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RESEARCH ARTICLE

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Reference interval and the role of soluble suppression of tumorigenicity 2 (sST2) in subclinical cardiac dysfunction at health checkups

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Abstract

Background: Soluble ST2 (sST2) is known to predict adverse outcomes and death in individuals with established heart failure. However, the role of sST2 testing in the general population has not been established. The aims of this study were to determine the reference interval (RI) and the clinical utility of sST2 in subclinical cardiac dysfunction in general population.

Methods: This cross-sectional study consecutively selected 41,806 general subjects at health checkups who underwent echocardiography and sST2 testing at 16 health promotion centers in 13 Korean cities. The reference subjects were obtained among those with normal findings in echocardiography. Sex-specific RIs were established according to the CLSI C28-A3 guidelines. sST2 was measured using immunoassay with the Presage ST2 assay (Critical Diagnostics).

Results: In the general subjects, age, sex, BMI, systolic blood pressure, blood glucose, creatinine, liver function, and triglycerides were associated with the sST2 levels. The RI for sST2 was higher in males (≤49.6 ng/mL, 95% CI = 48.5-51.5) than in females (≤44.5 ng/mL, 95% CI = 43.5-45.6) and higher in subjects aged < 40 years than ≥ 40 years in both sexes. The sST2 levels were 29.1 ± 10.7 (mean ± SD) and 29.1 ± 14.4 ng/mL in the groups with normal cardiac function and subclinical cardiac dysfunction, respectively. The sST2 level was not associated with subclinical cardiac dysfunction (odd ratio = 1.002, $P = .13$).

Conclusions: RIs obtained from a large and echocardiography-proven healthy community-based sample are presented. Subclinical cardiac dysfunction was associated with older age, male sex, and metabolic factors but not with the sST2 level.

KEYWORDS

cardiac dysfunction, echocardiography, reference interval, soluble ST2

1 | INTRODUCTION

The wide range of cardiovascular disorders that result in an impaired ability of the heart to fill or to pump blood may eventually lead to the clinical syndrome of heart failure (HF). The incidence of cardiovascular diseases is increasing (including in younger subjects) due to changes in lifestyles and dietary patterns, and there have also been increases in the rates of progression to HF.[1] Patients with HF often present with non-specific signs and symptoms. Moreover, systolic dysfunction is frequently present in community-dwelling individuals without recognized symptoms of HF.[2] Accordingly, biomarkers for identifying the presence of HF before it is fully developed are needed in community-dwelling individuals.

Suppression of tumorigenicity 2 (ST2) is the receptor for interleukin-33 (IL-33), which is an IL-1-like cytokine that is secreted by cardiac cells in response to myocardial stress. ST2 has two main isoforms: (a) transmembrane or cellular (ST2L) and (b) soluble or circulating (sST2). Interactions between IL-33 and ST2L are cardioprotective since they reduce myocardial fibrosis, cardiomyocyte hypertrophy, and apoptosis. However, when the soluble receptor is shed in cases of cardiac distress, sST2 binds to IL-33 in competition with ST2L, blocking the IL-33/ST2L system and eliminating the cardioprotective effects. Therefore, sST2 is considered a decoy receptor.[3-5]

Increased sST2 levels are clinically predictive of adverse outcomes in acute myocardial infarction,[6] acute decompensated HF,[7] and chronic HF.[8] The sST2 level has an impact in the prognosis and risk stratification of patients with established HF. However, the role of sST2 testing in the general population without apparent cardiac symptoms has not been established. Meanwhile, the sST2 level is also increased in several non-cardiac conditions such as asthma, pulmonary fibrosis, rheumatoid arthritis, collagen vascular disease, sepsis, trauma, malignancy, and helminthic infections.[9]

The aims of this study were to determine (a) the factors associated with sST2 and the reference interval (RI) in echocardiography-proven healthy reference subjects and (b) the utility of sST2 in preventive strategies at a population level through screening the sST2 level at health checkups.

2 | MATERIALS AND METHODS

This study was approved by the Institutional Review Board of the Korea Association of Health Promotion (approval no. 130750-202005-HR-008).

