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Nonmyocardial Production of ST2 Protein in Human Hypertrophy and Failure Is Related to Diastolic Load

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Objectives This study was designed to investigate: 1) relationships between serum ST2 levels and hemodynamic/neurohormonal variables; 2) myocardial ST2 production; and the 3) expression of ST2, membrane-anchored ST2L, and its ligand, interleukin (IL)-33, in myocardium, endothelium, and leukocytes from patients with left ventricular (LV) pressure overload and congestive cardiomyopathy.

Background Serum levels of ST2 are elevated in heart failure. The relationship of ST2 to hemodynamic variables, source of ST2, and expression of ST2L and IL-33 in the cardiovascular system are unknown.

Methods Serum ST2 (pg/ml; median [25th, 75th percentile]) was measured in patients with LV hypertrophy (aortic stenosis) ($n = 45$), congestive cardiomyopathy ($n = 53$), and controls ($n = 23$). ST2 was correlated to N-terminal pro-brain natriuretic peptide, C-reactive protein, and hemodynamic variables. Coronary sinus and arterial blood sampling determined myocardial gradient (production) of ST2. The levels of ST2, ST2L, and IL-33 were measured (reverse transcriptase-polymerase chain reaction) in myocardial biopsies and leukocytes. The ST2 protein production was evaluated in human endothelial cells. The IL-33 protein expression was determined (immunohistochemistry) in coronary artery endothelium.

Results The ST2 protein was elevated in aortic stenosis (103 [65, 165] pg/ml, $p < 0.05$) and congestive cardiomyopathy (194 [69, 551] pg/ml, $p < 0.01$) versus controls (49 [4, 89] pg/ml) and correlated with B-type natriuretic peptide ($r = 0.5$, $p < 0.05$), C-reactive protein ($r = 0.6$, $p < 0.01$), and LV end-diastolic pressure ($r = 0.38$, $p < 0.03$). The LV ST2 messenger ribonucleic acid was similar in aortic stenosis and congestive cardiomyopathy versus control ($p = NS$). No myocardial ST2 protein gradient was observed. Endothelial cells secreted ST2. The IL-33 protein was expressed in coronary artery endothelium. Leukocyte ST2L and IL-33 levels were highly correlated ($r = 0.97$, $p < 0.001$).

Conclusions In human hypertrophy and failure, serum ST2 correlates with the diastolic load. Though the heart, endothelium, and leukocytes express components of ST2/ST2L/IL-33 pathway, the source of circulating serum ST2 is extra-myocardial. (J Am Coll Cardiol 2008;52:2166–74) © 2008 by the American College of Cardiology Foundation

Inflammatory/innate immune signaling contributes to the pathophysiology of human hypertrophy and heart failure. Recently, serum ST2 protein has emerged as a new prognostic biomarker in patients with recent myocardial infarction and congestive heart failure (1–6). The ST2 protein, and its membrane-anchored counterpart, ST2L, belong to the Toll-interleukin (IL)-1 receptor family and production of both isoforms from a single messenger ribonucleic acid (mRNA) is transcriptionally regulated (7,8). Extracellular engagement of ST2L with its ligand, IL-33, leads to the induction of Th2 cytokines in T cells (9) and down-regulation of Toll-like receptor 4 signaling (10–12). Soluble ST2 protein is secreted in response to inflammatory signals. It inhibits the binding of IL-33 to ST2L (13) and has also been shown to bind to macrophages leading to down-regulation of proinflammatory cytokines (14–16). Separate

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studies identified IL-33 as an intracellular protein localizing to the nucleus in inflamed high endothelial venules (17,18).

Apart from prognostic information obtained from soluble ST2 serum levels, little is known about myocardial production of ST2 or regulation and expression of ST2, ST2L, and IL-33 (9) in the cardiovascular system. Serum ST2 strongly correlated with serum B-type natriuretic peptide (BNP) and pro-atrial natriuretic peptide (3), which are released from the myocardium in response to pressure or volume overload and relate to indexes of hemodynamic load (19). The ST2 levels also correlated with serum levels of norepinephrine (3), a marker of systemic neurohormonal activation in the failing heart. However, whether hemodynamic factors are directly related to serum ST2 levels in human hypertrophy and failure is unknown.

We tested the hypothesis that serum ST2 levels correlate to hemodynamic variables in patients with pressure overload hypertrophy and congestive cardiomyopathy (CCM). Both conditions represent 2 distinct pathophysiological mechanisms of heart failure allowing us to pin down hemodynamic variables related to elevated serum ST2. Furthermore, we evaluated the myocardium as the origin of serum ST2 by investigating expression levels in myocardial biopsies as well as protein production of ST2. Finally, we report new information on secretion of ST2 protein in endothelial cells and expression of ST2L and IL-33 in the myocardium and peripheral leukocytes from patients with pressure overload hypertrophy and CCM.

Methods

Patients. A total of 223 patients were studied. Serum ST2 levels were determined in 163 patients: 50 consecutive patients with symptomatic aortic stenosis (AS), 87 patients with congestive heart failure referred for elective diagnostic left-right heart catheterization (CCM), and a control group that consisted of 26 subjects in whom diagnostic cardiac catheterization showed normal coronary angiogram and left ventricular (LV) function. In addition, 14 patients presenting with diastolic heart failure defined by elevated filling pressures and preserved LV systolic function were included for ST2 determination. Patients presenting with acute coronary syndrome, systemic inflammation, or active infection were excluded. For determination of myocardial BNP and ST2 production, coronary sinus and arterial blood were sampled during cardiac catheterization from 24 patients with advanced congestive heart failure. In 38 patients (details follow) LV myocardial gene expression for ST2, ST2L, IL-33, and BNP was performed. The study was approved by the institutional ethics committee, and all patients gave informed consent.

