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ORIGINAL ARTICLE

Elevated circulating sST2 associated with subclinical atherosclerosis in newly diagnosed primary hypertension

Ihsan Ates[1], Nihal Ozkayar[2], Hale Ates[3], Uğur Nadir Karakulak[4], Oğuzhan Kursun[5], Canan Topcuoglu[6], Bayram Inan[1] and Nisbet Yilmaz[1]

The aims of this study were to measure the levels of interleukin-33 (IL-33) and soluble Suppression of Tumorigenicity 2 (sST2) in patients with newly diagnosed primary hypertension (HT) and to determine the relationship between carotid intima-media thickness (CIMT) and IL-33/sST2. Eighty-two patients with newly diagnosed primary HT and ninety healthy volunteers were included in the study. CIMT $\geqslant$ 0.9 mm was considered as significant for subclinical atherosclerosis. The sST2 levels of patients with primary HT were higher than those of the control group, whereas the IL-33 levels of these patients were much lower than those of the control group. The sST2 levels were higher in patients with subclinical atherosclerosis than in control subjects or patients with primary HT but not with subclinical atherosclerosis. In the primary HT group, sST2 had a positive correlation with CIMT, 24-h systolic–diastolic blood pressure, low-density lipoprotein and C-reactive protein, whereas sST2 had a negative correlation with the IL-33 level. A stepwise multivariable logistic regression analysis revealed that sST2 is an independent risk factor for subclinical atherosclerosis. Although the diagnostic predictive value of HT risk was determined as $>51.8\,pg\,l^{-1}$ in the receiver operating characteristic curve analysis in respect of the sST2 level, the diagnostic predictive value for subclinical atherosclerosis risk was determined to be $>107.2\,pg\,l^{-1}$. The sST2 level displays a positive correlation with atherosclerotic changes, and is an independent risk factor for subclinical atherosclerosis expressed as increased CIMT.
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INTRODUCTION

Hypertension (HT) is one of the most important known risk factors for atherosclerosis. HT can initiate the atherosclerotic process alone and can also accelerate the atherosclerotic process by contributing to other risk factors (hyperlipidemia, diabetes, smoking, etc.). Hypertrophy in vascular structures, which is caused by high blood pressure, and endothelial dysfunction, which appears in the advanced stage, appear to be the major risk factors involved in the pathogenesis of atherosclerosis.[1] In other words, the hypertrophic response of the vascular smooth muscle to high blood pressure is one of the most important indicators of subclinical atherosclerosis. Therefore, intima-media thickness, which measures the increase in large vessels such as the carotid, is an important biomarker of subclinical atherosclerosis.[2]

Atherosclerosis is a chronic disease in which all of the risk factors listed above, as well as the inflammatory process, have an active role from the beginning to the end.[3] In cases of HT where endothelial dysfunction has not developed, inflammatory cells begin to be produced in the endothelium and smooth muscle owing to increased levels of angiotensin II;[4–7] after endothelial dysfunction develops, inflammatory cells have an active role in the pathogenesis of atheromatous plaques.[8] The fact that inflammatory cells have a critical role in the early stages of atherosclerosis and in the process of atheromatous plaque development suggests that some inflammatory markers could be predictive biomarkers of subclinical atherosclerosis.[9,10] Recent studies have shown that inflammatory markers such as the highly sensitive C-reactive protein (CRP),[9] interleukin 6[11] and tumor necrosis factor α[12,13] can predict atherosclerosis.

Another inflammatory marker that has been shown to be associated with cardiovascular disease is Suppression of Tumorigenicity 2 (ST2). ST2 is a receptor of interleukin-33 (IL-33) that is associated with Th-2 and is a member of the IL-1 receptor family.[14] ST2 has two forms: soluble ST2 (sST2) and transmembrane ST2.[15] The form that has been