2.1 | Study subjects

This cross-sectional retrospective study consecutively selected subjects at health checkups who underwent echocardiography and sST2 testing at 16 health promotion centers in Korea between January 2018 and September 2019. The self-reported personal medical history, subjective symptoms and signs, and lifestyle information were obtained from all participants at time of health checkups. Their medical records were also reviewed. Individuals with non-cardiac conditions such as asthma, pulmonary fibrosis, rheumatoid arthritis, collagen vascular disease, sepsis, trauma, and malignancy were excluded through evaluation of medical records and personal medical history. Subjects who had echocardiography-detected HF, atrial fibrillation, or acute myocardial infarct or who were younger than 19 years were not eligible for inclusion. The general subjects comprised 41,806 individuals. Echocarciography-normal individuals defined as preserved left ventricular systolic function (LVEF > 50%) and those who do not have any abnormal findings in echocardiography, such as valvular insufficiency, diastolic dysfunction, atrial fibrillation, heart failure, pulmonary hypertension, or atrial enlargement. The 7090 reference subjects had normal findings in echocardiography and did not have diabetes, hypertension, obesity (body mass index [BMI] > 25 kg/m^2), renal disease (eGFR < 60 mL/min/1.73 m^2 or creatinine > 1.4 mg/dL), or hepatic dysfunction. Subclinical cardiac dysfunction in the general subjects was defined as any abnormal findings in echocardiography, such as a mild-to-moderate degree of valvular insufficiency, diastolic dysfunction, or atrial enlargement (Figure 1).

2.2 | Laboratory measurements and Echocardiography

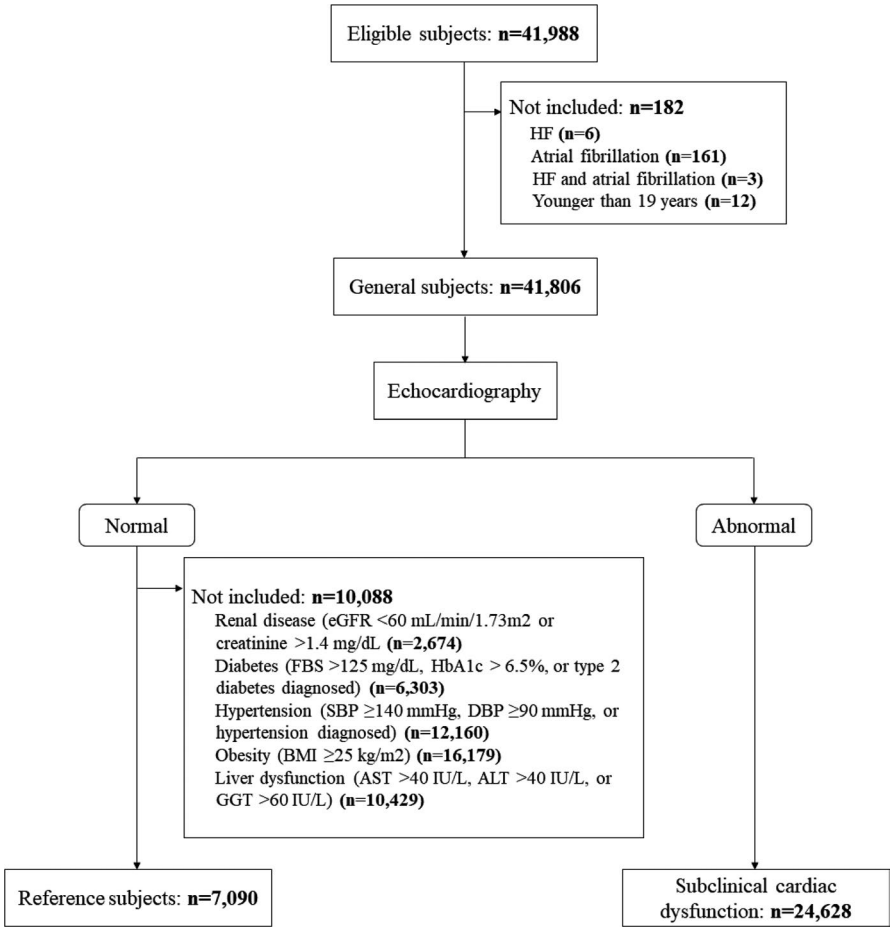
2.2.1 | Echocardiography

The echocardiographic investigations were carried out using a Philips/Hewlett-Packard Sono 5500 ultrasound device (Philips Ultrasound). M-mode, two-dimensional, and hemodynamic Doppler images were acquired using a standardized protocol with a 3.5-MHz transducer. The left ventricular ejection fraction was calculated using the modified Simpson method.[10]