Hemodynamics. At catheterization, pulmonary capillary wedge pressure was measured by a Swan-Ganz catheter, whereas LV pressure was recorded with a catheter positioned in the LV cavity. The LV volumes and ejection fraction were derived from the single-plane angiogram using the area-length method. Aortic valve area was calculated using the Gorlin formula.

Echo-Doppler was performed immediately before or after cardiac catheterization according to guidelines of the American Society of Echocardiography (20). The LV end-diastolic and -systolic meridional wall stress were calculated from M-mode in combination with pressure data as previously described (21). The LV mass was calculated by the Devereux et al. (22) equation. Increased diastolic load is defined as LV end-diastolic pressure (EDP) >16 mm Hg (23).

Abbreviations and Acronyms

AS = aortic stenosis
AU = arbitrary units
BNP = B-type natriuretic peptide
CCM = congestive cardiomyopathy
EDP = end-diastolic pressure
hsCRP = high sensitivity C-reactive protein
IL = interleukin
LV = left ventricular
mRNA = messenger ribonucleic acid
NT-proBNP = N-terminal pro-brain natriuretic peptide

Serum measurements. The ST2 protein was measured by enzyme-linked immunoadsorbent assay (MBL International, Inc., Woburn, Massachusetts). The enzyme-linked immunoadsorbent assay coefficient of variation and stability of ST2 have been described (1,4–6). N-terminal pro-brain natriuretic peptide (NT-proBNP) was measured using automated enzyme immunoassay (Roche Diagnostics, Basel, Switzerland). High-sensitivity C-reactive protein (hsCRP) was determined using an immunoturbidimetric method with latex beads coated with anti-CRP–specific antibody (Roche Diagnostics).

LV biopsies. The LV endomyocardial biopsies were obtained from 38 patients (9 control, 12 AS, and 17 CCM). Control biopsies were obtained from patients with normal LV function and stable angina pectoris referred for elective cardiac bypass surgery. In the control and AS groups, LV biopsies were obtained during cardiac surgery and in patients with congestive CCM during routine cardiac catheterization.

Cultured cells. Human saphenous vein endothelial cells were cultured in endothelial media at passages 3 to 5. Human coronary artery endothelial cells were obtained from Lonza (Allendale, New Jersey). Culture supernatant was assayed for ST2 16 h after treatment, or, for time course, when indicated. Phorbol ester (200 nmol/l), H_2O_2 (50 μmol/l), and brefeldin A (5 μmol/l) were obtained from Sigma (St. Louis, Missouri); IL-1-beta (5 ng/ml), and tumor necrosis factor-alpha (10 ng/ml) were obtained from R & D Systems (Minneapolis, Minnesota).

Ribonucleic acid (RNA) extraction. RNA was extracted using the RNeasy Mini Kit (Qiagen, Venlo, the Netherlands) and was deoxyribonuclease digested and reverse transcribed using High-Capacity cDNA Archive System (Applied Biosystems, Foster City, California).

Reverse transcriptase-polymerase chain reaction. Real-time reverse transcriptase-polymerase chain reaction on complementary deoxyribonucleic acids was performed in

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triplicate using Assay-on-Demand (ST2) or specific primers and TaqMan minor groove binder probes (ST2L-F: 5′-CAGGCGGCACATTTTCATC-3′, ST2L-R 5′-TGCTCGTAGGCAAACTCCTTATT-3′, ST2L-probe 6-FAM-ACCCCTCAGATCACTC-MGB and IL-33-F: 5′-CCAGGCCTTCTTTGTCCTTCATAAT-3′, IL-33-R 5′-CTCCAGGATCAGTCTTGCATTCAA-3′, IL-33-probe 6-FAM-CACTCCAACTGTGTTTCAT-MGB) (ABI Prism 7000 Sequence Detection System, Applied Biosystems). Expression of ST2, ST2L, and IL-33 was normalized to glyceraldehyde 3-phosphate dehydrogenase (Applied Biosystems) in the same sample.

Immunohistochemistry. Immunohistochemistry for IL-33 was performed on a human coronary artery using an affinity purified rabbit polyclonal antibody against an IL-33 peptide antigen (Affinity Bioreagents, Golden, Colorado) and diaminobenzidine staining with antigen retrieval (Vector Laboratories, Burlingame, California).

Statistical analyses. Data were analyzed using GraphPad Prism (La Jolla, California) and are presented as mean ± SD for normally distributed variables and as median [25th, 75th percentile] when non-Gaussian distributed. For 3 groups, data were analyzed by analysis of variance for parametric analysis or the Kruskal-Wallis test for nonparametric analysis and, when significant ($p < 0.05$), were followed by Dunn or Bonferroni multiple comparison testing, respectively. For 2 groups, data were analyzed using Student *t* test with $p < 0.05$ considered significant. When variables displayed heterogeneity of variance, data were transformed and are presented on a logarithmic scale. Pearson correlation was used for normally distributed variables and Spearman correlation was used for nonparametric variables.