[1]Department of Internal Medicine, Ankara Numune Training and Research Hospital, Ankara, Turkey; [2]Department of Nephrology, Ankara Numune Training and Research Hospital, Ankara, Turkey; [3]Department of Immunology and Allergy, Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital, Ankara, Turkey; [4]Department of Cardiology, Ankara Occupational Diseases Hospital, Ankara, Turkey; [5]Department of Neurology, Ankara Numune Training and Research Hospital, Ankara, Turkey and [6]Department of Biochemistry, Ankara Numune Training and Research Hospital, Ankara, Turkey
Correspondence: Dr I Ates, Department of Internal Medicine, Ankara Numune Training and Research Hospital, 06100, Sihhiye, Ankara, Turkey.
E-mail: dr.ihsanates@hotmail.com
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most frequently investigated in cardiovascular diseases is sST2.[16] By binding to IL-33, sST2 causes a decrease in levels of IL-33, which has cardioprotective effects.[17] Therefore, in previous studies, sST2 has been shown to be associated with coronary artery disease,[15] myocardial infarction[18] and ischemic heart disease.[19]

IL-33 is a member of the IL-1 family. IL-33 is known to be a cardioprotective marker, because it inhibits cardiomyocyte apoptosis and reduces myocardial fibrosis and myocyte hypertrophy.[17,20,21] In addition, in animal models, atherosclerotic lesions shrink in the aortic sinus after exogenous IL-33 administration, suggesting that IL-33 is also an anti-atherosclerotic agent.[20] Although the association of IL-33/ST2 with cardiovascular diseases and atherosclerosis is often discussed in the literature, no studies have investigated the association between IL-33/ST2 and asymptomatic organ damage (known as subclinical atherosclerotic changes) caused by HT.

On the basis of the above information, the aims of this study were to measure the IL-33 and sST2 levels in patients with newly diagnosed primary HT and to investigate the relationship between IL-33/sST2 and carotid intima-media thickness (CIMT), which is an important indicator of subclinical atherosclerosis.

METHODS

Study population, design and setting

This study used a cross-sectional design and was conducted in Ankara Numune Training and Research Hospital, Clinic of Internal Medicine between January and June 2015. Eighty-two patients (25 males and 57 females) over the age of 18 with newly diagnosed primary HT and who had not yet started treatment and 90 healthy volunteers (39 males and 51 females) without any known disease were included in the study. Primary HT patients were patients who attended the polyclinic with hypertensive symptoms, whose blood pressure measurements were determined to be high, and who had no secondary HT based on physical examination and laboratory findings. The control group consisted of 90 healthy volunteers with a normal mean 24-h blood pressure; the volunteers had attended the polyclinic for a check-up without having any known chronic disease or drug use. The demographic and characteristic features of the patients in the control group were obtained from the patients' files and patients' anamneses. Their body mass index (BMI) values were calculated by dividing the body dry weight by the square of the body height in meters ($BMI = kg\,m^{-2}$).

Patients with known cardiovascular, cerebrovascular or peripheral artery disease, diabetes, acute-chronic kidney or liver disease, or infective or rheumatic inflammatory disease were excluded from the study, as were those who smoked or took alcohol or regular medication. This study was designed in accordance with the 2013 Brasil version of the Helsinki Declaration, and was approved by the local Research Ethics Committee. All the participants provided written informed consent before the study.

Ambulatory blood pressure monitoring

For 24-h ambulatory blood pressure monitoring (ABPM), all participants wore a WatchBP 03 ABPM device (Microlife WatchBP AG, Widnau, Switzerland). The ABPM device was set to take 30-min measurements. The patients were allowed to continue their daily activities. Information necessary for the ABPM device to take accurate measurements was conveyed to the participants. As a result of the ABPM measurements, 24-h systolic blood pressure (SBP) and 24-h diastolic blood pressure (DBP) measurements were also obtained. Patients with mean 24-h blood pressure $\geqslant$130/80 mm Hg were considered to have primary HT. However, patients with mean 24-h blood pressure $<$130/80 mm Hg were considered to have normal blood pressure. The method was considered reliable if $>$70% of the measurements were valid.

CIMT measurement

Carotid Doppler ultrasonography was performed by an expert neurosonologist who was blinded to the clinical data of the patients. For carotid artery ultrasonography, patients were asked to lie down on the examination table in the supine position and rotate their neck slightly to the opposite side. Measurements were taken with a high-resolution B-mode ultrasound device (Xario, Toshiba, Japan) using a 7.5-MHz linear array transducer. Measurements were obtained from three different plaque-free areas for each individual carotid artery: the proximal carotid, distal carotid and proximal internal carotid artery. Longitudinal measurements were taken from the distances defined by the vessel lumen echogenicity and media-adventitia echogenicity at the posterior wall of each area. For each carotid (left and right), the mean of the measurements was taken. CIMT $\geqslant$0.9 mm was considered to represent subclinical atherosclerosis or asymptomatic organ damage.[22]

Laboratory parameters

Venous blood samples were drawn from all participants between 0800 and 1000 h after 8 h of fasting. The blood samples were then centrifuged for 10 min at 4000 r.p.m., and the serum and plasma samples were separated. The serum samples were stored at -80 °C before the sST2, IL-33, lipid parameters and CRP levels were measured.