2.2.2 | Laboratory measurements

Venous blood was drawn after an overnight fast for health checkups that included the complete blood count (CBC), biochemical measurements, and the sST2 level. The CBC and biochemical parameters were measured using the Sysmex XE-2100D analyzer (Sysmex) and the Hitachi 7600 analyzer (Hitach), respectively. Metabolic syndrome was defined in accordance with the National Cholesterol Education Program Adult Treatment Panel III.[11] The serum sST2 level was measured using a quantitative sandwich monoclonal ELISA in a 96-well plate format with the Presage ST2 assay (Critical Diagnostics). Presage ST2 ELISA was measured on GEMINI COMBO (Stratec Biomedical). Serum is loaded into appropriate wells in the anti-ST2 antibody-coated plate and incubated at room temperature (18-25°C) for 60 minutes. Following a series of steps where reagents are washed from plate, and additional reagents are added and subsequently washed out, the analyte is finally detected by addition of a colorimetric reagent, and the resulting signal is measured spectroscopically at 450 nm. The lower limit of detection

FIGURE 1 Study flow diagram



of the assay is 1.8 ng/mL. The assay has a within-run CV of 6.5% and a total CV of 9.1% at a mean concentration of 16.9 ng/mL, within-run CV of 3.4% and a total CV of 5.5% at a mean concentration of 33.1 ng/mL, and within-run CV of 2.4% and a total CV of 4.8% at a mean concentration of 159.1 ng/mL.

2.3 | Statistical analysis and calculation of RIs

Statistical analyses were performed using SAS version 9.4 (SAS Institute). Multivariate (adjusted) regression analysis was performed to determine the variables affecting an increased sST2 level. Q-Q plots were used to confirm normality of residuals, and Durbin-Watson D statistics was used to check non-autocorrelation. The variables considered in the analysis included age, sex, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose (FBS), triglyceride (TG) level, liver function tests, and creatinine.

The RI for sST2 was calculated for the 7,090 reference subjects. The sST2 levels were analyzed according to the CLSI C28-A3 guidelines.[12] Scatter and distribution plots were used to inspect the data. The data in each partition were transformed using the Box-Cox transformation method. RIs for all of the partitions were calculated using non-parametric methods. To analyze the variations in sST2 according to age and sex, the levels of sST2 for each sex were grouped into the following age groups: <30, 30-39, 40-49, 50-59, 60-69, and ≥70 years. The Wilcoxon rank-sum test was used to compare the levels of sST2 according to age groups.

Multivariate (adjusted) logistic regression analysis was performed to evaluate the association between the sST2 level and subclinical cardiac dysfunction. A P value of $< .05$ was considered statistically significant.

3 | RESULTS

3.1 | Demographic and clinical characteristics of the study subjects

Table 1 presents the characteristics of the general and reference subjects. The male and female general subjects were aged 56.7 ± 10.8 and 59.3 ± 10.0 years, respectively, and their serum levels of sST2 were 30.8 ± 13.7 and 27.5 ± 12.1 ng/mL.

3.2 | Variables associated with soluble ST2 in general subjects

Table 2 presents the variables associated with soluble ST2 in the general subjects. In these subjects, being male, and having higher SBP, FBS, serum creatinine, aspartate aminotransferase (AST), and gamma-glutamyl transpeptidase (GGT) levels were associated with

