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

Eun-Hee Nah¹ | Seon Cho¹ | Suyoung Kim¹ | Han-Ik Cho²

¹Department of Laboratory Medicine, Health Promotion Research Institute, Korea Association of Health Promotion, Seoul, Korea
²MEDicheck LAB, Korea Association of Health Promotion, Cheongju, Korea

Correspondence
Eun-Hee Nah, Health Promotion Research Institute, Korea Association of Health Promotion, 396, Gonghang-daero, Gangseo-Gu, Seoul 07649, Korea.
Email: cellonah@hanmail.net; cellonah@kahp.or.kr

Funding information
This research received no specific grant from any funding agency in the public or commercial sectors.

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. 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. Accordingly, biomarkers for identifying the presence of HF before it is fully developed are needed in community-dwelling individuals.

Suppression of tumorogenicity 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.

Increased sST2 levels are clinically predictive of adverse outcomes in acute myocardial infarction, acute decompensated HF, and chronic HF. 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.

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. Echocardiography-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/m²), renal disease (eGFR < 60 mL/min/1.73 m² 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.

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 (Hitachi), respectively. Metabolic syndrome was defined in accordance with the National Cholesterol Education Program Adult Treatment Panel III. 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 spectrophotometrically at 450 nm. The lower limit of detection
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.

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 (N = 20382) | Males (N = 2653)    |
|                  | Females (N = 21424) |
|                  | Females (N = 4437)  |
| Age, y           | 56.7 ± 10.8        |
|                  | 59.3 ± 10.0        |
|                  | 52.8 ± 11.3        |
|                  | 52.8 ± 10.3        |
| BMI, kg/m²       | 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         |
| Creatinine, %    | 6.0 ± 1.0          |
|                  | 5.9 ± 0.8          |
|                  | 5.6 ± 0.3          |
|                  | 5.5 ± 0.3          |
| eGFR, mL/min/1.73m² | 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/m²           | -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 $R^2 = .0474$. 

---

### Notes
- Table 1: Characteristics of the study subjects
- Table 2: Variables associated with sST2 in the general subjects
a higher 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, 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 2 Distribution of sST2 in reference subjects](image)

![FIGURE 3 Box plots of sST2 values according to age and sex in the reference subjects](image)
### TABLE 3 Reference limits for sST2 according to sex and age

| Variables | N   | 2.5th 95% CI | 25th 95% CI | 50th 95% CI | 75th 95% CI | 95th 95% CI | 97.5th 95% CI | 99th 95% CI |
|-----------|-----|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Total     | 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 | 45.7, 44.8, 46.7 | 47.7, 46.8, 47.8 | 50.8, 49.9, 51.0 |
| <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, 45.7 | 45.1, 44.7, 45.3 | 46.1, 45.7, 46.3 |
| ≥40 y     | 23,610 | 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, 45.7 | 45.1, 44.7, 45.3 | 46.1, 45.7, 46.3 |

**Male**

| Variables | N   | 2.5th 95% CI | 25th 95% CI | 50th 95% CI | 75th 95% CI | 95th 95% CI | 97.5th 95% CI | 99th 95% CI |
|-----------|-----|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| <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 | 54.5, 53.0, 64.6 | 64.1, 58.6, 75.6 |
| ≥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**

| Variables | N   | 2.5th 95% CI | 25th 95% CI | 50th 95% CI | 75th 95% CI | 95th 95% CI | 97.5th 95% CI | 99th 95% CI |
|-----------|-----|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| <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.

---

An association of sST2 with FBS was found in our study. Additionally, we found that sST2 levels were significantly higher in patients with chronic hepatitis C than in healthy controls. 

Roth et al. also demonstrated that sST2 levels were higher in female subjects than in male subjects. 