CRP was measured using the immunoturbidimetric method, and the total cholesterol and triglyceride levels were measured using the enzymatic colorimetric method. High-density lipoprotein (HDL) cholesterol was measured using the homogeneous enzymatic colorimetric method in a Hitachi Modular P800 (Roche Diagnostics, Indianapolis, IN, USA) auto analyzer. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald method.[23]

The serum IL-33 level was measured using a commercial ELİSA kit Bioscience made by the Affymetrix Company, Vienna, Austria (REF No: BMS2048, LOT No: 107276014). The sensitivity of the assay was 0.2 $pg\,l^{-1}$. The intra-study coefficient of variation (CV%) was 4.7%, and the and inter-study CV% was 6.9%.

The serum sST2 level was measured using a human IL-1 R4/ST2 commercial ELISA kit (Raybio, Raybiotech, Norcross, GA, USA, Cat No: ELH-IL-1 R4, LOT No: 022015 0296). The upper limit that the Rybio human ELISA kit could detect is 1200 $pg\,l^{-1}$, and the lower limit is 2 $pg\,l^{-1}$. The intra-study CV% was $<$10%, and the inter-study CV% was $<$12%.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS, Chicago, IL, USA) program was used for the statistical assessments. The Kolmogorov–Smirnov test was used to determine the distribution of the data. Continuous variables with normal distribution were expressed as the mean $\pm$ s.d., and continuous variables without normal distribution were expressed as the median (interquartile range). Categorical variables were presented as numbers and percentages. Continuous variables were compared with an independent sample *t*-test or Mann–Whitney *U*-test where appropriate, and the Bonferroni correction was applied to the two sub-group comparisons. The relationships between the numeric parameters were analyzed by Pearson and Spearman correlation analyses. A stepwise linear regression analysis was used to identify independent predictors of the CIMT and sST2 levels. Logarithmic transformation was applied to the sST2, CIMT, triglycerides and CRP levels before the regression analysis. A stepwise logistic regression analysis was used to identify independent predictors of subclinical atherosclerosis risk. A receiver operating characteristic curve analysis and the Youden index method were used to calculate the predictive values of the sST2 level for primary HT and subclinical atherosclerosis risk. The diagnostic predictive value with the highest Youden index level was considered to be the best diagnostic predictive value. $P<0.05$ was considered as significant for the statistical analyses.

RESULTS

Table 1 summarizes the demographic characteristics and laboratory findings of the study population. The age and sex ratios were similar in the primary HT and control groups ($P>0.05$). The BMI was high in the primary HT group compared with the control group ($30\pm6\,kg\,m^{-2}$ and $26\pm4\,kg\,m^{-2}$, respectively; $P<0.001$). The mean 24-h SBP and DBP levels (148 ± 18 mm Hg and 113 ± 8 mm Hg; $P<0.001$; 89 ± 9 mm Hg and 74 ± 8 mm Hg, respectively; $P<0.001$)