TABLE 1 Characteristics of the study subjects

	General subjects		Reference subjects	
	Males	Females	Males	Females
	(N = 20 382)	(N = 21 424)	(N = 2653)	(N = 4437)
Age, y	56.7 ± 10.8	59.3 ± 10.0	52.8 ± 11.3	52.8 ± 10.3
BMI, kg/m^2	25.0 ± 3.1	24.2 ± 3.3	22.5 ± 1.7	21.8 ± 1.9
WC, cm	87.1 ± 8.2	80.5 ± 9.0	80.4 ± 5.9	74.3 ± 6.4
SBP, mmHg	121.5 ± 13.6	119.6 ± 15.0	115.8 ± 11.0	111.8 ± 11.7
DBP, mmHg	76.6 ± 9.2	73.7 ± 9.0	72.9 ± 7.7	69.7 ± 7.5
sST2, ng/mL	30.8 ± 13.7	27.5 ± 12.1	30.5 ± 10.9	27.4 ± 9.8
AST, IU/L	32.9 ± 20.4	28.5 ± 18.1	25.5 ± 5.6	24.1 ± 5.5
ALT, IU/L	32.8 ± 23.4	23.9 ± 20.6	21.7 ± 6.9	17.9 ± 6.3
GGT, IU/L	57.7 ± 81.1	28.2 ± 40.0	28.5 ± 11.3	19.6 ± 8.7
TC, mg/dL	200.7 ± 40.7	206.4 ± 40.7	204.9 ± 36.1	210.5 ± 37.2
TG, mg/dL	147.2 ± 114.7	109.4 ± 69.8	112.4 ± 81.0	90.4 ± 64.0
HDL-C, mg/dL	50.6 ± 12.1	59.0 ± 13.6	53.6 ± 12.1	63.3 ± 14.0
LDL-C, mg/dL	121.9 ± 37.0	124.8 ± 37.5	128.2 ± 32.8	127.5 ± 33.8
FBS, mg/dL	106.4 ± 25.5	100.7 ± 20.8	94.9 ± 10.2	92.8 ± 9.6
$HbA1_C$, %	6.0 ± 1.0	5.9 ± 0.8	5.6 ± 0.3	5.5 ± 0.3
Creatinine, mg/dL	1.1 ± 0.2	0.8 ± 0.1	1.1 ± 0.1	0.8 ± 0.1
e-GFR, mL/min/1.73m^2	77.7 ± 13.4	77.7 ± 14.1	80.1 ± 11.9	81.2 ± 13.4
Metabolic syndrome	4972 (27.0)	3685 (19.9)	105 (4.8)	131 (3.5)

Note: Data are mean ± SD or N (%) values.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure.

FBS, fasting blood sugar; GGT, gamma-glutamyl transpeptidase; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol;

SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

TABLE 2 Variables associated with sST2 in the general subjects

	Coefficient	SE	Standard coefficient	*P*
Age, years	−0.037	0.006	−0.035	<.001
Sex(reference: female)	2.613	0.15	0.121	<.001
BMI, kg/m^2	−0.073	0.02	−0.022	.000
SBP, mm Hg	0.018	0.006	0.024	.004
DBP, mm Hg	−0.004	0.009	−0.004	.642
FBS, mg/dL	0.016	0.003	0.035	<.001
Creatinine, mg/dL	0.970	0.354	0.018	.006
AST, IU/L	0.075	0.005	0.127	<.001
ALT, IU/L	−0.020	0.004	−0.040	<.001
GGT, IU/L	0.010	0.001	0.061	<.001
TC, mg/dL	−0.002	0.001	−0.009	.098
TG, mg/dL	−0.004	0.001	−0.033	<.001
Metabolic syndrome	0.163	0.164	0.006	.321

Note: Durbin-Watson $D = 1.874$.

Adjusted $R^2 = .0474$.

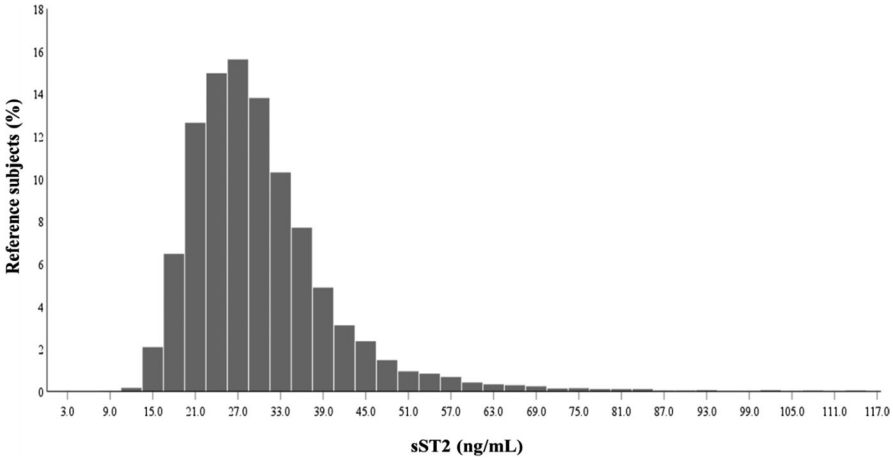


FIGURE 2 Distribution of sST2 in reference subjects

a higher serum sST2 levels, as were younger age and lower BMI, alanine aminotransferase (ALT), and TG levels ($P < .01$).