Results

Patient characteristics. Baseline characteristics of control, AS, and CCM patients undergoing serological analyses are shown in Table 1. The LV mass index was higher in AS and CCM patients compared with the index in control patients. The CCM patients had larger end-diastolic volumes, lower ejection fractions, and higher diastolic and systolic wall stress compared with the same measurements in control patients. The LV EDP was elevated in the AS and CCM groups compared with the LV EDP in the control group. Left ventricular developed pressure increased in the AS group and decreased in the CCM group compared with developed pressure in the control group. There were no differences in clinical or hemodynamic characteristics between both subpopulations. In both populations 64% and 52% of CCM patients, respectively, presented with acute decompensation or worsening of congestive heart failure symptoms.

Table 1 Clinical and Hemodynamic Parameters of Patients With Serological Analyses

	Control (n = 23)	AS (n = 45)	CCM (n = 53)
Age, yrs	60 ± 15	73 ± 8	65 ± 14
Female/male	10/13	24/21	20/33
NYHA functional class, I/II/III/IV, %	100/0/0/0	17/36/47/0	2/48/31/19
Acute heart failure	NA	6 (13%)	34 (64%)
Coronary artery disease	0	7 (15%)	13 (24%)
Beta-blockers	5 (22%)	20 (45%)	40 (75%)
ACE/AT_1 blockers	3 (13%)	19 (42%)	46 (87%)
Diuretics	2 (9%)	22 (49%)	50 (94%)
LV mass index, g/m^2	75 ± 28	128 ± 59*	136 ± 67*
EDVI, ml	80 ± 24	79 ± 30	127 ± 56†‡
LV ejection fraction, %	74 ± 10	68 ± 21	27 ± 11†§
LV developed pressure, mm Hg	133 ± 31	174 ± 44*	97 ± 30§‖
LV EDP, mm Hg	12 ± 4	18 ± 8*	23 ± 8†
Diastolic wall stress, kdyn/cm^3	14 ± 7	21 ± 10	40 ± 22†§
Systolic wall stress, kdyn/cm^3	93 ± 25	74 ± 35	120 ± 58§‖

Values are mean ± SD. Data were analyzed by analysis of variance followed by post-hoc analysis. *$p < 0.01$ versus control; †$p < 0.001$ versus control; ‡$p < 0.001$ versus AS; §$p < 0.01$ versus AS; ‖$p < 0.05$ versus control.

ACE = angiotensin-converting enzyme; AS = aortic stenosis; AT_1 = angiotensin II type 1; CCM = congestive cardiomyopathy; EDP = end-diastolic pressure; EDVI = end-diastolic volume index; LV = left ventricular; NYHA = New York Heart Association.

Serum ST2, hsCRP, and NT-proBNP levels. Serum levels of ST2 (Fig. 1A, left) were significantly increased in patients with AS (103 [65, 165] pg/ml, $p < 0.05$) and CCM (194 [69, 551] pg/ml, $p < 0.01$) compared with serum levels in control patients (49 [4, 89] pg/ml). Levels of NT-proBNP (Fig. 1A, middle) were significantly increased in AS (537 [253, 1,936] pg/ml, $p < 0.05$) and CCM patients (3,088 [629, 7,846] pg/ml, $p < 0.01$) versus control patients (43 [28, 100] pg/ml). Levels of hsCRP (Fig. 1A, right) were significantly increased in patients with AS (6.1 [1.3, 15] mg/l, $p < 0.05$) and CCM (12.0 [3.3, 29.2] mg/l, $p < 0.01$) versus control patients (2.2 [1.2, 3.0] mg/l). In the entire population, a significant positive relationship was noted between ST2 and NT-proBNP (Fig. 1B, left) ($r = 0.47$, $p < 0.05$) and between ST2 and hsCRP (Fig. 1B, right) ($r = 0.55$, $p < 0.001$).

Serum ST2 and hemodynamic parameters. No significant relationship was noted between serum ST2 levels and indices of LV remodeling including LV end-diastolic or -systolic volume, LV mass index, and LV relative wall thickness. Furthermore, ST2 levels did not correlate with LV systolic developed pressure or systolic wall stress. However, in the entire study population, a weak but significant, inverse relationship was noted between ST2 levels and LV ejection fraction ($r = -0.21$, $p < 005$). On the other hand, ST2 levels showed a stronger relationship with LV EDP ($r = 0.37$, $p < 0.01$).

ST2 and diastolic load. In the entire cohort (Fig. 2A), serum ST2 levels were higher in patients with moderately or severely elevated LV EDP as compared with patients with normal or mildly increased LV EDP. The ST2 levels were increased proportionally with the extent of diastolic load as assessed from elevated levels of LV EDP (Fig. 2B).

To further determine whether diastolic load is associated with ST2 levels independent of LV systolic function (ejection

