

# Prognostic value of suppression of tumorigenesis-2 (ST2) for cardiovascular events in coronary artery disease patients with and without diabetes mellitus

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## Original investigation

**Keywords:** ST2, Coronary artery disease, Atherosclerosis, Biomarker

**Posted Date:** November 17th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-63605/v2>

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**Version of Record:** A version of this preprint was published on February 19th, 2021. See the published version at <https://doi.org/10.1186/s12933-021-01244-3>.

# Abstract

**Background:** Suppression of tumorigenesis-2 (ST2) is implicated in myocardial overload and has long been recognized as an inflammation marker related to heart failure and acute coronary syndromes, but data on the prognostic value of ST2 in patients with coronary artery disease (CAD) remain limited. This study sought to investigate the prognostic value of ST2 in patients with established coronary artery disease and its predictive value in CAD patients with or without type 2 diabetes mellitus (T2DM).

**Methods:** A total of 3641 consecutive patients were included in this prospective cohort study. The primary end point was major adverse cardiovascular events (MACEs). The secondary end point was all-cause death. The association between ST2 and outcomes was investigated using multivariable Cox regression.

**Results:** During a median follow-up of 6.4 years, 775 patients had the occurrence of MACEs and 275 patients died. Kaplan-Meier survival estimates indicated that the patients with higher levels of ST2 (ST2 > 19 ng/ml) had a significantly increased risk of MACEs (log-rank  $p < 0.001$ ) and all-cause death (log-rank  $p < 0.001$ ). Multiple Cox regression models showed that higher level of ST2 was an independent predictor for MACEs developments (HR=1.36, 95% CI 1.17-1.56,  $p < 0.001$ ) and all-cause death (HR=2.01, 95% CI 1.56-2.59,  $p < 0.001$ ). The addition of ST2 to established risk factors significantly improved risk prediction of the composite outcome of MACEs and all-cause death (C-statistic, net reclassification index, and integrated discrimination improvement, all  $p < 0.05$ ). Subgroup analyses showed that ST2 remained a significant predictor of MACEs and all-cause death in patients with and without T2DM in multivariable models.

**Conclusions:** A higher level of ST2 is significantly associated with long-term MACEs and all-cause death in CAD patients with and without T2DM. ST2 may provide incremental prognostic value beyond traditional risk factors.

## 1 Background

Coronary artery disease (CAD) remains the leading cause of death worldwide [1]. Patients with previous coronary heart disease have a high probability of major adverse cardiac events (MACEs). The development of reliable prognostic biomarkers would be of vital importance to established CAD patients.

Suppression of tumorigenesis-2 (ST2) is an interleukin-1 (IL-1) receptor family member, that exists in two isoforms: membrane-bound (ST2L) and soluble isoforms (sST2) [2]. Previous studies have suggested that IL-33 acts as an “alarm” to alert potential tissue stress or damage [3, 4]. IL-33 promotes the production of inflammatory cytokines and Th2 immune responses by signaling through a heterodimer receptor complex composed of ST2L and IL-1 receptor attachment proteins, whereas sST2 is known to bind to IL-33 and acts as a “decoy” receptor for IL-33 to inhibit IL-33/ST2L signaling [5, 6]. The increase in the circulating sST2 concentration attenuates the systemic biological effects of IL-33. Therefore, ST2 has long been recognized as a marker of both the activation of inflammatory and hemodynamic overload [7-9]. Subsequently, soluble ST2 has been shown to be a powerful independent prognosticator for patients

with acute coronary syndrome (ACS) [10, 11] as well as heart failure (HF) [12-14]. However, in the long-term follow-up of CAD, whether ST2 is predictive of MACEs and all-cause death remains inconclusive [13, 15-18]. In addition, type 2 diabetes mellitus (T2DM) is a known predictor of elevated ST2 [19] [20], thus, we performed a large scale prospective study. The aim of the present study was to evaluate the prognostic value of ST2 for MACEs and all-cause mortality in established CAD patients with and without T2DM during a long-term follow-up.

## 2 Materials And Methods

### 2.1 The study population

The design, details, and primary results of the study have been reported previously [21]. In brief, the purpose of the study was to evaluate the prognostic value of different biomarkers for adverse cardiac events in patients with CAD. Briefly, from March 2011 to December 2015, 4078 patients who underwent coronary angiography at the Chinese PLA General Hospital were recruited for the study. These patients underwent coronary angiography examination because of angina-like chest pain or positive noninvasive tests (such as treadmill exercise test or coronary computed tomography angiography). On the basis of the angiography results, patients with at least one major coronary artery stenosis  $\geq 50\%$  were diagnosed as CAD. Patients were excluded if they had severe heart failure, atrial fibrillation, aortic dissection, active infectious disease, history of malignancy, or end stage of renal disease, or were in a deep coma. In the current study, patients were also excluded if their blood sample or detailed data were not available. Finally, a total of 3641 patients were included in the present study (Figure 1).

### 2.2 Data collection

Baseline data on demographic characteristics, lifestyle risk factors, cardiac history, and medications were collected at the time of enrollment. Blood samples were collected in the early morning. Based on the protocol, blood was collected into EDTA-anticoagulated plastic tubes. All blood samples were centrifuged at  $1000 \times g$  for 10 min and plasma samples were stored at  $-80^{\circ}\text{C}$ . Lipid profiles were measured by an automatic biochemistry analyzer (Hitachi 7150, Tokyo, Japan). Specifically, total cholesterol (TC) and triglyceride (TG) were analyzed by enzymatic methods. Low-density lipoprotein cholesterol (LDL-C) concentration was determined by the selective solubilization method (low-density lipid cholesterol test kit; Kyowa Medex, Tokyo). The high-density lipoprotein cholesterol (HDL-C) concentration was determined by a homogeneous method (Determiner L HDL; Kyowa Medex, Tokyo). Patients were considered to have hypertension with BP  $>140/90$  mmHg or use of antihypertensive medication. Hyperlipidemia was defined as one of the following criteria: LDL cholesterol  $\geq 160$  mg/dL; total cholesterol  $\geq 240$  mg/dL; triglyceride  $\geq 220$  mg/dL; HDL cholesterol  $\leq 35$  mg/dL or use of statin medication. Diabetes mellitus (DM) was defined as the presence of diabetes symptoms and a resting plasma glucose concentration  $\geq 200$  mg/dL, a fasting plasma concentration  $\geq 126$  mg/dL, a 2-h plasma glucose concentration  $\geq 200$  mg/dL in a 75 g oral glucose tolerance test, or use of a hypoglycemic agent or other medications for DM. Current smoking was defined as smoking if they reported any tobacco use in the last 30 days.