Table 1 Demographic characteristics and laboratory findings of study population

	Primary HT				P-*values*			
Variables	*All patients* (n = *82*)	*CIMT ⩾ 0.9 mm* (n = *26*)	*CIMT< 0.9 mm* (n = *56*)	*Control* (n = *90*)	*Primary HT vs. Control*	*CIMT⩾ 0.9 mm vs. Control*	*CIMT< 0.9 mm vs. Control*	*CIMT ⩾ 0.9 mm vs. CIMT< 0. 9 mm*
Age (years)	46 ± 12	46 ± 12	45 ± 12	46 ± 8	0.914	0.970	0.955	0.904
Gender, male, *n* (%)	25 (30.5)	7 (26.9)	18 (32.1)	39 (43.3)	0.082	0.173	0.223	0.798
BMI (kg m^{-2})	30 ± 6	29 ± 6	30 ± 6	26 ± 4	<0.001*	0.043*	<0.001*	0.530
24-h SBP (mm Hg)	148 ± 18	155 ± 20	145 ± 4	113 ± 8	<0.001*	<0.001*	<0.001*	<0.001*
24-h DBP (mm Hg)	89 ± 9	95 ± 8	87 ± 7	74 ± 8	<0.001*	<0.001*	<0.001*	<0.001*
CIMT (mm)	0.8 ± 0.2	1.1 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	<0.001*	<0.001*	0.456	<0.001*
Triglyceride (mg dl^{-1})	117 (73)	101 (69)	122 (79)	110 (101)	0.555	0.807	0.359	0.166
Total cholesterol (mg dl^{-1})	225 ± 54	225 ± 47	225 ± 53	209 ± 56	0.055	0.416	0.176	0.998
HDL cholesterol (mg dl^{-1})	54 ± 12	54 ± 15	53 ± 11	51 ± 12	0.198	0.559	0.538	0.973
LDL cholesterol (mg dl^{-1})	148 ± 38	151 ± 43	147 ± 36	115 ± 41	<0.001*	<0.001*	<0.001*	0.943
CRP (mg l^{-1})	4 (4)	4 (3)	2 (3)	2 (2)	0.001*	<0.001*	0.064	0.012*
sST2 (pg l^{-1})	80 (100)	157 (121)	75 (49)	46 (73)	<0.001*	<0.001*	0.002*	<0.001*
IL-33 (pg l^{-1})	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.6 ± 0.3	0.004*	0.034*	0.029*	0.999

Abbreviations: BMI, body mass index; CIMT, carotid intima-media thickness; CRP, C-reactive protein; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HT, hypertension; IL-33, interleukin-33; LDL, low-density lipoprotein; SBP, systolic blood pressure; sST2, soluble suppression of tumorigenicity 2.
*P<0.05 indicates statistical significance. In dual comparison of normally distributed numerical variables, Bonferroni-corrected *t*-test was applied, in dual comparison of numerical variables that do not display normal distribution, Bonferroni-corrected Mann–Whitney *U*-test was applied.

were high in the primary HT group compared with the control group. The mean total cholesterol was high in the primary HT group compared with the control group, and the statistical significance was borderline (225 ± 54 mg dl^{-1} and 209 ± 56 mg dl^{-1}, respectively; P=0.055). The mean LDL cholesterol level was also significantly higher in patients with primary HT (148 ± 38 mg dl^{-1} and 115 ± 41 mg dl^{-1}, respectively; P<0.001).

The median primary sST2 level was high in HT patients than in control subjects (80 and 46 pg l^{-1}, respectively; P<0.001), and the mean IL-33 value was low compared with the control group (2.5 ± 0.2 pg l^{-1} and 2.6 ± 0.3 pg l^{-1}, respectively; P=0.004). The mean CIMT level was also significantly higher in patients with primary HT than in control subjects (0.8 ± 0.2 mm and 0.6 ± 0.2 mm, respectively; P<0.001).

In patients with subclinical atherosclerosis, the BMI, 24-h SBP, 24-h DBP, LDL, CRP, CIMT and sST2 levels were higher than in the control group, whereas the IL-33 level was lower. Among patients with primary HT, the CRP and sST2 levels were significantly higher in patients who had subclinical atherosclerosis than in those who did not (Table 1).

The correlation analysis between CIMT, sST2, IL-33 and other demographic and laboratory findings in the primary HT patient group is shown in Table 2. The CIMT displayed a positive correlation with sST2 (r=0.733, P<0.001) (Figure 1), 24-h SBP (r=0.305, P<0.001), 24-h DBP (r=0.300, P<0.001), triglyceride (r=0.294, P=0.011), LDL cholesterol (r=0.393, P<0.001) and CRP (r=0.286, P<0.001), and a negative correlation with IL-33 (r=0.367, P=0.003). The sST2 level displayed a positive correlation with 24-h SBP (r=0.308, P=0.010), 24-h DBP (r=0.370, P<0.001), LDL cholesterol (r=0.347, P<0.001) and CRP (r=0.414, P<0.001), and a negative correlation with IL-33 (r=0.354, P=0.013). The IL-33 level displayed a negative correlation with LDL cholesterol (r=0.397, P=0.010) and 24-h DBP (r=-0221, P=0.004).

