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Magnitude of Soluble ST2 as a Novel Biomarker for Acute Aortic Dissection

Running Title: *Wang et al.; Soluble ST2 as a Novel Biomarker for Acute AD*

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Abstract

***Background*—**Misdiagnosis of acute aortic dissection (AAD) can lead to significant morbidity and death. Soluble ST2 (sST2) is a cardiovascular injury-related biomarker. The extent to which sST2 is elevated in AAD and whether sST2 can discriminate AAD from other causes of sudden-onset severe chest pain is unknown.

***Methods*—**We measured plasma concentrations of sST2 (R&D systems assay) in 1360 patients, including 1027 participants in the retrospective discovery set and 333 patients with initial suspicion of AAD enrolled in the prospective validation cohort. Measures of discrimination for differentiating AAD from other causes of chest pain were calculated.

***Results*—**In the acute phase, sST2 levels were higher in patients with AAD than those with either acute myocardial infarction (AMI) in the first case-control discovery set within 24h symptom onset or pulmonary embolism (PE) patients in the second discovery set (medians of 129.2 ng/mL *vs.* 14.7 with p<0.001 for AAD *vs*. AMI and 88.6 *vs.* 9.3 with p<0.001 for AAD *vs.* PE). In the prospective validation set, sST2 was most elevated in AAD patients (median [25, 75 percentile]: 76.4 [49.6, 130.3]) and modestly elevated in AMI (25.0 [15.5, 37.2]), PE (14.9 [10.2, 30.1]) and angina patients (21.5 [13.1, 27.6], all p<0.001 *vs.* AAD). The area under ROC curve for AAD patients versus all control patients within 24h presenting in emergency department were 0.97 (0.95, 0.98) for sST2, 0.91 (0.88, 0.94) for D-dimer, 0.50 (0.44, 0.56) for cTnI respectively. At a cutoff level of 34.6 ng/mL, sST2 had the sensitivity of 99.1%, specificity of 84.9%, positive predictive value of 68.7%, negative predictive value of 99.7%, positive likelihood ratio of 6.6 and negative likelihood ratio of 0.01.

Conclusions—Among patients with suspected aortic dissection in the emergency department, sST2 showed superior overall diagnostic performance than D-dimer or cTnI. Additional study is needed to determine if sST2 might be a useful “rule-out” marker for AAD in the emergency room.

Key Words: aortic dissection; acute myocardial infarction; pulmonary embolism; diagnosis; soluble ST2

Clinical Perspective

What is new?

- In the acute phase, sST2 was most elevated in acute aortic dissection (AAD) patients and modestly elevated in patients with other causes of acute chest pain.
- sST2 showed superior overall diagnostic performance for AAD over D-dimer or cTnI within 24 h of presentation to the emergency department.
- ST2 test with levels < 35ng/mL (measured by the R&D systems assay) appear to reliably rule out AAD in patients with suspicion of this disease if used within 24 hours after symptom onset.

What are the clinical implications?

- sST2 could be a useful biomarker for aortic dissection which can discriminate AAD from other diseases presenting with acute chest pain.
- sST2 may provide fast and inexpensive diagnostic test to exclude early aortic dissection.

Aortic aneurysm and dissection results in around 10,000 deaths in the United States annually[1]. As a life-threatening cardiovascular disease, acute aortic dissection (AAD) is a rapidly fatal clinical emergency, with an untreated mortality of approximately 1%–2% per hour following symptom onset[2]. Immediate early diagnosis is crucial and lifesaving for appropriate management of the condition[3]. One of the main challenges for a definitive diagnosis is to distinguish AAD from other sudden-onset severe chest pain diseases, especially acute myocardial infarction (AMI) and pulmonary embolism (PE), because these patients show similar symptoms but require different treatments. Misdiagnosis of AAD as myocardial infarction (MI) or PE often results in catastrophic hemorrhage or an exacerbation of AAD, especially when thrombolytic drugs are inappropriately used[4-6]. However, electrocardiograms (ECG) and chest X-rays lack sensitivity and specificity in these circumstances[7]. Definitive confirmatory investigation (e.g., computed tomography and magnetic resonance imaging) may be limited or not available in the emergency room. It is reported that variation in the presentation of AAD can result in misdiagnosis, with up to 40% of cases only being established at post-mortem[8, 9].