## 2.3 Plasma ST2 detection

Blood samples were collected within 24 h of hospital admission after at least 8 h of fasting. The ST2 levels were determined in plasma in single measurements by using a quantitative sandwich monoclonal enzyme-linked immunosorbent assay (Presage ST2 Assay, Critical Diagnostics, Inc., San Diego, California). A standard curve was constructed. Analysts were blinded to the patients' characteristics and endpoints of the study participants.

## 2.4 Outcome assessment

Patients were followed-up until May 2020 or until the occurrence of cardiovascular events. All participants were followed-up by analyses of clinical materials and telephone contact semiannually. The primary endpoint was MACEs, and the second endpoint was all-cause death. MACEs was defined as cardiac death, myocardial infarction, unstable angina and unplanned revascularization. All deaths were considered cardiac unless a definitive non-cardiac cause was established. Unstable angina pectoris was defined as new or accelerating symptoms of myocardial ischemia accompanied by new ischemic ST-T changes. Myocardial infarction was defined as the increase in cardiac biomarkers with evidence of myocardial ischemia. Unplanned revascularization was diagnosed if the patient underwent percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) with evidence of myocardial ischemia. We obtained follow-up for all patients until the primary outcome or date of censoring. All-cause death was defined as death from any cause. The follow-up time was calculated from the date of cardiac event onset to the date of event occurrence or the date of the last follow-up. Written informed consent was obtained from all study participants, and the study was approved by the ethics committee of the Chinese PLA General Hospital.

## 2.5 Statistical analysis

Differences in baseline characteristics between the two groups were evaluated by chi-square tests (categorical variables), and analysis of variance as appropriate. Variables with a normal distribution are presented as mean  $\pm$  standard deviation (SD), whereas in case of non-normality, the medians are presented. Categorical data are presented as counts or percentages. Kaplan-Meier curves were used to estimate the cumulative incidence risks of outcomes across baseline ST2 levels and compared by log-rank tests. Cox proportional hazards models were used to evaluate the association of baseline ST2 levels with the study endpoints. The results are presented as the hazard ratios (HRs) and 95% confidence intervals (CIs) according to levels of ST2. We fitted two multivariate proportional hazards models. Model 1 was adjusted for clinical variables including age, sex, BMI, current smoking status, hypertension, hyperlipidemia, DM, previous myocardial infarction (MI), previous PCI/CABG, TC, TG, HDL-C, and LDL-C. Model 2 was based on model 1, with the addition of ST2. The relation of ST2 levels with outcomes is presented with Cox proportional hazard models both with ST2 as a continuous variable and with ST2 as a categorical variable. The area under the receiver operating curve (AUC) was used to compare the predictive ability of the parameters of interest. Furthermore, continuous net reclassification index (NRI) and integrated discrimination improvement (IDI) were generated to evaluate any improvement in

prognostic prediction when ST2 was added to the established model. SPSS (Version 22.0) and R 4.0.0 (R Foundation for Statistical Computing) were used for descriptive data analysis. All statistical tests were 2-tailed, and p values <0.05 were considered statistically significant.

## 3 Results

### 3.1 Baseline characteristics.

Baseline measurements of ST2 were available for 3641 patients. The median concentration of ST2 was 19 ng/ml. The baseline characteristics of the consecutive CAD patients are shown in Table 1. We divided the patients into two groups based on the median concentrations of ST2. Those patients with higher concentrations of ST2 were older and more often men, with a higher prevalence of previous PCI/CABG, and the proportion of ACS patients was higher. They also had a higher level of TG (Table 1).

**Table 1 Baseline clinical and laboratory characteristics of the study patients according to ST2 levels**

	Total n=3641	ST2(<math>\lt; 19\text{ng/ml}</math>) (n=1818)	ST2(>math>\geq 19\text{ng/ml}</math>) (n=1823)	p value for trend
Age, years	61.40(27-95)	61.03(26-93)	61.86(30-95)	0.031
Male, n%	2632(72.29)	1226(67.44)	1406(77.13)	0.000
BMI(kg/m <sup>2</sup> )	25.64(13.30-41.00)	25.70(13.30-42.19)	25.60(14.50-39.70)	0.230
Risk factors for atherosclerosis				
Current smokers, n (%)	1668(45.81)	810(44.55)	858(47.07)	0.086
Hypertension, n (%)	2370(65.09)	1160(69.81)	1210(66.37)	0.090
Hyperlipidemia, n (%)	1120(30.76)	581(31.96)	539(29.57)	0.099
Diabetes mellitus, n (%)	1163(31.94)	590(32.45)	573(31.43)	0.943
Cardiac history				
Previous MI, n (%)	254(6.98)	125(6.88)	129(7.08)	0.085
Previous PCI/CABG, n (%)	299(8.21)	127(6.99)	172(9.43)	0.003
Laboratory data				
TC (mmol/L)	4.03±1.08	4.03±1.07	4.03±1.09	0.816
HDL-C (mmol/L)	1.07±0.68	1.07±0.71	1.07±0.65	0.951
LDL-C (mmol/L)	2.40±0.91	2.38±0.85	2.41±0.91	0.314
TG (mmol/L)	1.62±1.21	1.54±1.40	1.70±0.98	0.000
Medications				
Aspirin, n (%)	3415(93.79)	1712(94.17)	1703(93.42)	0.138
ACEI, n (%)	1503(41.28)	720(39.60)	813(44.60)	0.530
β-blocker, n(%)	1821(50.01)	891(49.01)	930(51.01)	0.960
Statins, n (%)	3442(94.53)	1725(94.88)	1717(94.19)	0.153
CAD classification				
SAP	899(24.69)	486(26.73)	413(22.65)	0.950
ACS	2742(75.3)	1128(62.05)	1614(88.54)	0.030