Table 2 Demographic and clinical findings regarding CIMT, sST2 and IL-33 levels in the primary HT group

	sST2		*IL-33*	
Variables	r	P	r	P
CIMT	0.733	<0.001*	−0.367	0.003*
sST2	—	—	−0.354	0.013*
IL-33	−0.354	0.013*	—	—
Age	−0.011	0.882	−0.045	0.556
BMI	0.082	0.287	−0.061	0.425
24-h SBP	0.308	0.010*	−0.118	0.124
24-h DBP	0.370	<0.001*	−0.221	0.004*
Triglyceride	−0.142	0.064	−0.022	0.778
Total cholesterol	0.032	0.676	−0.088	0.257
HDL cholesterol	0.131	0.090	0.019	0.801
LDL cholesterol	0.347	<0.001*	−0.397	0.010*
CRP	0.414	<0.001*	−0.033	0.671

Abbreviations: BMI, body mass index; CIMT, carotid intima-media thickness; CRP, C-reactive protein; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HT, hypertension; IL-33, interleukin-33; LDL, low-density lipoprotein; SBP, systolic blood pressure; sST2, soluble Suppression of Tumorigenicity 2.
*P<0.05 indicates statistical significance.

The independent predictors of CIMT, sST2 and the risk of subclinical atherosclerosis are shown in detail in Table 3. The independent predictors that predicted the log(CIMT) level were log(sST2) (β ± s.e. = 1.922 ± 0.189; P<0.001), 24-h SBP (β ± s.e. = 1.595 ± 0.527; P=0.003) and LDL cholesterol (β ± s.e. = 0.905 ± 0.391; P=0.022), whereas the independent predictors of log(sST2) level were log(CIMT) (β ± s.e. = 1.900 ± 0.180; P<0.001) and log(CRP) (β ± s.e. = 0.129 ± 0.027; P<0.001), and the independent risk factor of subclinical atherosclerosis was sST2 (odds ratio = 1.160; P=0.001).

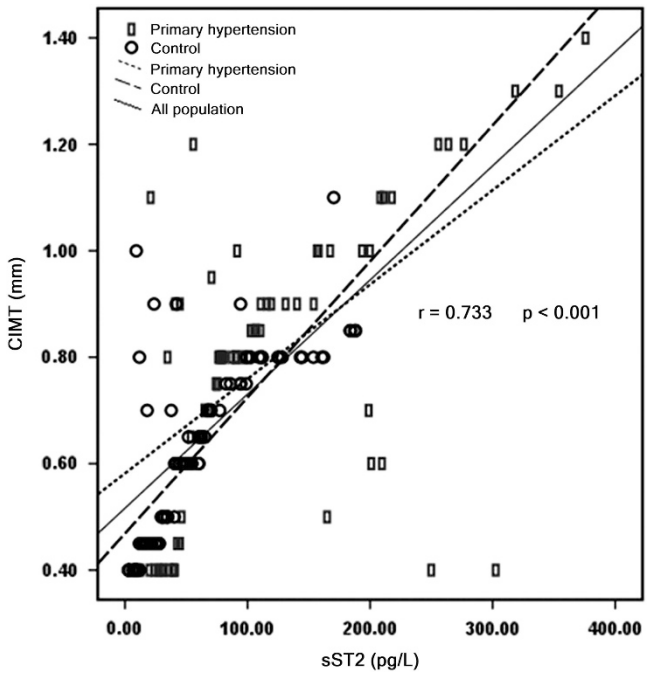

Figure 1 The correlation between CIMT and sST2 level shown on a scatter plot.

The diagnostic predictive value for primary HT risk was $>51.8\ pg\ l^{-1}$ (area under curve ± s.e. = 0.717 ± 0.039; $P<0.001$; sensitivity = 79.3%, specificity = 58.9%) on the receiver operating characteristic curve analysis of sST2 level, and the diagnostic predictive value for subclinical atherosclerosis risk the value was $>107.2\ pg\ l^{-1}$ (area under curve ± s.e. = 0.795 ± 0.061; $P<0.001$; sensitivity = 76.9%, specificity = 87.5%) (Figure 2).