To accelerate diagnosis of AAD, which is a disease of the aortic medial layer, the search for potential biomarkers has focused on those markers associated with injury to either vascular smooth muscle (smooth muscle myosin)[10], vascular interstitium (calponin)[11], elastic laminae of the aorta (soluble elastin fragments)[12], or endothelial turnover (CD40 ligand)[13], and markers associated with exposure of blood to non-intimal vascular surfaces (D-dimer)[14]. At present, only D-dimer has a clinically relevant role in suspected aortic dissection[14, 15]. However, low specificity was reported for D-dimer in patients with false lumen thrombosis, less extensive disease, and younger age groups[16]. Though significantly elevated D-dimer levels were reported in patients with AAD compared with AMI, D-dimer cannot discriminate patients with AAD from

those with PE. A biomarker that can provide additional information to help in early diagnosis and to either reliably include or exclude AAD as a diagnostic possibility would be valuable, particularly if it can be assessed at the time of presentation.

ST2 is an interleukin-1 receptor family member with transmembrane (ST2L) and soluble isoforms (sST2). sST2, a soluble truncated form of ST2L, is secreted into the circulation and functions as a "decoy" receptor for IL-33. Blood concentrations of sST2 increase in many inflammatory diseases and heart diseases[17, 18], emerging as a clinically useful prognostic biomarker in patients with heart failure[19-24]. However, the extent to which sST2 is elevated in AAD is unknown.

We measured plasma concentrations of sST2 in patients with aortic dissection, other acute chest pain diseases (e.g. AMI, PE, or angina), and compared them with healthy participants to evaluate the diagnostic performance of different levels of sST2 at discriminating AAD from other diagnoses, and to assess whether sST2 is a potential novel biomarker for AAD under different circumstances.

Methods

Study sample

The overall study design is shown in **Figure 1**. The study comprised a retrospective discovery set and a prospective validation cohort. The data that support the findings of this study are available from the corresponding author upon reasonable request. The detailed study design and population samples of the discovery set were described in the **eAppendix 1** and **Supplemental Figure A1**. In short, two case-control sets were established to evaluate the diagnostic performance of sST2 for discriminating AAD from AMI or PE respectively. The reason to set up

two separate case-control groups was mainly due to the consideration of time from symptoms onset and numbers of patients available. To discriminate AAD from AMI, all patients with AAD within 24-hour symptom onset were enrolled and frequency-matched with AMI patients within the same time frame. Because of the limited number of PE cases within 24-hour symptom onset, we established the second retrospective, case-control study set including all patients with AAD or PE available within 14-day symptom onset to evaluate the diagnostic performance of sST2 at discriminating AAD from PE. All patients were subject to the same exclusion criteria described in the **eAppendix 1**. All AAD patients in the study set for AAD vs AMI were also eligible and included in the study set for AD vs PE.

To further evaluate the diagnostic value of sST2, we designed a validation cohort including all consenting patients with a suspicion of acute AD within the first 24 hours of presentation to the emergency department (ED) in Anzhen Hospital (Beijing, China). Patients with initial suspicion of having AAD were prospectively enrolled in the validation cohort between October 2016 and March 2017. From the total 3618 chest pain patients presenting in the ED, we excluded patients in whom there was little or no suspicion of a life-threatening disease (1386 patients), patients with confirmed AMI when presenting (656 patients, e.g. those transferred from other hospitals or diagnosed by ECG), confirmed angina (974 patients), confirmed PE (30 patients), or confirmed AAD (126 patients), and those if the symptoms were clearly not related to AD (113 patients, e.g. pleurisy, pneumonia, acute abdominal diseases) (**Supplemental Figure 1**). Exclusion of these patients was based on the guidelines on the diagnosis and treatment of aortic diseases[25-27]. All remaining 333 patients, i.e. suspected AAD or not immediately ruled out to have AAD, were finally eligible and included for the validation cohort without further selection of patients based on the final diagnosis. Patients suspected to