3.3 | Reference intervals

A non-normal distribution of sST2 values was found in the reference subjects by visual inspection and the Shapiro-Wilk test ($P < .05$) (Figure 2). To analyze the variations in sST2 according to age and sex, box plots of sST2 values were depicted in both sexes according to the following age groups: <30, 30-39, 40-49, 50-59, 60-69, and ≥70 years. The levels of sST2 were higher in subjects aged 30-39 years than in those aged 40-49 years in both sexes ($P < .01$) (Figure 3). The sex-specific and age-specific (<40 and ≥40 years) RIs for sST2 are presented in Table 3. The one-side upper 95th percentiles of the sST2 level were 49.6 ng/mL (95% confidence interval [CI] = 48.5-51.5) and 44.5 ng/mL (95% CI = 43.5-45.6) in males and females, respectively. The sST2 levels were generally higher in males than females.

3.4 | Logistic regression of the association between sST2 and cardiac dysfunction in the general subjects

Subclinical cardiac dysfunction was detected using echocardiography in 24,628 (58.9%) of the general subjects. These subjects were older and had higher BMI, blood pressure, FBS, HbA1c, and TG levels and a lower high-density lipoprotein cholesterol (HDL-C) levels. However, the sST2 level did not differ significantly between subjects with and without subclinical cardiac dysfunction. While older age, female sex, and higher BMI, HbA1c, and TG levels were associated with subclinical cardiac dysfunction ($P < .001$), this was not associated with the sST2 level in multiple logistic regression analysis ($P = .130$) (Table 4).

4 | DISCUSSION

This study determined that sex, age, serum creatinine, AST, and GGT were associated with the sST2 level. RIs were established in the reference subjects who had echocardiography-proven normal cardiac function. We have further demonstrated that subclinical cardiac dysfunction is associated with older age, female sex, and higher BMI, HbA1c level, and TG levels but not with a higher sST2 level.

The biological and clinical roles of sST2 have been widely studied in patients with existing cardiovascular disease,[13,14] but far less in apparently healthy general populations. As the use of sST2 increases, an understanding of its roles in this general population is needed along with the establishment of RIs. This study found that sex was a strong factor affecting the sST2 level, and the RI was also lower in females than in males. This finding was consistent with

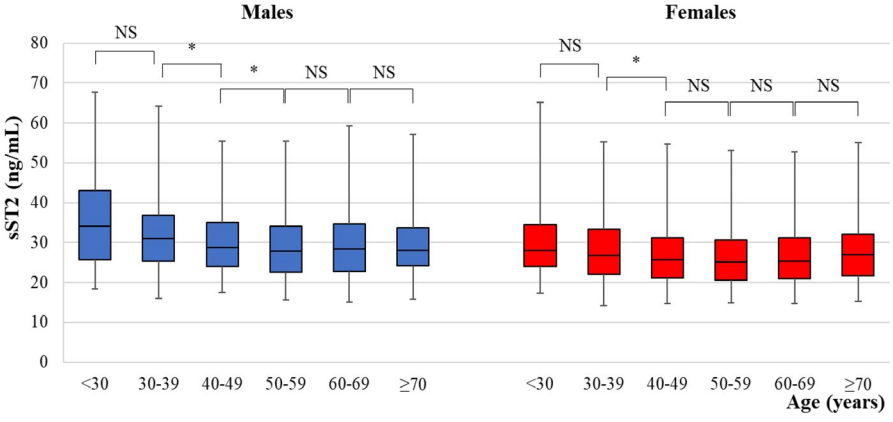


FIGURE 3 Box plots of sST2 values according to age and sex in the reference subjects. Box limits and horizontal lines within boxes represent interquartile ranges and medians, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively. The significance of the difference in median values between each age group for each sex was determined using the Wilcoxon rank-sum test: $^{*}P < .01$; NS, not significant