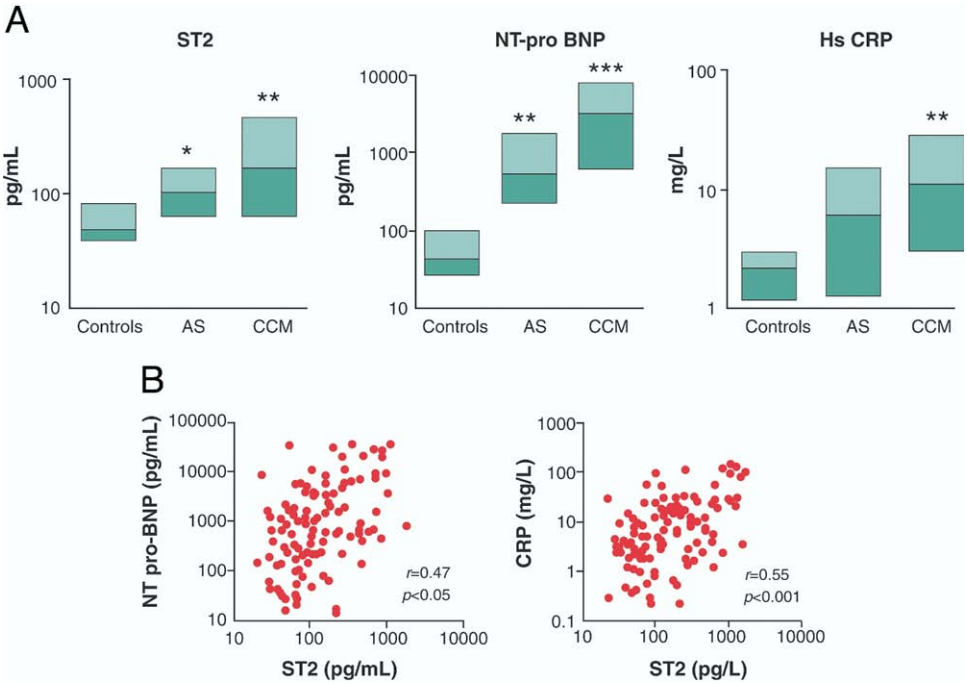

Figure 1 **Serum Levels of Humoral Markers**

(A) Serum levels of ST2, CRP, and BNP in AS, CCM, and control patients. Box plots show median [25th, 75th percentile]. **(B)** Scatterplot between NT-proBNP and ST2 **(left)** and CRP and ST2 **(right)** in all patients. *p < 0.05 versus control; **p < 0.001 versus control; ***p < 0.005 AS versus control. AS = aortic stenosis; BNP = B-type natriuretic peptide; CCM = congestive cardiomyopathy; hsCRP = high sensitivity C-reactive protein; NT-proBNP = N-terminal pro-brain natriuretic peptide.

fraction), ST2 was examined in patients (n = 14) presenting with isolated diastolic heart failure and normal LV systolic function (Fig. 3A, left). Serum ST2 was higher in these patients compared with ST2 levels in control patients (383 ± 149 pg/ml vs. 75 ± 12 pg/ml, p < 0.001). Likewise, NT-proBNP also increased compared with the levels in control patients (901 ± 293 pg/ml vs. 88 ± 31 pg/ml, p = 0.001) (Fig. 3A, right). The corresponding LV EDP (18 ± 10 mm Hg vs. 12 ± 4 mm Hg, p = 0.03) and ejection fraction (73 ± 13% vs. 74 ± 7%, p = NS) are shown (Fig. 3B). These results suggest that diastolic load is a hemodynamic factor that modulates ST2 production.

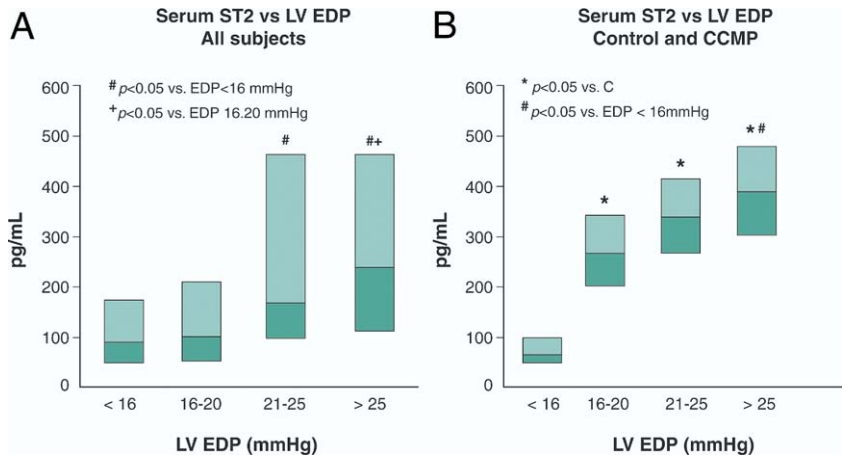

Figure 2 **Relationship Between Serum ST2 Levels and Extent of Diastolic Load as Assessed From LV EDP**

Serum ST2 levels according to LV EDP in all study subjects **(A)** and in control and CCM patients **(B)**. Box plots show median [25th, 75th percentile]. CCMP = congestive cardiomyopathy patients; LV EDP = left ventricular end-diastolic pressure; other abbreviations as in Figure 1.