have heart failure were included. The suspicion of AAD generally causes clinicians to order a D-dimer test which is currently recommended in the Chinese clinical guidelines for diagnosing AAD[27] and implemented in our hospital ED, though this may not be routine practice in other countries. All patients in our validation cohort had confirmatory medical imaging examination regardless of the result of the D-dimer test.

For all participants, whole blood was drawn into sodium citrate tubes, processed immediately into plasma, and stored at −80°C. Blood samples were handled using the same procedures for the different case/control groups. We used the first blood sample from participants after they entered the hospital and before surgery. Baseline characteristics and surgical information of patients were collected from medical records and confirmed by the study physicians.

The study was approved by the Beijing Anzhen Hospital Ethics Review Board. All patients provided written informed consent. For AAD patients with sudden death soon after admission or diagnosed at autopsy, consent was obtained from family members.

Outcome

All patients with AD had image information from echocardiograms and computed tomography to confirm the final diagnosis. D-dimer was not used for confirmation of the final diagnosis of aortic dissection. AD patients were classified according to the site of the intimal tear or the part of the aorta affected, irrespective of the position of the tear. Anatomical classification was made using the Stanford system. Patients with AD were classified as acute dissection if their time from onset of symptoms was ≤14 days. In unusual circumstances, where the time of symptom onset was unclear, acuteness of dissection was determined from other clinical features, such as the characteristics of the dissecting membrane on imaging and the appearance of the aortic wall

during the operation. Patients were diagnosed with AMI if they had chest pain lasting >20 min, diagnostic serial ECG changes comprising new pathological Q waves or ST-segment and T-wave changes, and a plasma creatine kinase-MB elevation greater than twice the normal level or cardiac troponin I (cTnI) level greater than 0.1 ng/mL. Diagnosis of PE was confirmed by positive spiral computed tomography or pulmonary angiography, a high probability on ventilation perfusion scintigraphy, or a proximal deep vein thrombosis documented on compression ultrasonography or angiography. AMI patients who were suspected to be accompanied by heart failure were based on the Killip classification where Killip class > 1 indicates patients with certain clinical sign of heart failure. PE patients with the ratio of right ventricle over left ventricle (RV/LV) ≥0.9 implies presence of RV strain.

Measurements of sST2 and D-dimer

Circulating sST2 was measured using a DuoSet ELISA kit (DY523B-05; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions[28-32]. The limit of detection for sST2 is 0.019 ng/mL, with mean intra-assay coefficient of variation of <6.0% and mean inter-assay coefficient of variation of <9.5%. Detailed procedures as well as the comparison between assay methods for sST2 is described in the supplementary material (**eAppendix 2** and **Supplemental Figure B1**). Levels of D-dimer were measured using the commercially available automated latex-enhanced immunoturbidimetric assay (HemosIL D-dimer HS; Instrumentation Laboratory, Bedford, MA, USA). The limit of detection for D-dimer is 21 ng/mL, with mean intra-assay coefficient of variation of <8.3% and mean inter-assay coefficient of variation of <11.0%. Technicians were blind to the settings of the different case/control groups.