TABLE 3 Reference limits for sST2 according to sex and age

Variables	N	Percentile (95% CI)												
		2.5th	95% CI	25th	95% CI	50th	95% CI	75th	95% CI	95th	95% CI	97.5th	95% CI	99th	95% CI
Total															
<40 y	822	15.2	14.2, 16.2	23.6	22.8, 24.2	28.7	28.1, 29.6	35.5	34.6, 36.8	**50.8**	**47.7, 55.2**	61.4	54.2, 65.5	68.0	65.1, 82.0
≥40 y	6,268	15.2	15.0, 15.4	21.7	21.4, 21.9	26.4	26.2, 26.7	32.4	32.0, 32.7	**46.1**	**45.1, 47.5**	54.4	53.0, 56.2	65.4	62.4, 69.4
Male															
<40 y	350	16.4	15.6, 18.4	25.4	24.3, 26.4	31.4	30.1, 32.9	38.4	36.2, 40.6	**53.0**	**49.5, 64.1**	64.3	54.2, 75.3	75.3	65.5, 82
≥40 y	2,303	15.9	15.3, 16.3	23.3	22.9, 23.6	28.3	27.9, 28.7	34.4	33.8, 35.1	**49.0**	**47.6, 50.9**	56.6	54.3, 60.3	67.9	63.5, 77.9
Female															
<40 y	472	14.2	13.2, 15.8	22.4	21.9, 23.3	27.3	26.6, 28.2	33.9	32.4, 35.1	**47.2**	**44.5, 54.7**	55.6	50.8, 66.5	66.5	61.4, 83.3
≥40 y	3,965	15.0	14.5, 15.2	20.9	20.7, 21.2	25.3	25.1, 25.7	31.0	30.6, 31.3	**44.0**	**42.7, 45.4**	53.1	51.5, 54.8	63.7	60.0, 68.8

Note: Age partitionings of reference values were significant using Harris and Boyd's method after Box-Cox transformation.
Bold values to emphasize the 95th percentile.

the Framingham Heart Study[15] finding considerable differences between male and female subjects. The reason and possible consequences of these sex-specific differences are that sST2 synthesis or secretion might be partially under androgen control. Framingham Heart Study showed that the sST2 level was lowest in females taking estrogen when the investigators stratified the analyses according to estrogen-replacement status.

An association of sST2 with FBS was found in our study. Although the underlying mechanisms are not known, animal studies have shown that sST2 signaling may be important in modulating the autoimmune effects on the pancreas associated with diabetes.[16] Miller et al[17] also reported that the sST2 level was strongly associated with markers of diabetes, including triglyceride, liver function, and blood glucose. We also found that the sST2 level was associated with AST and GGT. It was found previously[18] that the sST2 level was significantly higher in patients with chronic hepatitis C than in healthy controls. Roth et al[19] reported that the serum sST2 level was higher in patients with acute liver failure than in patients with chronic liver failure and healthy controls, which suggests that sST2 is an early biomarker of liver injury.

We determined the RIs of sST2 using a well-characterized reference population. Our large sample of reference subjects was demonstrated echocardiographically to be healthy. In addition to echocardiography, the clinical history and laboratory tests allowed us to avoid including individuals with subclinical cardiovascular dysfunction in the group of reference subjects. Our RIs were similar to those of Coglianese et al[15] but higher than those reported by Dieplinger et al.[20] The association between the sST2 level and age seems to be controversial among studies. Some previous studies[21,22] showed that links between the sST2 level, age, and male sex, whereas Lu et al[23] did not find any association of sST2 with age. In our study, the RIs of sST2 were higher in the subjects aged <40 years than in those aged ≥40 years in both sexes.