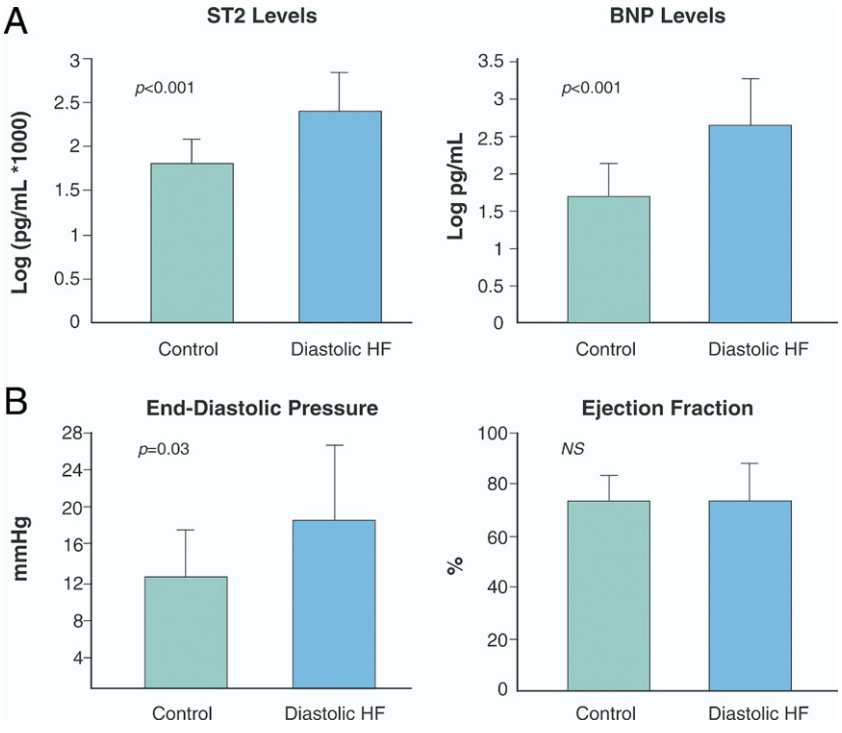

Figure 3 Serum ST2 and BNP in Patients With Isolated Diastolic HF

(A) Serum ST2 **(left)** and BNP **(right)** levels in patients with isolated diastolic failure. Data were transformed due to heterogeneity of variance into the logarithmic scale. **(B)** The LV EDP **(left)** and LV ejection fraction **(right)** in patients with diastolic failure versus control patients. HF = heart failure; other abbreviations as in Figures 1 and 2.

Myocardial production of BNP and ST2. To determine myocardial production of BNP and ST2, the coronary sinus minus arterial blood concentration gradient was determined in 24 patients with chronic heart failure undergoing implantation of biventricular pacemakers. Hemodynamic characteristics of these patients with corresponding LV EDP (22 ± 9 mm Hg vs. 23 ± 9 mm Hg, p = NS) and ejection fraction (27 ± 9% vs. 27 ± 9%, p = NS) were similar to the CCM group undergoing serological analyses. Arterial and coronary sinus levels of NT-proBNP were 1,069 ± 933 pg/ml and 1,798 ± 1,178 pg/ml, respectively, with a difference of 729 ± 410 pg/ml, p < 0.0001 sinus versus arterial (Fig. 4A, left). Arterial and sinus levels of ST2 were 275 ± 195 pg/ml and 264 ± 171 pg/ml, respectively, with a difference of −11 ± 39 pg/ml, p = NS (Fig. 4A, right). These results demonstrate myocardial production of BNP but not of ST2. In LV myocardial biopsies (characteristics presented in Table 2), BNP mRNA levels were 4.4-fold increased in AS (log arbitrary units [AU]: 0.98 ± 1.16) and 43-fold in CCM (log AU: 2.71 ± 0.41) patients compared with the levels in control patients (log AU: 0.27 ± 1.11, p < 0.001) (Fig. 4B, left). Serum NT-proBNP levels correlated with their corresponding myocardial BNP mRNA levels (samples from the same patients, r = 0.54, p = 0.01). The ST2 mRNA levels were not significantly increased in AS (log AU: 0.73 ± 0.66) and CCM patients (log AU: 0.85 ± 0.39) compared with the levels in control patients (log AU: 0.55 ± 0.20, p = NS) (Fig. 4B, right) with no correlation between serum ST2 and myocardial ST2 mRNA.

ST2 secretion by venous and arterial endothelial cells. Phorbol-ester induced ST2 secretion in venous endothelial cells compared with ST2 secretion in control patients (299 ± 22 pg/ml vs. 92 ± 6 pg/ml, p < 0.001) (Fig. 5). Interleukin-1-beta induced ST2 secretion in venous and arterial endothelial cells (venous: 125 ± 6 pg/ml vs. 92 ± 6 pg/ml, p < 0.001; arterial: 103 ± 48 pg/ml vs. 50 ± 24 pg/ml, p < 0.05). Tumor necrosis factor-alpha also induced ST2 secretion (115 ± 4 pg/ml) compared with secretion in control cells (96 ± 6 pg/ml, p < 0.001). The H_2O_2 blocked basal ST2 secretion in endothelial cells (16 ± 4 pg/ml vs. 50 ± 24 pg/ml, p < 0.05), demonstrating that local oxidative stress can inhibit ST2 secretion. The ST2 secretion was elevated by 3 h compared with secretion in control patients (150 ± 20 pg/ml vs. 52 ± 12 pg/ml, p < 0.001), reached peak levels at 6 h (304 ± 14 pg/ml vs. 51 ± 10 pg/ml, p < 0.001) (Fig. 5, right), and declined afterward (not shown). Brefeldin A, a blocker of protein secretion through the endoplasmic reticulum and Golgi apparatus, in the presence of phorbol ester completely blocked ST2 secretion (Fig. 5, right). Data represent 3 to 4 experiments with duplicate samples.