The baseline characteristics of study participants according to T2DM status were summarized in Table 2. There were 1163 (31.94%) CAD patients who had T2DM. After adjusting for age and sex, the diabetic group still had a significantly higher level of ST2, a higher rate of hypertension and a higher TG level and BMI, a higher rate of angiotensin-converting enzyme inhibitor (ACEI) and  $\beta$ -blocker medication use, and a higher rate of previous history of ACS than the nondiabetic group. Diabetes patients also have a lower rate of smoking history and lower levels of TC and LDL-C.

Table 2 The baseline characteristics of the diabetic group and nondiabetic group

	Diabetic (n=1163)	Nondiabetic (n=2478)	p value for trend
Age, years	62.10(27-92)	61.10(28-95)	0.019
Male, n%	809(69.56)	1823(73.57)	0.017
BMI(kg/m <sup>2</sup> )	26.06±3.40	25.45±3.56	0.000
Risk factors for atherosclerosis			
Current smokers, n (%)	494(42.48)	1174(47.38)	0.007
Hypertension, n (%)	839(72.14)	1531(61.78)	0.000
Hyperlipidemia, n (%)	342(29.41)	778(31.40)	0.234
Cardiac history			
Previous MI, n (%)	81(6.94)	173(6.98)	0.288
Previous PCI/CABG, n (%)	95(8.17)	204(8.23)	0.312
Laboratory data			
TC (mmol/L)	3.96±1.06	4.07±1.10	0.004
HDL-C (mmol/L)	1.05±0.87	1.59±0.58	0.093
LDL-C (mmol/L)	2.32±0.85	2.44±0.94	0.000
TG (mmol/L)	1.75±1.32	1.57±1.15	0.000
ST2	19.98±13.97	18.43±13.73	0.000
Aspirin, n (%)	1085(93.29)	2330(94.03)	0.591
ACEI, n (%)	537(46.17)	966(38.98)	0.000
$\beta$ -blocker, n(%)	865(74.40)	1761(71.07)	0.039
Statins, n (%)	1097(94.33)	2345(94.63)	0.918
CAD classification			
SAP	285(24.51)	614(24.78)	0.065
ACS	954(82.03)	1788(72.15)	0.000

### 3.2 Association between plasma ST2 and prognosis of MACEs and all-cause death

#### Primary endpoint

During the median follow-up of 6.4 years, MACEs occurred in 775 (21.29%) patients. Patients with higher ST2 levels had a significantly higher rate of MACEs than patients with lower levels (24.90% vs 17.66%,  $p<0.001$ ). After adjusting for the established factors included in Model 1 and using the lower level of ST2

as a reference, we found that patients with ST2 $\geq$ 19 ng/ml had a significantly higher risk of experiencing a primary outcome (HR=1.36, 95% CI 1.17-1.56, p<0.001) (Table 3). Kaplan-Meier curves showed that the cumulative event curves for MACEs stratified according to ST2 levels: patients with higher levels of ST2 were more likely to have a higher MACEs rates (log-rank test, p < 0.001) (Figure 2a).

## Secondary endpoint

During the follow-up, 275 (7.55%) patients died. Compared with participants with a lower level of ST2, the higher level group had a significantly higher incidence of all-cause death (10.15% versus 4.95%). After adjusting for the established factors included in Model 1 and using the lower level of ST2 as reference, we found that patients with ST2  $\geq$ 19 ng/ml had a higher risk of experiencing all-cause death (HR=2.01, 95% CI 1.56-2.59, p<0.001) (Table 3). Kaplan-Meier curves illustrated the cumulative event curves for all-cause death stratified according to ST2 levels. Patients with higher levels of ST2 were more likely to experience all-cause death (log-rank test, p < 0.001) (Figure 2b).

**Table 3 HRs and 95% CI of outcomes according to the categories of plasma ST2 in model 2**

Outcomes	ST2 level		
	$\leq$ 19 ng/ml	$\geq$ 19 ng/ml	P trend
Primary outcome: MACEs	775		
Number of cases (%)	321 (17.66)	454 (24.90)	$\leq$ 0.001
Model 2 HR	1.00 (REF)	1.36 (1.17-1.56)	$\leq$ 0.001
Cardiac death	163		
Number of cases (%)	48 (2.64)	115 (6.31)	$\leq$ 0.001
Model 2 HR	1.00 (REF)	1.70 (1.50-1.98)	0.033
MI	57		
Number of cases (%)	21 (1.1)	36 (1.8)	0.066
Model 2 HR	REF	1.17 (0.67-2.04)	0.579
Unstable angina	550		
Number of cases (%)	245 (13.48)	305 (16.73)	0.280
Model 2 HR	REF	1.42 (1.25-1.98)	$\leq$ 0.001
Revascularization treatment	5		
Number of cases (%)	2 (0.11)	3 (0.16)	0.343
Model 2 HR	REF	1.08 (0.94-1.17)	0.265
Secondary outcome: all-cause death	275		
Number of cases (%)	90 (4.95)	185 (10.15)	$\leq$ 0.001
Model 2 HR	1.00 (REF)	2.01 (1.56-2.59)	$\leq$ 0.001