However, sST2 was initially described in the context of cell proliferation, inflammatory states, and autoimmune diseases. sST2 levels have overlapped in diverse clinical conditions, including all cardiac diseases. It is known to be induced when cardiac fibroblasts or cardiomyocytes are subjected to mechanical stresses and appear to be intimately involved in cardiac remodeling and fibrosis in heart failure. 

The association between sST2 and inflammatory states, and autoimmune diseases was significantly higher in patients with chronic hepatitis C than in healthy controls. 

Moreover, sST2 was initially described in the context of cell proliferation, inflammatory states, and autoimmune diseases. It is known to be induced when cardiac fibroblasts or cardiomyocytes are subjected to mechanical stresses and appear to be intimately involved in cardiac remodeling and fibrosis in heart failure. 

However, ST2 was initially described in the context of cell proliferation, inflammatory states, and autoimmune diseases. It is known to be induced when cardiac fibroblasts or cardiomyocytes are subjected to mechanical stresses and appear to be intimately involved in cardiac remodeling and fibrosis in heart failure. 

However, ST2 was initially described in the context of cell proliferation, inflammatory states, and autoimmune diseases. It is known to be induced when cardiac fibroblasts or cardiomyocytes are subjected to mechanical stresses and appear to be intimately involved in cardiac remodeling and fibrosis in heart failure.
Multiple logistic regression

Further with older age, male sex and cardiometabolic factors but not with the ST2 level. This suggests ST2 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.

ACKNOWLEDGMENTS

The authors thank the Central Data Center at Korea Association of Health Promotion for collecting health information data.

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.

ORCID

Eun-Hee Nah https://orcid.org/0000-0003-0637-4364
Seon Cho https://orcid.org/0000-0002-6432-5897
Suyoung Kim https://orcid.org/0000-0003-0512-1189
Han-Ik Cho https://orcid.org/0000-0001-6075-7636

REFERENCES

1. Lloyd-Jones D, Adams RJ, Brown TM, et al. Heart disease and stroke statistics–2010 update: a report from the American Heart Association. Circulation. 2010;121(7):e46-e215.
2. Davies M, Hobbs F, Davis R, et al. Prevalence of left-ventricular systolic dysfunction and heart failure in the Echocardiographic Heart of England Screening study: a population based study. Lancet. 2001;358(9280):439-444.
3. Tominaga S. A putative protein of a growth specific cDNA from BALB/c-3T3 cells is highly similar to the extracellular portion of mouse interleukin 1 receptor. FEBS Lett. 1989;258(2):301-304.
4. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. Nat Rev Immunol. 2010;10(2):103-110.
5. Schmitz J, Owyang A, Oldham E, et al. 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(5):479-490.

| Variables | Cardiac dysfunction | Multiple logistic regression |
|-----------|---------------------|-----------------------------|
|           | Absent (N = 1718)   | 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 | 1.032 (0.987, 0.987) | .017 |
| Males     | 8616 (42.3)         | 11 766 (57.7)               | <.001 | 1.031 (1.026, 1.035) | <.001 |
| BMI, kg/m²| 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 ST2 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 ST2 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 ST2 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 ST2 level. This suggests ST2 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.
6. Sabatine MS, Morrow DA, Higgins Lj, et al. Complementary roles for biomarkers of biomechanical strain ST2 and N-terminal prohormone B-type natriuretic peptide in patients with ST-elevation myocardial infarction. *Circulation.* 2008;117(15):1936-1944.

7. Rehman SU, Muller T, Januzzi JL Jr. Characteristics of the novel interleukin family biomarker ST2 in patients with acute heart failure. *J Am Coll Cardiol.* 2008;52(18):1458-1465.

8. Latini R, Masson S, Anand IS, et al. Prognostic value of very low plasma concentrations of troponin T in patients with stable chronic heart failure. *Circulation.* 2007;116(11):1242-1249.

9. Mueller T, Jaffe AS. Soluble ST2—analytical considerations. *Am J Cardiol.* 2015;115(7):8B-21B.

10. Folland ED, Parisi AF, Moynihan PF, et al. Assessment of left ventricular ejection fraction and volumes by real-time, two-dimensional echocardiography. A comparison of cineangiographic and radionuclide techniques. *Circulation.* 1979;60(4):760-766.

