelines[11]. Main exclusion criteria were an acute cardiovascular admission or change in prognostically indicated medication within 4 weeks of recruitment, a planned cardiovascular admission, significant renal impairment (estimated glomerular filtration rate (eGFR) <20), or the inability or unwillingness to consent.

Clinical review and blood sampling took place at 5 time points – baseline, 1 hour, 1 month, 3 months and 6 months. Reviews took place at the same time of day for each visit. Vital signs and NYHA class were recorded together with medications and details of any hospital admissions.

Blood samples were obtained by venepuncture after 30 minutes of semi-recumbent rest. Serum samples for creatinine were analysed immediately. After centrifugation at 3000 rpm, 2 mL aliquots of plasma were stored at -30^{o}C until analysed.

sST2 was measured by enzyme linked immunosorbent assay (ELISA) (R&D Systems Europe, Ltd., Abingdon, UK). The sST2 assay contains NS0-expressed recombinant human sST2 and has been shown to accurately quantitate the recombinant factor. The intra-assay precision was 5.6, 4.4 and 4.5% and the inter-assay precision was 7.1, 5.4 and 6.3% at 5.4, 12.6 and 20.6 μg/L respectively. The limit of detection was 0.005 μg/L. Reference range is 6.74 – 20.4 μg/L

NTproBNP was measured by two-site chemiluminescence immunoassay (Immulite 2000, Siemens Healthcare Diagnostics Ltd, Camberley, Surrey UK). The intra-assay precision was 5.4, 3.0 and 4.1% and the inter-assay precision was 6.4, 4.0 and 4.7% at 35.6, 1430 and 29725 ng/L respectively. The limit of detection was 10 ng/L. Reference range is 125 ng/L and 450 ng/L at age <75 and >75 respectively.

The total coefficient of variation (CV_T) is composed of both analytic and biologic variation. Each patient's 1 hour, 1 month, 3 month and 6 month CV_T for both NTproBNP and sST2 was calculated from the standard deviation of the respective values at baseline and 1 hour, baseline and 1 month, baseline and 3 months and baseline and 6 months. As concentrations of all biomarkers were not normally distributed, data was log transformed prior to analysis.

Analytical coefficient of variation (CV_A) describes the reproducibility of the measurement of an analyte. Where possible, samples were analysed in a single series to minimise the contribution of inter-assay analytical variation.

CV_I is the random variation that occurs around a homeostatic setting point in an individual. CV_I was calculated according to the formula:

$$CV_I = (CV_T^2 - CV_A^2)^{1/2}.$$

RCVs at a 95% confidence level were calculated from median CV_I values according to the formula:

$$RCV = Z \times 2^{1/2}(CV_I^2 + CV_A^2)^{1/2}$$

where Z (the 95% confidence interval Z score) is 1.96.

Categorical variables are described as proportions. Continuous variables are described with mean and standard deviation for normally distributed variables and median and interquartile range (IQR) for non-normally distributed variables. Data was log transformed prior to calculation for BV. Differences in 1 hour, 1 month, 3 month and 6 month CV_I for NTproBNP and sST2 were analysed using the paired t test. SPSS v21 was used for all analyses.

The study was performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practice. The East Midlands National Research Ethics Service Committee approved the study protocol. All patients were given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Patients provided signed and dated informed consent before any study specific procedure was conducted.

Results

Characteristics of the 50 patients enrolled in this study are shown in table 1. Four patients withdrew prior to completion of follow-up. Blood samples from 1 patient at the 1 hour time point and 1 patient at the 6 month time point were misplaced after arrival in the lab. Eight patients required hospital admission during the study period with decompensated heart failure.

Concentrations of NTproBNP and sST2 at each visit are detailed in table 2. There were no significant differences from baseline across the time points for either NTproBNP (1 month p=0.883, 3 months p=0.144, 6 months p=0.279) or sST2 (1 month p=0.958, 3 months p=0.857, 6 months p=0.752).