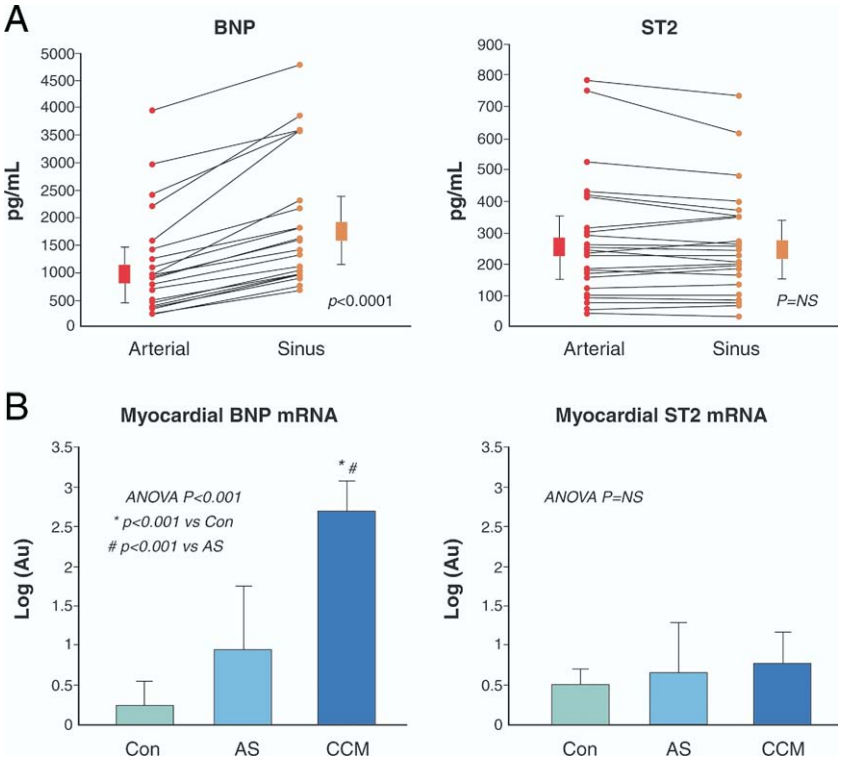

Figure 4 Transmyocardial Gradient of and Myocardial mRNA Levels of BNP and ST2

(A) Transmyocardial production of BNP **(left)** and ST2 **(right)** in individual patients (n = 24) with heart failure. Mean ± SD are also shown in addition to individual values. **(B)** The LV BNP mRNA levels **(left)** in control, AS, and CCM cardiac biopsies. The LV ST2 mRNA levels **(right)** are not significantly different in all groups. AU = arbitrary units; mRNA = messenger ribonucleic acid; other abbreviations as in Figure 1.

Expression of IL-33 and ST2L in the heart and endothelium. The IL-33 mRNA levels were modestly lower in LV biopsies from AS patients (0.80 ± 0.33 AU) compared with levels in biopsies from CCM patients (1.33 ± 0.48 AU, p = 0.005) (Fig. 6A, left). Levels of membrane-anchored ST2L were significantly decreased in AS patients compared with levels in control patients (0.27 ± 0.22 AU vs. 1.00 ± 0.60 AU; $p < 0.05$), but levels were similar between CCM (0.92 ± 0.72 AU) and control patients (p = NS) (Fig. 6A, left). The IL-33 protein localizes to endothelial cells in the human coronary artery (Fig. 6B). A weak correlation between IL-33 and ST2L mRNA expression in myocardial biopsies was observed (r = 0.35, p = 0.045).

Peripheral leukocyte expression of ST2, ST2L, and IL-33. Levels of soluble ST2 mRNA in peripheral leukocytes were similar in all groups (control: 2.0 ± 1.4 AU; AS: 4.3 ± 3.6 AU; CCM: 4.2 ± 2.8 AU; p = NS) as were levels of ST2L (control: 20.1 ± 15.9 AU; AS: 23.8 ± 17 AU; CCM: 16.0 ± 14 AU; p = NS) and IL-33 (control: 0.94 ± 0.84 AU; AS: 1.13 ± 1.1 AU; CCM: 0.63 ± 0.77 AU; p = NS). However, a strong correlation was noted between IL-33 and ST2L mRNA in leukocytes over the range of all values (r = 0.996; $p < 0.001$) (Fig. 7).

Discussion

The present study shows that serum ST2 protein is elevated in patients with chronic LV pressure overload due to AS as well as in patients with congestive heart failure. The ST2 positively correlated with NT-proBNP and the inflammatory marker CRP. The ST2 levels were proportionally related to the extent of diastolic load. A dominant role of diastolic load in relationship to ST2 levels is further supported by findings in a subgroup of patients with normal systolic function and isolated diastolic failure. In this group, ST2 levels were increased as were NT-proBNP levels. Thus, ST2 induction appears to be sensitive primarily to diastolic load. However, it is interesting that unlike BNP induction (19,23), ST2 production did not show a significant relationship with systolic wall stress.

The source of serum ST2 in cardiovascular disease was presumed to be myocardial following in vitro data showing load induction of ST2 mRNA in neonatal rat cardiac myocytes (24). We provide direct evidence that the adult, human myocardium is not the source of increased serum ST2 in pressure overload hypertrophy and congestive cardiomyopathy. First, it is well established that ST2 is transcriptionally regulated (mRNA induction) (7,8), yet

Table 2 Clinical and Hemodynamic Parameters of Patients With Gene Expression Analysis