Statistical analysis

Demographic and medical information of AAD and other chest pain diseases were summarized by mean (SD) or median (interquartile range (IQR)) for skewed variables (e.g. sST2 and D-dimer). Two-sample t test was used to compare mean levels of log-transformed sST2 or other continuous risk factors (log-transform where appropriate) by different disease outcomes. The χ^2 test was used for assessing difference in distribution of a categorical variable by different disease outcomes. For the time course of sST2 levels according to the time from symptom onset, we fit the linear regression model on log-sST2 with the continuous time from symptom onset. Linear trend test was used to test the association. Compared with D-dimer, the diagnostic performance of sST2 for distinguishing AAD from all other diseases, AMI, PE or angina was assessed using receiver operating characteristic (ROC) curve analysis. The area under the ROC curve (AUROC), sensitivity, specificity, accuracy, two likelihood ratios suggested by Choi for a positive and a negative test result[33], positive predictive value (PPV), and negative predictive value (NPV) were calculated. Nonparametric ROC analyses was performed using only continuous variable of sST2, D-dimer or cTnI, without the adjustment of other risk factors. Wald test was used to assess the significance of the difference between AUROCs. Because the prevalence of AD in patients presenting with suspicion of AD is poorly understood, to ease the generalization of our estimations, we used 25% (i.e. 1 in 4 patients) for calculating PPV and NPV, as applied in other studies with similar study design for D-dimer[14]. The optimal cutoff point from the study was the threshold leading to the maximum summation of sensitivity and specificity, i.e. the Youden index[34]. The recommended pre-defined cutoff point for D-dimer was 500 ng/mL, which is widely used in published literatures[14]. Analyses were made using Stata (ver. 14.0) software (Stata Statistical Software, USA). All P-values were two-tailed and have not been adjusted for multiple

testing. A P-value < 0.05 was considered statistically significant. Two-sided P-values and 95% confidence intervals (95% CI) were used.

Results

Patient demographics and sST2 distribution

In the discovery set, data were available for 1027 participants, which included 677 AD cases (including 443 acute AD cases), 234 AMI cases, 49 PE cases, and 67 healthy control participants (**Figure 1**). In the first case-control set to discriminate AAD from AMI within 24 h of symptom onset, baseline characteristics of patients are shown in **Supplemental Table 1**. There were 245 patients with AAD and 234 patients with AMI. Both sST2 and D-dimer were significantly higher in patients with AAD compared with AMI. Levels of sST2 in patients with AMI were also higher than those of healthy participants (**Figure 2a**). sST2 levels were elevated at 129.2 ng/mL (median, IQR: 71.3–197.5) in patients with AAD compared with 14.7 ng/mL (median, IQR: 9.9–23.3) in AMI patients (p<0.001). Of total 234 AMI patients, 184 patients had Killip class I which indicated no sign of heart failure and 50 patients had Killip class II, III and IV. sST2 concentration were higher in AMI patients with Killip class >1 at 23.9 (median, IQR: 15.6 – 44.7) than those with Killip = 1 at 13.3 (median, IQR: 8.6 – 19.8) (p<0.001). However, both were significantly lower than those with AAD (both p<0.001 *vs.* AAD). sST2 concentrations positively correlated with D-dimer concentrations. Pearson's correlation coefficients were highest at 0.39 in patients with dissection and 0.27 in patients with AMI (**Supplemental Figure 2**). Meanwhile, sST2 concentrations were positively correlated with brain natriuretic peptide (BNP) in AMI patients but not in AD patients (**Supplemental Figure 3**). Pearson's correlation coefficients were 0.30 in patients with AMI and only -0.04 in patients with AD.

In the second case-control set to discriminate AAD from PE, baseline characteristics of 443 patients with AAD and 49 patients with PE are shown in **Supplemental Table 2**. Levels of D-dimer were similar among patients with both AAD and PE (median: 1431 *vs.* 1594, p=0.19). sST2 was significantly elevated in patients with AAD compared with PE (median: 88.6 *vs.* 9.3, p<0.001) regardless of whether patients underwent a type A or a type B dissection (**Figure 2b**). sST2 levels in patients with PE were also higher than those in healthy participants. Pearson's correlation coefficients in patients with PE were 0.14 for sST2 with D-dimer and 0.21 for sST2 with BNP respectively (**Supplemental Figure 2&3**). Among 49 PE patients, 12 (24.5%) had RV/LV ≥ 0.9 which indicated presence of RV strain[35]. Similar to AMI patients with Killip class > 1, sST2 concentration were higher in PE patients with RV/LV ≥0.9 at 18.1 (median, IQR: 6.8 – 27.9) than others at 9.0 (median, IQR: 7.2 – 14.3) (p=0.006). Again, both were significantly lower than those with AAD (both p<0.001 *vs.* AAD). The additional dissection patients in AAD *vs.* PE set were generally comparable to those in AAD vs AMI set, except that levels of both sST2 and D-dimer were slightly lower, probably due to longer time from symptoms onset of these patients (**Supplemental Table 3**).