The mean intra-assay coefficient of variation was used as an estimate of overall CV_A. Using this calculation, the CV_As for NTproBNP and sST2 were 4.17% and 4.83% respectively. Mean CV_I and corresponding RCVs for NTproBNP and sST2 at each time-point are shown in table 3.

Paired t-tests were used to examine differences in mean CV_I across the time points. Compared with 1 hour $CV_{I,}$ significant variability was seen across all time points for NTproBNP; 1 hour to 1 month p=0.003, 1 hour to 3 months p<0.001, and 1 hour to 6 months p=0.003. Variability for sST2 existed only between 1 hour and 6 months p=0.019. No significant difference was demonstrated between CV_I for NTproBNP and sST2 at 1 hour (p=0.076). Significant differences were, however, observed between CV_I for NTproBNP and sST2 at all other points; 1 month $p<0.001$, 3 months $p<0.001$ and 6 months $p<0.001$.

Repeated analysis following removal of the data from the eight patients with clinical decompensation during the study period did not result in any significant change to mean CV_I for either NTproBNP (1 month 36.15 (RCV 101%) vs. 36.75 (RCV 103%) (p=0.889); 3 months 41.54 (RCV 115%) vs. 40.98 (114%) (p=0.631); 6 months 42.92 (RCV (120%) vs. 46.02 (RCV 128%) (p=0.610)) or sST2 (1 month 12.44 (RCV 37%) vs. 12.02 (RCV 36%) (p=0.968); 3 months 12.98 (38%) vs. 12.23 (RCV 36%) (p=0.492); 6 months 13.54 (RCV 39%) vs.16.41 (RCV 47%) (p=0.281)).

Discussion

This is the first reported study assessing and directly comparing the BV and RCVs of sST2 with NTproBNP in a stable CHF population. In contrast to NTproBNP, sST2 does not exhibit significant variation either in the short or long term with RCVs for sST2 ranging from 31% to 47%, compared with 52% to 128% for NTproBNP.

The implications of these results are clear, especially when taken in conjunction with previously reported data. Studies have shown that standard heart failure therapies reduce average NTproBNP and BNP by up to 50%[12-16], whilst reductions in sST2 >50% have been shown to be indicative of therapeutic success of heart failure therapy[17]. Thus, based on the results obtained in this study, a reduction of 50% in NTproBNP cannot be attributed to a therapeutic intervention alone, as this reduction is less than the RCV. In contrast, the same changes in sST2 would be above that of the observed

RCV and thus more likely due to clinical intervention than variability alone. sST2 may therefore be better than NTproBNP for serial monitoring to guide therapy.

Questions remain, however, as to the exact pathophysiological process such changes in sST2 represent. Although there is much evidence to support its use as a marker of fibrosis, studies also indicate that it plays a vital role in immune modulation and inflammatory responses[18-20]. In the shorter-term, changes in sST2 may therefore reflect other underlying processes associated with decompensation - such as infection and renal dysfunction. Like CHF, chronic kidney disease (CKD) is a multifactorial disorder, occurring in the context of chronic co-morbid conditions, many of which are related to inflammation and inflammatory responses. sST2 has previously been shown to be associated with disease severity in CKD[21] and variations in renal function across the time points measured could arguably influence the observed BV.. This study was insufficiently powered to account for such variables but, repeated calculations performed after the removal of any patients exhibiting a >25% change in creatinine did not result in a significant change in median CV_I or RCV (data not shown).