We attempted to evaluate the utility of sST2 in screening subclinical cardiac dysfunction in terms of preventive strategies at a population level. The subjects with subclinical cardiac dysfunction were older and had a higher BMI, blood pressure, FBS, and triglyceride levels, and a lower HDL-C level. However, the sST2 level did not differ significantly between subjects with and without subclinical cardiac dysfunction. To investigate this relationship further, we created a model that included covariates that are associated with cardiac function. We observed associations of subclinical cardiac dysfunction with cardiovascular risk factors such as higher BMI, DBP, HbA1c, triglycerides, and low-density lipoprotein cholesterol levels, but not with the sST2 level. The ST2 system is known to be induced when cardiac fibroblasts or cardiomyocytes are subjected to mechanical stresses[24] and appear to be intimately involved in cardiac remodeling and fibrosis in HF. However, ST2 was initially described in the context of cell proliferation, inflammatory states, and autoimmune disease.[25] The sST2 levels have overlapped in diverse clinical conditions, including allergy, autoimmune disease, cancer, diabetes, inflammation, and cardiac diseases.[26] These observations mean that sST2 lacks the

TABLE 4 Logistic regression of the association between sST2 and cardiac dysfunction in the general subject

	Cardiac dysfunction			Multiple logistic regression		
Variables	Absent (N = 17 178)	Present (N = 24 628)	*P*	OR	(95% CI)	*P*
Age, y	54.5 ± 10.6	60.5 ± 9.7	<.001	1.066	(1.062, 1.069)	<.001
Sex,						
Females	8562 (40.0)	12 862 (60.0)	<.001		1	
Males	8616 (42.3)	11 766 (57.7)		0.931	(0.878, 0.987)	.017
BMI, kg/m^2	24.3 ± 3.3	24.8 ± 3.2	<.001	1.041	(1.032, 1.051)	<.001
SBP, mm Hg	118.4 ± 13.5	122.0 ± 14.8	<.001	0.993	(0.990, 0.996)	<.001
DBP, mm Hg	73.8 ± 8.8	76.0 ± 9.4	<.001	1.031	(1.026, 1.035)	<.001
FBS, mg/dL	102.1 ± 22.9	104.4 ± 23.7	<.001	0.992	(0.990, 0.994)	<.001
HbA1c, %	5.8 ± 0.8	6.0 ± 0.9	<.001	1.326	(1.258, 1.398)	<.001
TC, mg/dL	206.5 ± 40.2	201.5 ± 41.1	<.001	0.987	(0.984, 0.990)	<.001
TG, mg/dL	127.0 ± 97.7	129.2 ± 96.1	.034	1.002	(1.002, 1.003)	<.001
HDL-C, mg/dL	55.8 ± 13.8	54.1 ± 13.2	<.001	1.009	(1.005, 1.013)	<.001
LDL-C, mg/dL	125.2 ± 36.5	120.2 ± 37.8	<.001	1.012	(1.008, 1.015)	<.001
sST2, ng/mL	29.1± 10.7	29.1 ± 14.4	.702	1.002	(1.000, 1.004)	.130

Data are mean ± SD or N (%) values, except where indicated otherwise.
Multivariate models included age, sex, BMI, blood pressure, FBS, HbA1c, blood lipid, and sST2.

specificity needed for a diagnostic test, and hence, it might not be a useful diagnostic marker for HF. Moreover, our general subjects with subclinical cardiac dysfunction were apparently normal with a mild-to-moderate degree of cardiac dysfunction. Our findings suggest that sST2 is not useful for detecting mild-to-moderate degree of heart failure evolution.

Our study has some limitations. First, it employed a cross-sectional design to investigate the role of the serum sST2 level in screening subclinical cardiac dysfunction at health checkups, and so, future prospective studies are necessary to support the present findings. Second, the inhomogeneity of the subclinical cardiac dysfunction group in terms of underlying etiology might have prevented a meaningful analysis of the association between the potential biomarker and cardiac function. This means that careful interpretation of the present results is needed. Further research is required to investigate whether this biomarker can detect the subclinical cardiac dysfunction within a more narrowly defined etiology.

In conclusion, the RIs of sST2 obtained from a large and echocardiography-proven healthy community-based sample have been revealed in this study. Subclinical cardiac dysfunction was associated with older age, male sex and cardiometabolic factors but not with the sST2 level. This suggests sST2 will not be useful for screening subclinical cardiac dysfunction in primary healthcare units. Further studies are needed to explore the usefulness of this biomarker in determining the long-term outcomes in the general population.

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AUTHOR'S CONTRIBUTION

All of the authors participated in designing this study. SC performed data collection. SK undertook the statistical analyses. EN and SK analyzed and interpreted the data. EN wrote the first draft of the manuscript, which was reviewed by all of the other authors, who also provided further contributions and suggestions.

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