Time course was examined in the discovery set using box plot analysis according to time from symptom onset Of the 677 patients who underwent dissection, the peak level of sST2 was within 24 h from symptom onset. A moderate decline was observed over time (linear trend test p<0.001, **Figure 3**).

In the validation cohort, there were 333 patients, including 114 patients with AAD and 219 patients with different final diagnoses. Of the non-AD controls, there were 72, 24, 54, and 69 patients with AMI, PE, angina and other diseases respectively (**Figure 1**). Baseline characteristics of patients are shown in **Supplemental Table 4.** sST2 levels were elevated in

AAD at 76.4 ng/mL (median, IQR: 49.6 - 130.3). These were 3-fold, 5-fold, 3.6-fold, and 4-fold higher than levels for AMI (median at 25.0 ng/mL), PE (median at 14.9 ng/mL), angina (median at 21.5 ng/mL) and other diseases (median at 18.6 ng/mL) respectively (**Figure 2c**). Similar to the results in the discovery set, 22% of AMI patients (n=16) had Killip class >1 and 25% of PE patients (n=6) had RV/LV≥0.9. Though sST2 levels were higher in those patients with suspected heart failure than other patients, they were still lower than those patients with AAD (**Figure 2c**).

Diagnostic performance for discriminating AAD

In the prospective validation cohort, the AUROC for 114 AAD patients versus all non-AD control patients within 24 hours presenting in the emergency department were 0.97 (0.95, 0.98) for sST2, 0.91 (0.88, 0.94) for D-dimer, 0.50 (0.44, 0.56) for cTnI respectively (**Figure 4a**). For different control diseases, the AUROC of sST2 to discriminate AAD were 0.92 (0.88, 0.96) from AMI patients, 0.96 (0.91, 1) from PE patients, and 0.995 (0.990, 1) from angina patients respectively (**Figure 4b**). Thus, sST2 showed superior overall diagnostic performance compared with D-dimer and cTnI when all these conditions with sudden-onset severe chest pain were present in the emergency department.

Classification of patients using levels of sST2

In the validation cohort o, sST2 at cutoff levels of 34.6 ng/mL and D-dimer at 323 ng/mL were the thresholds leading to the maximum summation of sensitivity and specificity in discriminating AAD from all other control diagnoses (**Figure 4 & Table 1**). Corresponding sensitivities were 99.1% for sST2 and 93.9% for D-dimer, and specificities were 84.9% for sST2 and 78.5% for D-dimer, resulting in 89.8% of patients for sST2 and 83.8% of patients for D-dimer being correctly classified. Though predictive values are widely used from a clinical prospective, their estimations relied on a known prevalence in the tested population, i.e. proportion of confirmed

AD in all patients with suspicion of AAD. Assuming 1 in 4 patients who would have AD as used in other studies[14], positive and negative predictive value for sST2 at 34.6 ng/mL were 68.7% and 99.7% respectively. Positive and negative likelihood ratios for sST2 were 6.6 and 0.01. Negative predictive value was > 90% and negative likelihood ratios was <0.1, indicating that sST2 at ≈35 ng/mL was a good "rule-out" tool for AAD. Moreover, higher cutoff values were associated with higher positive predictive values. For a sST2 of 40 ng/mL, sST2 had the highest accuracy of 90.1% with sensitivity of 87.7%, specificity of 91.3%, positive predictive value of 77.1%, negative predictive value of 95.7%, positive likelihood ratio of 10.1 and negative likelihood ratio of 0.13. Diagnostic performance of sST2 at either 34.6, 36 or 40 ng/mL was also superior than D-dimer at pre-defined cutoff level of 500 ng/mL.