Several issues including pre-analytic factors, analytical variability and biological variability all contribute to total variability. In order to minimise pre-analytic factors we adopted strict entry criteria in order to establish stability at baseline. This is reflected by the low median values that, in the case of sST2, did not exceed the normal reference range. In addition, patient preparation and blood collection protocol was standardised across the group. Blood samples were stored and assays performed in the minimum number of batches in order to reduce analytical variability. Although we collected only single samples at each time point, clinical chemistry practice has suggested that although duplicate samples result in a reduction of CV_A, this will result in double the cost of reagents and is only useful if the CV_A is >50% of the CV_I. Under these conditions, it is estimated that the assay imprecision will add only 10% to the BV. In our study the CV_A of sST2 was 48% of CV_I at 1 hour, 40% at 1 month, 39% at 3 months and 29% at 6 months. For NTproBNP CV_A was 23% of CV_I at 1 hour, 11% at 1 month, 10% at 3 months and 9% at 6 months.

Currently there are 3 commercially available assays to sST2; the MBL assay, the Presage assay and the R&D assay. Whilst the majority of studies looking at ADHF have employed the Presage assay, studies looking at sST2 in CHF have used a variety of all 3. Mueller et al[22] performed a comparison of plasma concentrations by these assays and found considerable differences between concentrations obtained. Results between the methods are, therefore, not directly comparable. They were not, however, able to demonstrate any superiority of any assay over another. Although such differences will impart changes to the CV_A, CV_I should be consistent regardless of the assay utilised. To date, there are no comparative studies assessing the BV of sST2 in CHF, however previous studies looking at the natriuretic peptides demonstrated similar BV and RCV across both healthy individuals and those with CHF. In support of our findings, Wu et al[9] recently examined the BV of sST2 in healthy individuals, using the Presage assay. In this they demonstrate a CV_I of 11% and RCV of 30% for sST2 at 2 months.

Acknowledgements

The research was supported by the National Institute for Health Research (NIHR) Clinical Research Facility at King’s College Hospital, London and NIHR Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust, London and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Funding

The research was funded by a British Heart Foundation Pump Priming Grant (RE/08/003), together with the support of the National Institute for Health Research, through the Comprehensive Clinical Research Network.

Conflict of Interest

Professor McDonagh has received honoraria from Alere for participation in advisory boards. All other authors have no conflict of interest pertaining to this research to declare.

1. Januzzi JL, Jr., Peacock WF, Maisel AS, Chae CU, Jesse RL, Baggish AL, O'Donoghue M, Sakhuja R, Chen AA, van Kimmenade RR, Lewandrowski KB, Lloyd-Jones DM, Wu AH. Measurement of the interleukin family member ST2 in patients with acute dyspnea: results from the PRIDE (Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department) study. *J Am Coll Cardiol* 2007;50:607-613.

2. Manzano-Fernandez S, Mueller T, Pascual-Figal D, Truong QA, Januzzi JL. Usefulness of soluble concentrations of interleukin family member ST2 as predictor of mortality in patients with acutely decompensated heart failure relative to left ventricular ejection fraction. *Am J Cardiol* 2011;107:259-267.

3. Shah RV, Chen-Tournoux AA, Picard MH, van Kimmenade RR, Januzzi JL. Serum levels of the interleukin-1 receptor family member ST2, cardiac structure and function, and long-term mortality in patients with acute dyspnea. *Circ Heart Fail* 2009;2:311-319.

4. Boisot S, Beede J, Isakson S, Chiu A, Clopton P, Januzzi J, Maisel AS, Fitzgerald RL. Serial sampling of ST2 predicts 90-day mortality following destabilized heart failure. *J Card Fail* 2008;14:732-738.

5. Bayes-Genis A, Pascual-Figal D, Januzzi JL, Maisel A, Casas T, Valdes Chavarri M, Ordonez-Llanos J. Soluble ST2 monitoring provides additional risk stratification for outpatients with decompensated heart failure. *Revista Espanola De Cardiologia* 2010;63:1171-1178.

6. Manzano-Fernandez S, Januzzi JL, Pastor-Perez FJ, Bonaque-Gonzalez JC, Boronat-Garcia M, Pascual-Figal DA, Montalban-Larrea S, Navarro-Penalver M, Andreu-Cayuelas JM, Valdes M. Serial monitoring of soluble interleukin family member ST2 in patients with acutely decompensated heart failure. *Cardiology* 2012;122:158-166.