DISCUSSION

In this study, the median sST2 level in patients with newly diagnosed primary HT was higher than that in the control group, and the mean IL-33 level was lower. In addition, a positive correlation was discovered between sST2 levels and cardiovascular risk factors such as 24-h SBP and DBP, CRP, LDL cholesterol and CIMT. Moreover, in this patient group, sST2 was found to be an independent predictor of subclinical atherosclerosis.

In experimental studies, IL-33 has been shown to improve cardiac function and to contribute to cardiac cell survival, especially in post-MI patients, by inhibiting apoptosis in myocardial cells.[24] sST2, when combined with IL-33, which is an important cardioprotective molecule in acute and chronic diseases, impedes the realization of the effects of IL-33.[20] In previous studies, sST2 levels were found to be increased in coronary artery disease, acute myocardial infarction and coronary failure.[15,18,25]

Although the role of IL-33/sST2 in cardiovascular diseases is well known, its relationship with subclinical atherosclerosis is not clear. In our study, the IL-33 level was low and the sST2 level was high in patients with primary HT compared with the control group. In the correlation analysis, a positive correlation was found between sST2 and vascular and metabolic risk parameters such as 24-h SBP, 24-h DBP, LDL cholesterol, CIMT and CRP. In the regression analysis, CRP, which is an inflammatory marker, and CIMT, which is a vascular indicator, were shown to predict sST2. Therefore, sST2 is a versatile marker of vascular, atherogenic and metabolic characteristics.

Table 3 The determination of independent predictors for CIMT, sST2 and CIMT ⩾ 9 mm with stepwise regression analysis

		95% CI		
Variables	*β ± s.e.*	*Lower*	*Upper*	P
CIMT[a]				
sST2	1.922 ± 0.189	1.549	2.296	<0.001*
24-h SBP	1.595 ± 0.527	0.555	2.635	0.003*
LDL cholesterol	0.905 ± 0.391	0.132	1.678	0.022*
$R^2=0.476$; $P<0.001$*				
sST2[b]				
CIMT	1.990 ± 0.180	1.634	2.345	<0.001*
CRP	0.129 ± 0.027	0.077	0.182	<0.001*
$R^2=0.553$; $P<0.001$*				
	OR			
CIMT ⩾ 9 mm[c]				
sST2	1.160	1.065	1.264	0.001*
Nagelkerke $R^2=0.321$; $P<0.001$*				

Abbreviations: β ± s.e., non-standardized regression number ± s.e.; BMI, body mass index; CI, confidence intervals; CIMT, carotid intima-media thickness; CRP, C-reactive protein; DBP, diastolic blood pressure; IL-33, interleukin-33; LDL, low-density lipoprotein; OR, odds ratio; SBP, systolic blood pressure; sST2, soluble Suppression of Tumorigenicity 2.
*$P<0.05$ indicates statistical significance. Before regression analysis, logarithmical transformation was performed on sST2, CIMT, triglyceride and CRP levels.
[a]Stepwise multivariable linear regression analysis was used. Regression model age, gender, BMI, 24-h SBP, 24-h DBP, laboratory findings and log (sST2) and IL-33 were composed of risk factors.
[b]Stepwise multivariable linear regression analysis was used. Regression model age, gender, BMI, 24-h SBP, 24-h DBP, laboratory findings and CIMT were composed of risk factors.
[c]Stepwise multivariable logistic regression analysis was used. Regression model age, gender, BMI, 24-h SBP, 24-h DBP, laboratory findings and sST2 and IL-33 were composed of risk factors.

Previous studies indicated a positive correlation between SBP and sST2.[26,27] In these studies, it was discovered that stress caused by high blood pressure increased sST2 protein secretion from endothelial cells. Similarly, the study by Demyanets *et al.*[28] showed that cardiac, aortic and coronary endothelial cells produced sST2. In a different study, it was shown that circulating IL-33 increases as a result of increasing myocardial pressure caused by HT.[29] It is possible that as a response to the stress-induced increase in IL-33 levels, sST2 levels increase and reduce IL-33 levels by binding free IL-33 molecules. Increased production of reactive sST2 and IL-33 binding would change the balance of sST2/IL-33 in favor of sST2. In our study, the determination of a negative correlation between IL-33 and sST2 levels also supports this hypothesis.