11. Lipsy RJ. The National Cholesterol Education Program Adult Treatment Panel III guidelines. *J Manag Care Pharm.* 2003;9(1 Suppl):2-5.

12. Horowitz GL, Atlaie S, Boyd JC, et al. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline. C28-A3c. Wayne, PA: Clinical and Laboratory Standards Institute. 2010.

13. Weinberg EO, Shimo M, Hurwitz S, et al. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. *Circulation.* 2003;107(5):721-726.

14. Yu J, Oh PC, Kim M, et al. Improved early risk stratification of patients with ST-segment elevation myocardial infarction undergoing primary myocardial infarction undergoing primary percutaneous coronary intervention using a combination of serum soluble ST2 and NT-proBNP. *PloS One.* 2017;12(8):e0182829.

15. Coglianese EE, Larson MG, Vasan RS, et al. Distribution and clinical correlates of the interleukin receptor family member soluble ST2 in the Framingham Heart Study. *Clin Chem.* 2012;58(12):1673-1681.

16. Zdravkovic N, Shahin A, Arsenijevic N, et al. Regulatory T cells and ST2 signaling control diabetes induction with multiple low doses of streptozotocin. *Mol Immunol.* 2009;47(1):28-36.

17. Miller AM, Purves D, McConnachie A, et al. Soluble ST2 associates with diabetes but not established cardiovascular risk factors: a new inflammatory pathway of relevance to diabetes? *PloS One.* 2012;7(10):e47830.

18. Wang J, Zhao P, Guo H, et al. Serum IL-33 levels are associated with liver damage in patients with chronic hepatitis C. *Mediators Inflamm.* 2012;2012:819636.

19. Roth GA, Zimmerman M, Lubsczyk BA, et al. Up-regulation of interleukin 33 and soluble ST2 serum levels in liver failure. *J Surg Res.* 2010;163(2):e79-e83.

20. Dieplinger B, Januzzi JL Jr, Steinmair M, et al. Analytical and clinical evaluation of a novel high-sensitivity assay for measurement of soluble ST2 in human plasma—the Presage ST2 assay. *Clin Chim Acta.* 2009;409(1–2):33-40.

21. Dhillon OS, Narayan HK, Quinn PA, et al. Interleukin 33 and ST2 in non-ST-elevation myocardial infarction: comparison with Global Registry of Acute Coronary Events Risk Scoring and NT-proBNP. *Am Heart J.* 2011;161(6):1163-1170.

22. Kohl P, Bonaca MP, Kakkar R, et al. Role of ST2 in non-ST-elevation acute coronary syndrome in the MERLIN-TIMI 36 trial. *Clin Chem.* 2012;58(1):257-266.

23. Lu J, Snider JV, Grenache DG. Establishment of reference intervals for soluble ST2 from a United States population. *Clin Chim Acta.* 2010;411(21–22):1825-1826.

24. Weinberg EO, Shimo M, De Keulenaer GW, et al. Expression and regulation of ST2, an interleukin-1 receptor family member, in cardiomyocytes and myocardial infarction. *Circulation.* 2002;106(23):2961-2966.

25. Xu D, Chan WL, Leung BP, et al. Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. *J Exp Med.* 1998;187(5):787-794.

26. Pascual-Figal DA, Januzzi JL. The biology of ST2: the International ST2 Consensus Panel. *Am J Cardiol.* 2015;115(7 Suppl):3B-7B.

How to cite this article: Nah E-H, Cho S, Kim S, Cho H-I. Reference interval and the role of soluble suppression of tumorigenicity 2 (sST2) in subclinical cardiac dysfunction at health checkups. *J Clin Lab Anal.* 2020;34:e23461. [https://doi.org/10.1002/jcla.23461]