7. Pascual-Figal DA, Ordonez-Llanos J, Tornel PL, Vazquez R, Puig T, Valdes M, Cinca J, de Luna AB, Bayes-Genis A. Soluble ST2 for predicting sudden cardiac death in patients with chronic heart failure and left ventricular systolic dysfunction. *J Am Coll Cardiol* 2009;54:2174-2179.

8. Weinberg EO, Shimpo M, Hurwitz S, Tominaga S, Rouleau JL, Lee RT. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. *Circulation* 2003;107:721-726.

9. Wu AH, Wians F, Jaffe A. Biological variation of galectin-3 and soluble ST2 for chronic heart failure: Implication on interpretation of test results. *American Heart Journal* 2013;165:995-999.

10. Piper SE, Sherwood RA, Amin-Youssef GF, Shah AM, McDonagh TA. Serial soluble ST2 for the monitoring of pharmacologically optimised chronic stable heart failure. *International Journal of Cardiology* 2015;178:284-291.

11. McMurray JJ, Adamopoulos S, Anker SD, Auricchio A, Bohm M, Dickstein K, Falk V, Filippatos G, Fonseca C, Gomez-Sanchez MA, Jaarsma T, Kober L, Lip GY, Maggioni AP, Parkhomenko A, Pieske BM, Popescu BA, Ronnevik PK, Rutten FH, Schwitter J, Seferovic P, Stepinska J, Trindade PT, Voors AA, Zannad F, Zeiher A, Bax JJ, Baumgartner H, Ceconi C, Dean V, Deaton C, Fagard R, Funck-Brentano C, Hasdai D, Hoes A, Kirchhof P, Knuuti J, Kolh P, McDonagh T, Moulin C, Reiner Z, Sechtem U, Sirnes PA, Tendera M, Torbicki A, Vahanian A, Windecker S, Bonet LA, Avraamides P, Ben Lamin HA, Brignole M, Coca A, Cowburn P, Dargie H, Elliott P, Flachskampf FA, Guida GF, Hardman S, Iung B, Merkely B, Mueller C, Nanas JN, Nielsen OW, Orn S, Parissis JT, Ponikowski P. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2012;14:803-869.

12. Troughton RW, Frampton CM, Yandle TG, Espiner EA, Nicholls MG, Richards AM. Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. *Lancet* 2000;355:1126-1130.

13. Johnson W, Omland T, Hall C, Lucas C, Myking OL, Collins C, Pfeffer M, Rouleau JL, Stevenson LW. Neurohormonal activation rapidly decreases after intravenous therapy with diuretics and vasodilators for class IV heart failure. *J Am Coll Cardiol* 2002;39:1623-1629.

14. Takeda Y, Fukutomi T, Suzuki S, Yamamoto K, Ogata M, Kondo H, Sugiura M, Shigeyama J, Itoh M. Effects of carvedilol on plasma B-type natriuretic peptide concentration and symptoms in patients with heart failure and preserved ejection fraction. *Am J Cardiol* 2004;94:448-453.

15. Hara Y, Hamada M, Shigematsu Y, Suzuki M, Kodama K, Kuwahara T, Hashida H, Ikeda S, Ohtsuka T, Hiasa G, Hiwada K. Effect of beta-blocker on left ventricular function and natriuretic peptides in patients with chronic heart failure treated with angiotensin-converting enzyme inhibitor. *Japanese Circulation Journal* 2000;64:365-369.

16. Latini R, Masson S, Anand I, Judd D, Maggioni AP, Chiang YT, Bevilacqua M, Salio M, Cardano P, Dunselman PH, Holwerda NJ, Tognoni G, Cohn JN. Effects of valsartan on circulating brain natriuretic peptide and norepinephrine in symptomatic chronic heart failure: the Valsartan Heart Failure Trial (Val-HeFT). *Circulation* 2002;106:2454-2458.