	Control (n = 9)	AS (n = 12)	CCM (n = 17)
Age, yrs	61 ± 13	71 ± 9	58 ± 16
Female/male	1/8	6/6	4/13
NYHA functional class, I/II/III/IV, %	100/0/0/0	25/67/8/0	6/42/35/17
Acute heart failure	NA	2 (12%)	9 (52%)
Coronary artery disease	6 (66%)	3 (25%)	3 (17%)
Beta-blockers	4 (44%)	1 (8%)	10 (57%)
ACE/AT_1 blockers	4 (44%)	5 (42%)	17 (100%)
Diuretics	2 (22%)	5 (42%)	10 (57%)
LV mass index, g/m^2	52 ± 18	117 ± 40*	120 ± 25*
EDVI, ml	77 ± 14	83 ± 25	145 ± 58†‡
LV ejection fraction, %	72 ± 11	67 ± 18	24 ± 11†§
LV developed pressure, mm Hg	117 ± 26	178 ± 46‖	90 ± 25§‖
LV EDP, mm Hg	13 ± 3	17 ± 6*	21 ± 7†
Diastolic wall stress, kdyn/cm^3	18 ± 9	19 ± 9	38 ± 15†§
Systolic wall stress, kdyn/cm^3	70 ± 25	69 ± 32	134 ± 27§‖

Values are mean ± SD. Data were analyzed by analysis of variance followed by post-hoc analysis. *$p < 0.01$ versus control; †$p < 0.001$ versus control; ‡$p < 0.001$ versus AS; §$p < 0.01$ versus AS; ‖$p < 0.05$ versus control.
Abbreviations as in Table 1.

ST2 mRNA levels were not significantly increased in myocardial biopsies. This is in contrast with BNP expression, which was increased in the same RNA pool of LV samples reflecting increased levels of serum NT-proBNP in the same patients. Second, direct measurements of the BNP and ST2 protein concentration in coronary sinus and arterial blood samples demonstrated a transmyocardial gradient (production) of BNP, but no transmyocardial gradient of ST2. These surprising findings prompted us to examine ST2 protein production in extra-myocardial, nonmyocyte cell types. We present the novel findings that human venous and arterial endothelial cells secrete ST2 protein. The ST2 protein synthesis and release was rapid, peaked within 6 h, and was blocked by brefeldin, which blocks intracellular protein transport (25), confirming that ST2 exits endothelial cells through a secretion pathway responsive to inflammatory signals. This finding, together with the relationship between serum ST2 and indexes of diastolic load, suggests that the vascular endothelium, sensing hemodynamic and inflammatory status, is a potential source of elevated serum ST2 levels in hemodynamic overload and heart failure.

In this regard, it is noteworthy that ST2 levels failed to provide prognostic information in patients with acute coronary syndrome (26). Interestingly, those authors noticed the highest serum ST2 levels in chronic alcoholics, patients with systemic sepsis, and concomitant lung diseases, consistent with studies reporting elevated serum ST2 in pulmonary diseases (13,27). Serum ST2 is also elevated in patients with dengue virus infection (28), sepsis and trauma (29), and in cerebrospinal fluid following subarachnoid hemorrhage (30). The broad specificity of ST2 induction corroborates its nonmyocardial production and may explain why ST2 performs well as an independent biomarker in multimarker studies, providing information that is distinct from BNP in cardiovascular disease.

In addition to a cardiovascular biomarker, ST2/ST2L signaling is likely to be important in cardiovascular disease modulation. At the cellular level, soluble ST2 binds to macrophages and down-regulates proinflammatory cytokines, IL-1, IL-6, and tumor necrosis factor-alpha (14,15). Soluble ST2 can bind to IL-33, the ST2L ligand, to diminish mitogen-activated protein kinase and nuclear factor kappa B signaling (13). Membrane-anchored ST2L also inhibits IL-1 receptor and innate

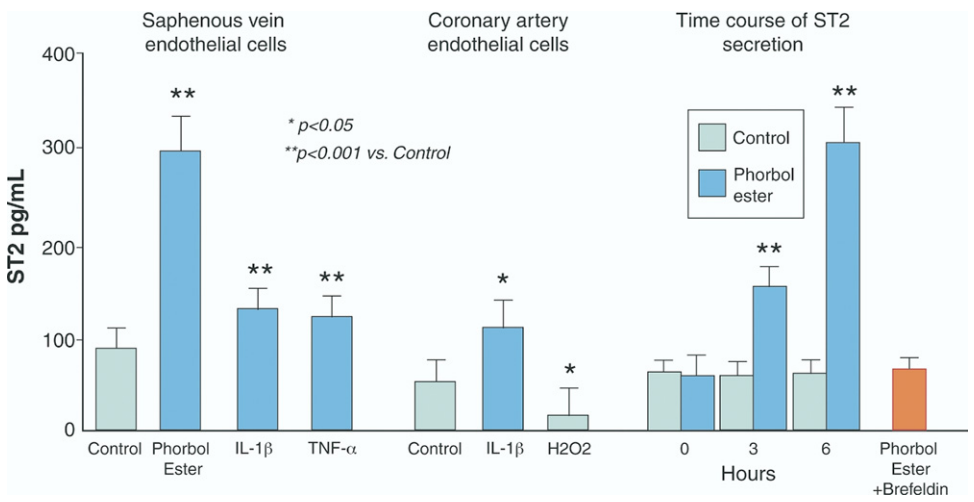

Figure 5 ST2 Protein Secretion by Human Venous and Arterial Endothelial Cells

Phorbol ester, IL-1-beta, and TNF-alpha induced ST2 secretion in venous endothelial cells **(left)**. IL-1-beta induced secretion but H_2O_2 blocked secretion in arterial endothelial cells **(middle)**. Time course shows ST2 is rapidly synthesized and secreted with significantly elevated levels at 3 h and reaches peak values at 6 h **(right)**. Brefeldin A blocked ST2 secretion **(right)**. IL = interleukin; TNF = tumor necrosis factor.