## 3.3 Incremental value of ST2 over conventional risk factors

For MACEs, we further examined whether adding ST2 to the clinical model consisting of traditional risk factors could improve the risk model prediction performance. As shown in Table 4, adding ST2 significantly improved the C-statistic from 0.586 (95% CI 0.559–0.603) to 0.619 (95% CI 0.605–0.638). There was a significant difference compared to the clinical model with ST2 ( $p=0.001$ ) (Figure 3a). Furthermore, adding ST2 categories to model 1 significantly improved NRI=0.178 (95% CI 0.094-0.262,  $p<0.001$ ) and IDI=0.009 (95% CI 0.003-0.014,  $p<0.001$ ) (Table 4). For all-cause mortality, adding ST2 significantly improved the C-statistic from 0.642 (95% CI 0.594–0.701) to 0.766 (95% CI 0.717–0.806). There was a significant difference compared to the clinical model with ST2 ( $p<0.001$ ) (Figure 3b). Moreover, adding ST2 categories to model 1 significantly improved NRI=0.342 (95% CI 0.118-0.547  $p<0.001$ ) and IDI=0.012 (95% CI 0.004-0.013,  $p<0.001$ ) (Table 4).

**Table 4. Reclassification and discrimination statistics for clinical outcomes by plasma ST2**

Clinical outcomes	Model	C-statistics		Continuous NRI,%		IDI,%	
		Estimate (95%CI)	P value	Estimate (95%CI)	P value	Estimate (95%CI)	P value
MACEs	Model1	0.586(0.559-0.603)	$\leq 0.001$	REF	$\leq 0.001$	REF	$\leq 0.002$
	Model1+ST2	0.619(0.605-0.638)		17.8(9.4-26.2)		0.9(0.3-1.4)	
Cardiac death	Model1	0.746(0.703-0.789)	$\leq 0.001$	REF	$\leq 0.001$	REF	$\leq 0.001$
	Model1+ST2	0.783(0.743-0.823)		27.9(15.9-34.5)		0.8(0.2-1.6)	
MI	Model1	0.644(0.564-0.715)	0.352	REF	0.561	REF	0.721
	Model1+ST2	0.655(0.582-0.729)		15.3(9.7-20.0)		1.2(0.8-2.8)	
Unstable angina	Model1	0.583(0.561-0.608)	$\leq 0.001$	REF	0.015	REF	0.001
	Model1+ST2	0.601(0.583-0.617)		18.2(10.4-31.4)		0.07(0.01-0.26)	
Revascularization	Model1	0.575(0.545-0.596)	$\leq 0.001$	REF	$\leq 0.001$	REF	$\leq 0.001$
	Model1+ST2	0.584(0.572-0.609)		16.7(3.9-22.3)		1.4(0.6-1.8)	
All-cause death	Model1	0.642(0.594-0.701)	$\leq 0.001$	REF	$\leq 0.001$	REF	$\leq 0.001$
	Model1+ST2	0.766(0.717-0.806)		34.2(11.8-54.7)		1.2(0.4-1.3)	

### 3.4 The prognostic value of ST2 in CAD patients with or without diabetes

The predictive value of ST2 for MACEs and all-cause death in CAD patients with or without diabetes are presented in Figure 4. For MACEs, in multivariable Cox regression analyses, ST2 remained a significant predictor of MACEs in patients both with and without T2DM after adjusting for age, sex and other confounders. Among patients with T2DM, the AUC increased from 0.675 (95% CI 0.639–0.711) to 0.737 (95% CI 0.704–0.771),  $p<0.001$ . In patients without T2DM, the AUC increased from 0.581 (95% CI 0.552–0.610) to 0.620 (95% CI 0.591–0.649),  $p<0.001$  (Figure 4a, b). For all-cause death, in multivariable Cox

regression analyses, ST2 remained a significant predictor of all-cause death in patients with and without T2DM after adjusting for age, sex and other confounders. Among patients with T2DM, the AUC increased from 0.896 (95% CI 0.870–0.922) to 0.923 (95% CI 0.890–0.960)  $p < 0.001$ . In patients without T2DM, the AUC increased from 0.744 (95% CI 0.700–0.787) to 0.789 (95% CI 0.748–0.831),  $p < 0.001$  (Figure 4c, d).

## 4 Discussion

Our study established that a higher level of ST2 was a significant and independent predictor of cardiovascular events. In our study, we found that higher concentrations of ST2 ( $\geq 19$ ng/ml) were associated with an increased risk of MACEs and all-cause death in patients with CAD. Higher concentrations of ST2 remained an independent indicator of MACEs and all-cause mortality after adjusting for established traditional risk factors for cardiovascular disease. Furthermore, our study confirmed the incremental prognostic value of ST2 for MACEs and all-cause mortality beyond the clinical model. In the subgroup analysis depending on diabetes status, ST2 remained a significant predictor of MACEs and all-cause death in patients with and without T2DM after adjusting for age, sex and other confounders. In summary, our results suggest that the addition of plasma ST2 measurements to established cardiovascular risk factors may further improve risk stratification in patients with CAD and our results provide updated information on the long-term prognostic role of ST2 in established CAD patients with and without T2DM.

### 4.1 Prognostic value of biomarkers in CAD patients

Over the past two decades, biomarkers have become increasingly important tools that help to improve patient outcome prognosis [22-24]. Numerous biomarkers have been identified in the diagnosis, prognosis and risk prediction of cardiovascular disease, but few have made their way to clinical practice [25]. The most extensively used cardiovascular biomarkers are natriuretic peptides in the diagnosis and prognosis of heart failure and cardiac troponins in the diagnosis of acute myocardial infarction. Deeper experimental studies of the pathophysiology of atherosclerosis have identified a large number of molecules as potential prognostic biomarkers in cardiovascular disease [26]. To date, however, no marker has been shown to predict cardiovascular events with high accuracy. Therefore, the investigation of potential markers for predicting cardiovascular events is still of great value. There have been only 2 small studies reporting the prognostic value of ST2 in patients with CAD [16, 27]. A study showed that ST2 and IL-33 were associated with mortality in patients with ST elevation myocardial infarction (STEMI) but not patients with non-STEMI (NSTEMI) or stable angina (SAP) [27]. Another study showed that increased concentrations of ST2 were an independent predictor of all-cause mortality in patients with stable CAD [16]. Therefore, a large sample study including SAP and ACS is urgently needed to further demonstrate the predictive value of ST2 in CAD patients during long-term follow-up.