Our study is the first to show that sST2 is an independent predictor of CIMT. The mechanistic explanation for the relationship between sST2 and CIMT could include several possibilities. The secretion of IL-33 into the circulation increases when endothelial cells are subjected to mechanical stress caused by an increase in systemic blood pressure.[30,31] As a response to increasing IL-33 levels, the sST2 level would also begin to rise, and this increase would block the antihypertrophic and atheroprotective effects of IL-33.[15] Moreover, it has been shown that sST2 can be secreted directly from the endothelial cells of vascular structures such as the aorta.[28] In this case, increased CIMT due to high blood pressure becomes a significant source of sST2. sST2, which is a stimulant for atheroma plaques and has been associated with various atherosclerotic risk factors, is a risk factor for CIMT, and increased CIMT is a resource for sST2; this relationship explains the dual interaction and association between

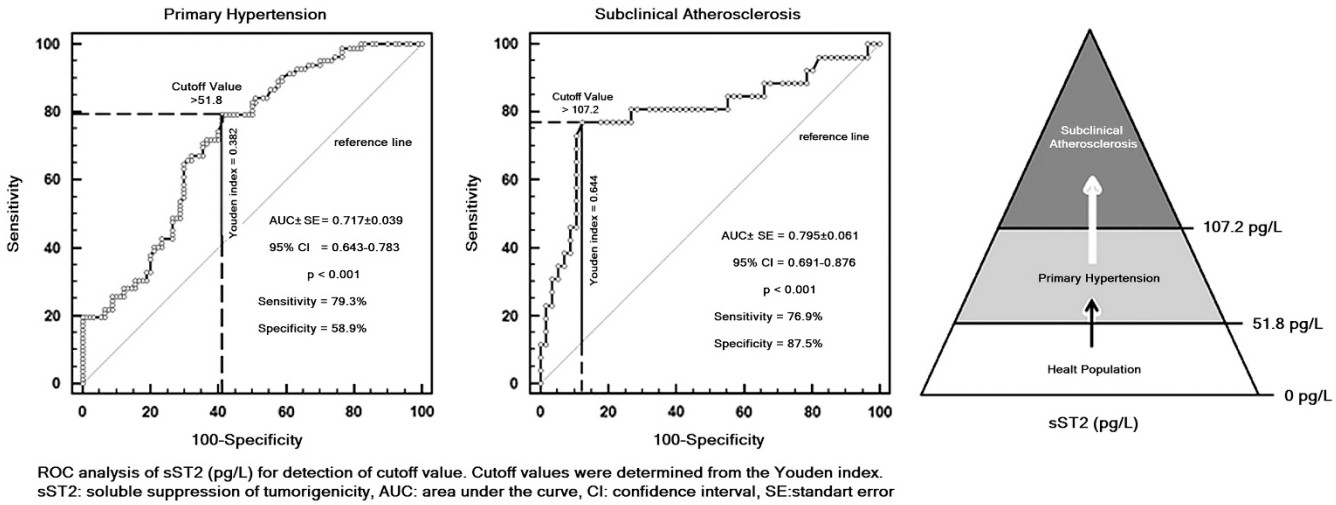

Figure 2 Determination of the diagnostic predictive value of sST2 for the risk of primary hypertension and subclinical atherosclerosis by receiver operating characteristic (ROC) curve analysis.

CIMT and sST2. This study and previous studies have shown that sST2 and CIMT are two separate atherogenic and vascular parameters that are effective in increasing the levels of each other. In addition to important clinical, metabolic and inflammatory markers including BMI, 24-h SBP, 24-h DBP, lipid profile and CRP, sST2 has been identified as an independent risk factor for subclinical atherosclerosis represented by increased CIMT, as shown by regression analysis.

The main limitations of our study are its cross-sectional design and the lack of follow-up in terms of the cardiovascular outcomes of patients. There is a need for a prospective study with a higher number of study subjects and a need for investigations of the association between sST2 and CIMT with prospective randomized controlled studies. Additionally, understanding how this association would respond to both HT and atherosclerosis would lead to a better understanding of the mechanism.

This study showed that in patients with primary HT, the IL-33 level was low and sST2 was high compared with control subjects, the sST2 level displayed a positive correlation with atherosclerotic changes, and sST2 is an independent risk factor for subclinical atherosclerosis represented by increased CIMT.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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