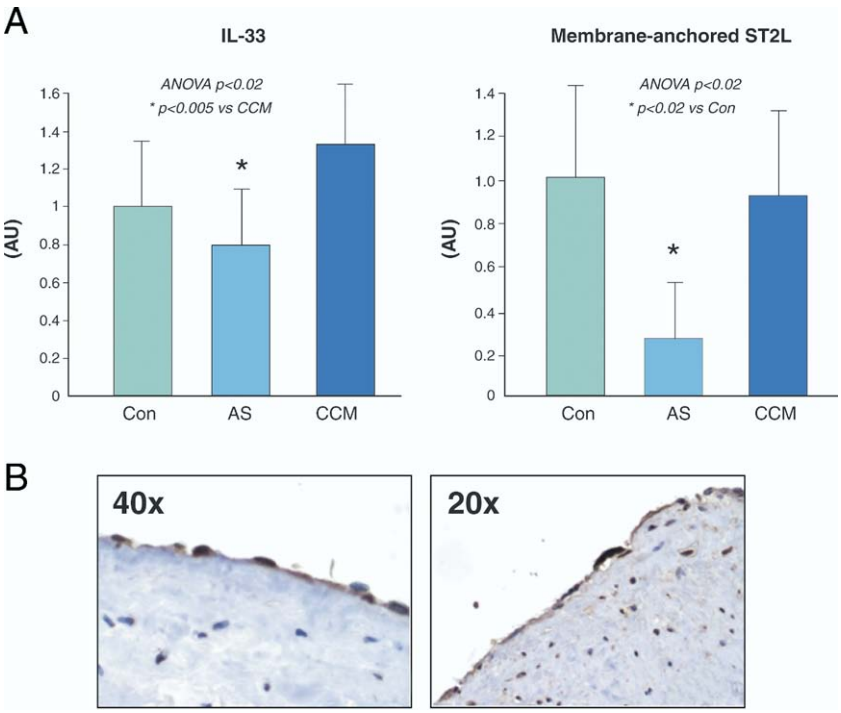

Figure 6 IL-33 and ST2 mRNA Levels in Myocardial Biopsies

(A) The IL-33 mRNA levels were decreased in AS hearts compared with the levels in CCM hearts. The ST2L mRNA levels were significantly decreased in AS hearts compared with the levels in control hearts. The ST2L levels were similar in CCM hearts compared with the levels in control hearts. **(B)** The IL-33 protein was expressed in human coronary artery endothelium (**brown** staining). ANOVA = analysis of variance; CON = control; other abbreviations as in Figures 1, 4, and 5.

immunity Toll-like receptor 4 signaling (11,12,31). Hence, both ST2L and soluble ST2 negatively regulate IL-1 receptor and Toll-like receptor 4 signaling (12,32). We present the novel findings that ST2L and IL-33 mRNA are expressed in normal and diseased human myocardium and both are modestly but significantly decreased in patients with AS. The mechanism is unknown, although it is recognized that soluble versus membrane-anchored ST2L are regulated separately (7,8). In addition, we found that IL-33 protein is expressed in endothelial cells within coronary arteries suggesting that IL-33 may have a similar function in vascular endothelium that may be regulated by soluble ST2. We report a remarkable pronounced correlation between ST2L and IL-33 levels in peripheral blood leukocytes, suggesting that they are highly coregulated in these cells. Further studies are needed to determine the biological relevance of these observations.

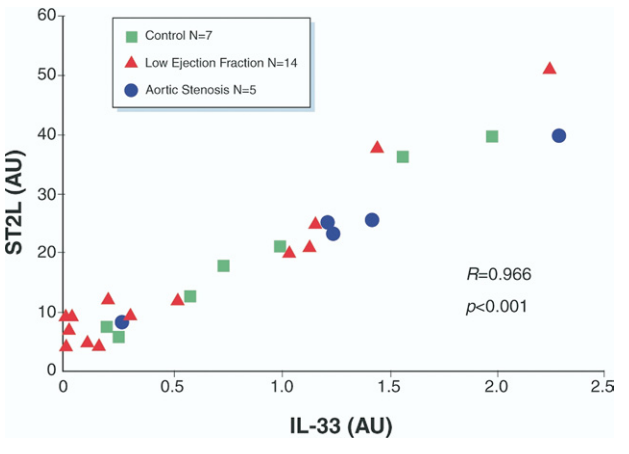

Figure 7 Correlation Between ST2L and IL-33 in Leukocytes

Relationship between ST2L and IL-33 mRNA levels in leukocytes from control, AS, and CCM patients. Abbreviations as in Figures 1, 4, and 5.

Study limitations. The present study is limited to patients with chronic AS or heart failure. It would be interesting to interrogate myocardial production of ST2 protein in acute myocardial infarction following which serum ST2 levels are also increased. Although the lack of transmyocardial ST2 gradient argues against cardiac production of serum ST2, ST2 protein production in human adult cardiac myocytes was not directly tested.

Conclusions

This study describes elevated serum ST2 in patients with pressure overload hypertrophy and congestive or diastolic

heart failure. Our data support diastolic load as a predominant hemodynamic factor that contributes to ST2 production in heart disease. Note that our data indicate that overloaded myocardium is not a major source of increased serum ST2. We showed that endothelial cells possess a functional ST2 secretory system in vitro, providing proof-of-concept for ST2 production by the vascular endothelium in vivo, which can be investigated in future studies.

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Key Words: interleukins ■ ST2 ■ hypertrophy ■ heart failure ■ natriuretic peptides ■ endothelium-derived factors.