### 4.2 Prognostic value of ST2 in cardiovascular disease

Previous studies suggested that ST2 may be a potential biological marker for mechanical overload in the heart. ST2 was markedly upregulated in mechanically- stimulated cardiomyocytes. Furthermore, ST2 has

been proven to be a predictor of outcome in patients with HF [12, 14, 17, 18]. Recent evidence suggests that ST2 may be predictive in patients with ACS [28, 29]. According to Eggers KM's research, ST2 levels are elevated early in NSTEMI-ACS and predict 1-year mortality [10]. Wang YP's research showed that serum levels of ST2, IL-33 and BNP were positively correlated with MACEs in patients with AMI after PCI [30]. However, no study has investigated the long-term value of ST2 in the prediction of MACEs or all-cause death in patients with CAD in a large population.

### **4.3 The underlying mechanisms**

The inflammatory hypothesis of atherosclerosis suggests that inflammatory cell signaling drives the formation, development, and eventual instability of atherosclerotic plaques [31]. IL-33 was originally reported as a modulator of inflammation, tipping the balance towards CD4+ T helper-cell type 2 mediated immune responses [32]. The effect of IL-33 on the function of foam cells indicated the protective role of IL-33 in atherosclerosis [33]. ST2 acts as a decoy receptor for IL-33, thus blocking its protective effects. It has been reported that ApoE (-/-) mice treated with soluble ST2 developed significantly larger atherosclerotic plaques in the aortic sinus compared with the control mice [34]. Researchers have found that ST2 is particularly expressed in arterial endothelial cells and is involved in the progression of atherosclerosis [35]. These results suggested that ST2 may be proposed as a marker of plaque burden and predictor of future cardiovascular events [36]. In this respect, the IL-33-ST2 pathway deserves consideration. Although the above data suggest that ST2 has a role in the prognosis of patients presenting with ACS, whether ST2 contributes to cardiovascular risk prediction in a large scale CAD patients during a long-term follow-up remains uncertain.

To evaluate the prognostic value of a biomarker in CVD, researchers must demonstrate the elevated risk of cardiovascular events associated with higher levels of the new biomarker with adjustment for other established risk factors. The results should be presented as hazard ratio relative risk estimates from a Cox model and a probability value test of significance of the marker in the multivariable models [37]. Our results indicated that after incorporating age, sex, and other clinically relevant covariates, the adjusted HRs for MACEs and all-cause death were 1.36 and 2.01, respectively, in the Cox proportional-hazards models. Moreover, in previous studies, the follow-up time for the predictive value of ST2 was relatively short. Brown et al assessed the prognostic value of ST2 during a short-term follow-up of 30 days for acute MI, ACS, and MACEs [38], Aldous et al revisited the prognostic value of ST2 in patients with chest pain with a longer follow-up of 18 months [39]. Two reports were based on data from 3 clinical trials in STEMI that provided data on the prognostic value of plasma ST2 for 30 days after MI for adverse events, and a further article reported prognostic performance over an average follow-up time of 20 months [29, 40, 41]. Our results demonstrated that in a median follow-up of 6.4 years, a higher level of ST2 is significantly associated with all-cause death and MACEs and provides incremental prognostic value beyond traditional risk factors.

### **4.4 Prognostic value of ST2 in CAD patients with and without diabetes**

Based on Lin's research, ST2 levels were significantly elevated in patients with diabetes compared with normal subjects and each SD log ST2 was associated with a 1.57-fold increased risk of atherosclerosis [19]. Another study proved that ST2 is regulated by the p75 neurotrophic receptor and predicts mortality in diabetic patients [20]. Durga's research suggested that elevated levels of ST2 were able to predict mortality and MACEs in ACS patients, along with an increased risk of MACEs and mortality in ACS patients with diabetes [42]. Hasan's research indicated that circulating ST2 may be used to establish a cutoff value for cardiometabolic risk/disease in individuals with glycemia in the normal/prediabetes range [43]. However, on the other hand, subclinical cardiac dysfunction was associated with older age, male sex, and metabolic factors but not with the ST2 level [44]. IL-33 serves as an important local link between tissue injury or metabolic disturbances and a physiological response of limiting or repairing tissue damage [45]. It has been shown that circulating ST2 is associated with markers of liver function and lipid metabolism in severely obese patients and a reduction in ST2 occurs after successful bariatric surgery, most prominently in diabetic patients [46]. IL-33 and ST2 are abundantly expressed in adipose tissues and IL-33 levels are correlated with high body mass index, suggesting an association of IL-33 with obesity and diabetes [47, 48]. Based on the above studies, we think ST2 may affect metabolism and further affect the prognosis of diabetic patients, but the underlying mechanism has not been confirmed. In our study, among patients with T2DM, the AUC increased from 0.896 to 0.923, so our results indicated that ST2 had a higher predictive value for the prognosis of CAD patients with diabetes and provided new evidence for the role of ST2.

## Limitations

Our study still has several important limitations. First, while the study provides a large, well-characterized study sample with adjudicated outcomes, the research is limited to a single center, and these data represent the results of an observational analysis. As in any observational study, we cannot exclude residual confounding. Second, other promising new biomarkers, such as high-sensitivity troponin I and high-sensitivity troponin T, interleukin 6 and hs-CRP were not included in our study. The combination of these biomarkers may have higher prognostic value in established CAD patients. Third, based on our research, higher values of ST2 confer a markedly adverse prognosis, and the underlying mechanisms still need to be studied. Fourth, the results from the present study including only Chinese patients cannot be extrapolated to other ethnic groups, and further studies will be required.