17. Breidthardt T ST, Drexler B, Noveanu M, Haaf P, Balmelli, Cattelan C IA, Reichlin T, Potocki M, Mueller C. Interleukin family member ST2 rapidly responds to heart failure treatment and its changes predict one-year mortality. *Eur Heart J* 2011;32.

18. Coyle AJ, Lloyd C, Tian J, Nguyen T, Erikkson C, Wang L, Ottoson P, Persson P, Delaney T, Lehar S, Lin S, Poisson L, Meisel C, Kamradt T, Bjerke T, Levinson D, Gutierrez-Ramos JC. Crucial role of the interleukin 1 receptor family member T1/ST2 in T helper cell type 2-mediated lung mucosal immune responses. *The Journal of Experimental Medicine* 1999;190:895-902.

19. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, Gorman DM, Bazan JF, Kastelein RA. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005;23:479-490.

20. Trajkovic V, Sweet MJ, Xu D. T1/ST2--an IL-1 receptor-like modulator of immune responses. *Cytokine Growth Factor Rev* 2004;15:87-95.

21. Bao YS, Na SP, Zhang P, Jia XB, Liu RC, Yu CY, Mu SH, Xie RJ. Characterization of interleukin-33 and soluble ST2 in serum and their association with disease severity in patients with chronic kidney disease. *Journal of Clinical Immunology* 2012;32:587-594.

22. Mueller T, Zimmermann M, Dieplinger B, Ankersmit HJ, Haltmayer M. Comparison of plasma concentrations of soluble ST2 measured by three different commercially available assays: the MBL ST2 assay, the Presage ST2 assay, and the R&D ST2 assay. *Clin Chim Acta* 2012;413:1493-1494.

Clinical	
Age (years) mean (SD; Range)	67.3 (11.6; 45-87)
Men	n=41 (82%)
Body mass index (kg/m^2) mean (SD; Range)	30 (5.7; 20-45)
Hypertension	n=25 (50%)
Diabetes Mellitus	n=8 (16%)
Coronary heart disease	n=24 (48%)
Atrial fibrillation	n=12 (24%)
QRS (ms) mean (SD; Range)	130.4 (38.4; 75-222)
Cardiac Resynchronisation Therapy	n=14 (28%)
Implantable Cardiac Defibrillator	n=7 (14%)
New York Heart Association Class I	n=5 (10%)
New York Heart Association Class II	n=35 (70%)
New York Heart Association Class III	n=10 (20%)
Estimated Glomerular Filtration Rate mean (SD; Range)	64.2 (17.9; 26-91)
Medication	
Angiotensin Converting Enzyme Inhibitor	n=37 (74%)
Angiotensin Receptor Blocker	n=13 (26%)
Beta-blocker	n=45 (90%)
Mineralocorticoid Receptor Antagonist	n=34 (68%)
Digoxin	n=8 (16%)
Regular Loop Diuretics	n=37 (74%)
Echo	
Ejection Fraction mean (SD; Range)	30.7 (6.7; 14-40)
Moderate-Severe Valve disease	n=12 (24%)

Table 1: Baseline patient characteristics

	Baseline	One Month	Three Months	Six Months
NTproBNP (µg/L)	300 (80.8-1282)	466 (80.2-1171)	291 (46.5-1006)	356 (61.9-1469)
sST2 (µg/L)	17.6 (13.3-22.5)	17.3 (13.6-21.5)	17.1 (13.8-22.5)	16.9 (13.7-21.6)

Table 2: Median (IQR) biomarker concentrations at each time point

	One Hour		One Month		Three Months		Six Months	
	NTproBNP	sST2	NTproBNP	sST2	NTproBNP	sST2	NTproBNP	sST2
Mean CV_i	18.47	9.99	36.75	12.02	40.98	12.23	46.02	16.41
RCV (%)	52	31	103	36	114	36	128	47

Table 3: Mean CV_I and corresponding RCV at each time point