## Conclusions

Higher values of ST2 confer a markedly adverse prognosis characterized by a large excess risk of MACEs and all-cause death over a long period of follow-up. Our study demonstrated that ST2 was a useful predictor for adverse clinical outcomes in patients with CAD, suggesting that elevation of ST2 might provide long-term prognostic information in CAD patients. Measurement of ST2 should be considered part of the approach to risk stratification in CAD patients during long-term follow-up.

# Declarations

## Ethics approval and consent to participate

This study was approved by the Ethics Board of the Chinese PLA General Hospital

## Consent for publication

Written informed consent for publication was obtained from each author and each patient.

## Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interests

## Funding

This work was supported by the Key Projects of Logistics Scientific Research Project of Chinese PLA (17BJZ48) and the National Key Research Program of China (2017YFC0840103).

## Authors' contributions

Hongbin Liu contributed to substantial contributions to the conception or design of the work. Man Li contributed to data collection, data interpretation, and critical review of the manuscript drafting the manuscript. Lei Duan, Yulun Cai, Benchuan Hao, Jianqiao Chen, Huiying Li contributed to data collection. All authors read and approved the final manuscript.

## Acknowledgements

Not applicable

# List Of Abbreviations

ACS	acute coronary syndrome
AUC	area under the curve
CABG	coronary artery bypass grafting
CAD	coronary artery disease
CAG	coronary angiography
CVD	cardiovascular disease
DM	diabetes mellitus
HF	heart failure
HDL-C	high-density lipoprotein cholesterol
HR	hazard ratio
IDI	integrated discrimination improvement
IL-1	interleukin-1
IL-33	interleukin-33
IHD	ischemic heart disease
LDL-C	low-density lipoprotein cholesterol
MACEs	major adverse cardiovascular events
MI	myocardial infarction
NRI	net reclassification index
NEST-ACS	non-ST segment elevation acute coronary syndrome
NSTEMI	Non-ST elevation MI
PCI	percutaneous coronary intervention
ROC	receiver operating characteristic curves
SD	standard deviation
STEMI	ST elevation MI
ST2	suppression of tumorigenesis-2
TC	total cholesterol
TG	triglycerides

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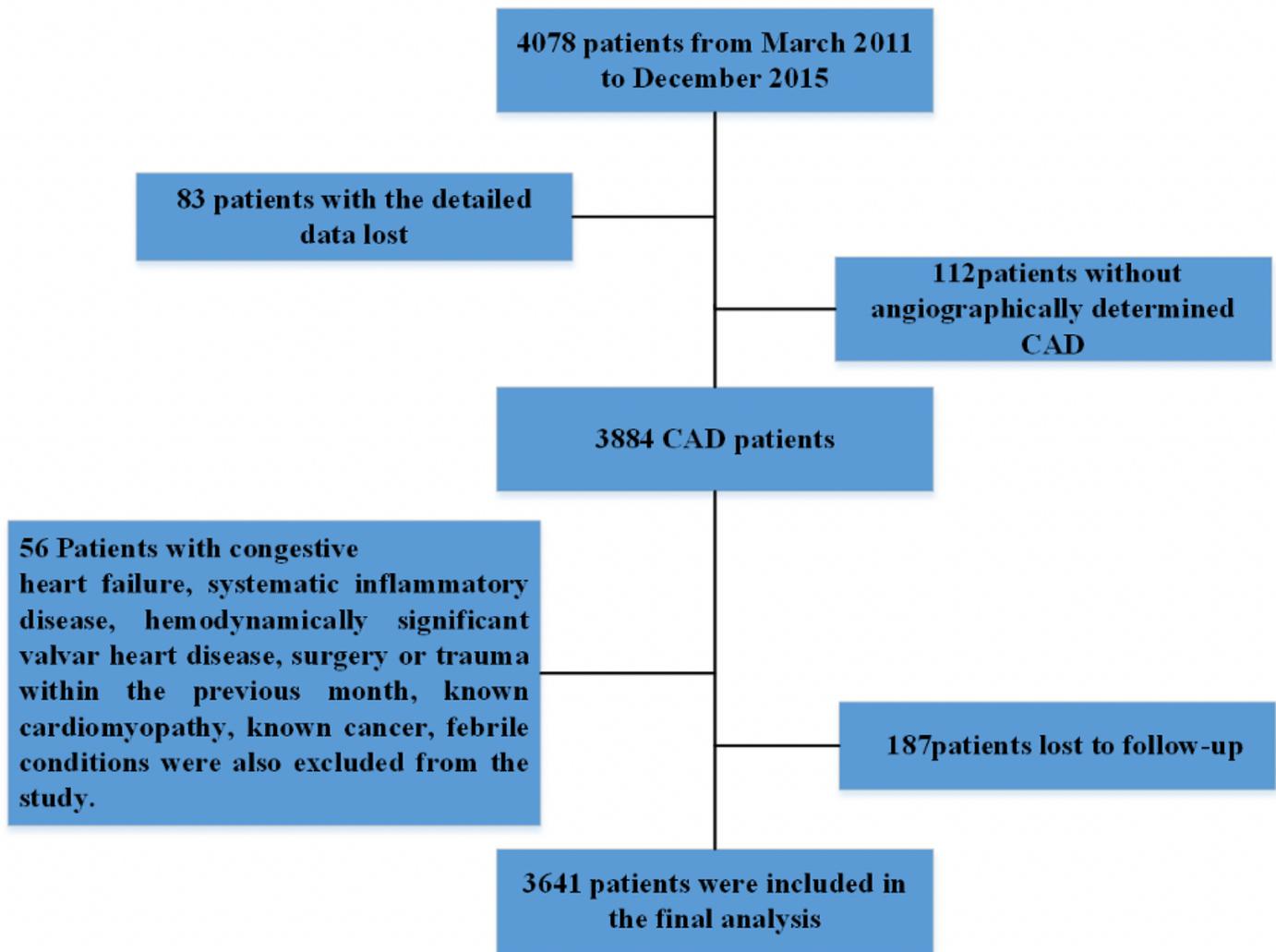
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## Figures



**Figure 1**

Flowchart of the study

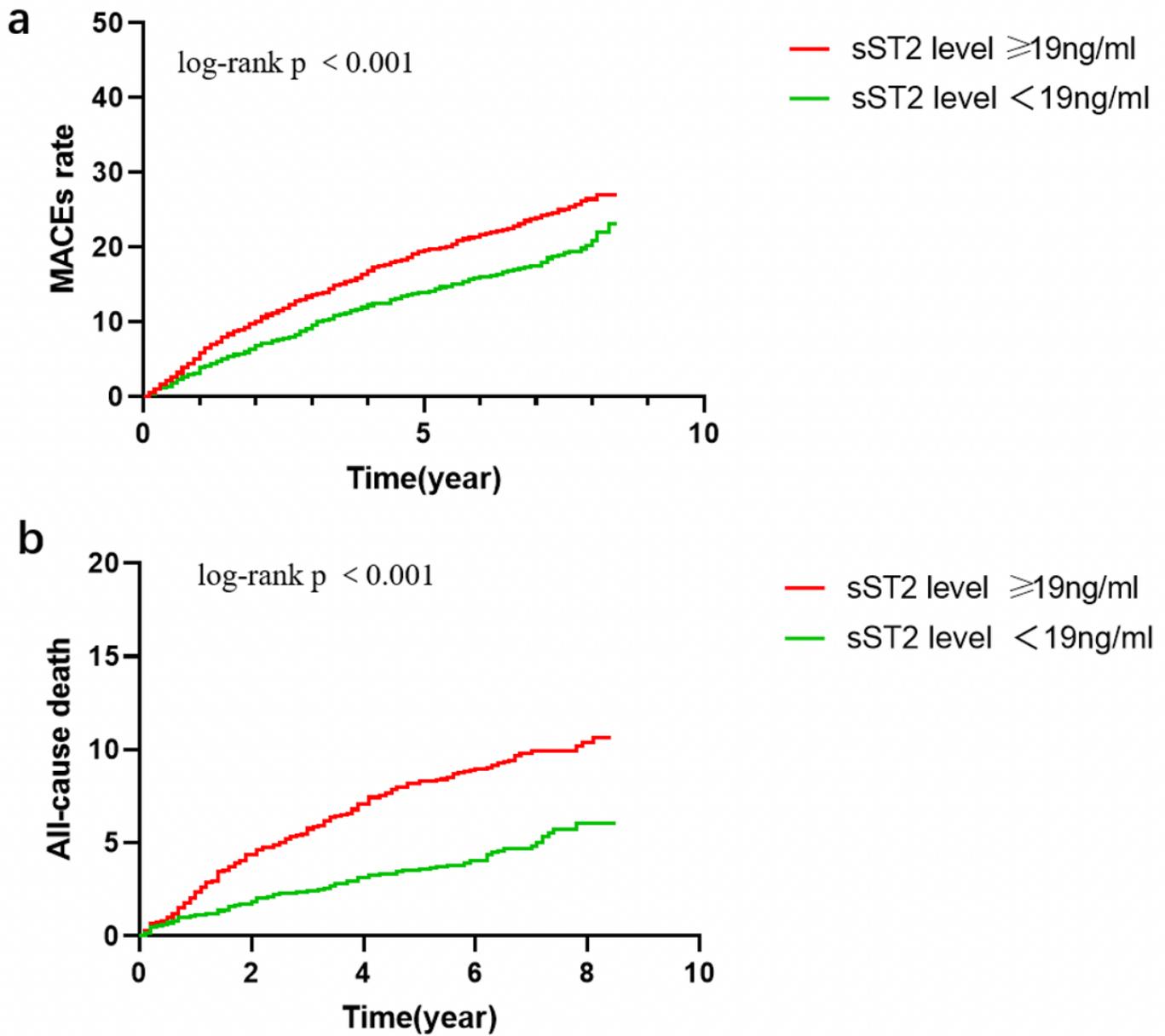
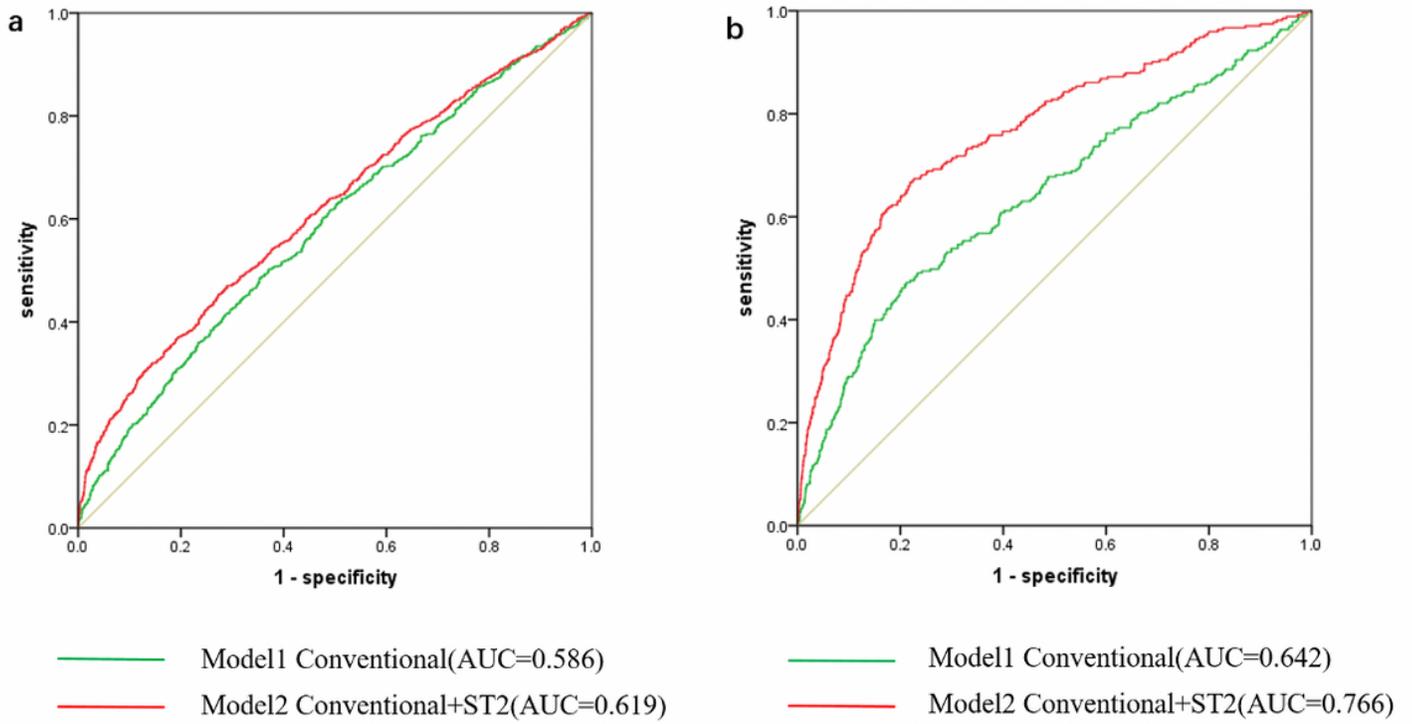


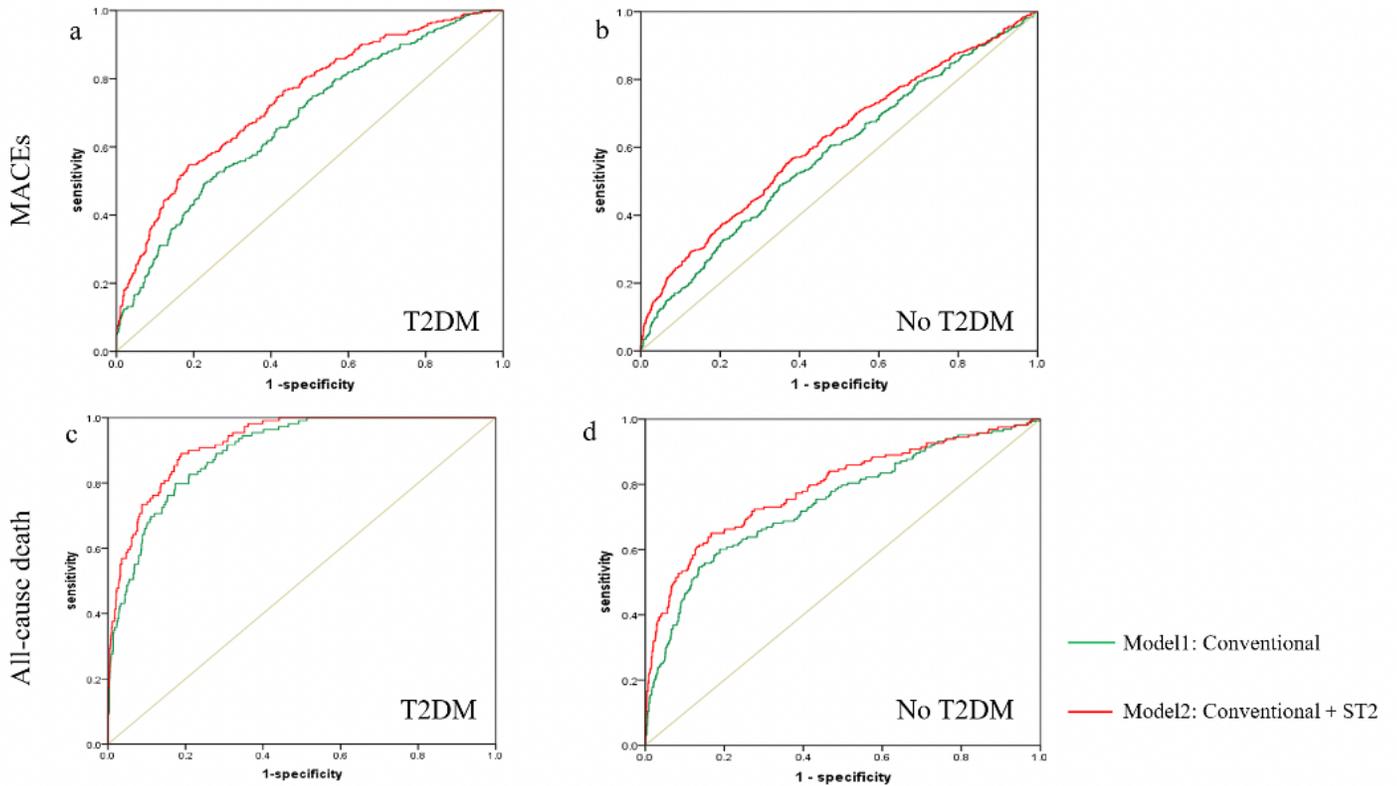
Figure 2

Kaplan-Meier curves for prediction of MACEs (a) and all-cause death (b) in patients with higher levels of ST2 (ST2 $\geq$ 19ng/ml) and lower levels of ST2 (ST2 $<$ 19ng/ml)



**Figure 3**

ROC curve analyses that relate ST2 levels to MACEs (a) and all-cause death (b).



**Figure 4**

ROC curve analyses that relate ST2 levels to MACEs and all-cause death in patients with diabetes (a, c) and without diabetes (b, d)