Discussion

This study analyzed data from total 1360 participants, including 1027 participants in the retrospective discovery set and 333 patients with initial suspicion of having AAD in the prospective validation cohort. Several promising results suggest that the sST2 may be a potential novel biomarker for discriminating AAD from other severe chest pain diseases, and therefore might help in the early diagnosis of AAD.

We found that the magnitude of elevated sST2 can distinguish patients with AAD from patients with AMI within 24 h after symptom onset. When aortic dissection occurs, disruption to the aortic media immediately changes aorta hemodynamics, with intramural hemorrhage leading to propagation (especially when the intimal layer is also disrupted) and tracking of blood within the media. Vascular smooth muscle cells are directly exposed to blood flow, shear stress and local inflammation[36]. In patients with dissection, smooth muscle cell stretch and vascular injury

occurred in large area of aorta, the largest artery, which may induce higher levels of sST2 in the circulation than that in small or medium vessels of AMI or PE. Our results suggested that the degree of the elevation of sST2 levels is associated with the different magnitudes of vascular injury among AAD, AMI and PE. In fact, though sST2 levels was higher in AMI or PE patients with suspected heart failure, they were still lower than that in patients with AAD in our study, suggesting that the increment of sST2 in AAD may mainly due to smooth muscle cell stretch and vascular injury, rather than myocardial strain.

Besides AMI, we discovered that the magnitude of elevated sST2 can distinguish patients with AAD from patients with PE, whereas D-dimer, as a fibrin degradation product and present in the circulation following fibrinolysis of thrombus, was found to be significantly increased similarly in a number of diseases, including both PE and AAD. Currently, D-dimer aids clinical diagnosis for PE only as a “rule-out” tool when the test result is negative.

In contrast, sST2 is not considerably elevated in patients with PE. This observation suggests that sST2 could be a potential biomarker for discriminating AAD from PE, providing additional valuable information beyond that provided by D-dimer. This is strongly supported by our results from the prospective validation cohort which included all suspicious AD patients with acute chest pain within the first 24 h presenting to the emergency department. A high proportion of PE patients would limit the performance of D-dimer for evaluation of AAD but cause no influence on sST2.

In addition, we found that using sST2 at around 35 ng/mL can exclude aortic dissection with a negative likelihood ratio <0.1 and a negative predictive value of >90%. It is noted that the cutoff point of 35 ng/mL for sST2 was also recommended in the emergency department for heart failure. However, this value for heart failure is proposed using the Presage assay, currently

recommended by the US Food and Drug Administration. The Presage assay was not used in our study because it is not validated with our retrospective citrated plasma. We therefore compared sST2 plasma concentrations measured with the R&D Systems assay *vs.* the Presage assay in the evaluation set with 67 patients available (**eAppendix 2**). 35 ng/mL using R&D assay was equivalent to 71 ng/mL using Presage assay in our evaluation set. Furthermore, higher cutoff values of sST2 were associated with higher positive predictive values. sST2 at 40 ng/mL had the highest accuracy of 90% in our study with positive predictive value of 77% and positive likelihood ratio of 10.. Therefore, sST2 could be a practical and promising tool to guide the need for further imaging diagnoses of AAD.

Two isoforms, a soluble (sST2) and a membrane bound form (ST2L), are produced from a dual promoter system through differential mRNA processing[37]. Both ST2 forms are expressed in vascular cells and thoracic aorta tissues, with sST2 residing in the extracellular matrix of the integumentary system[38]. ST2 mRNA expression is induced by mechanical strain, TNF, IL-1α, and IL-1β. With stress and pro-inflammatory stimuli, the proximal (active sST2), not the distal, promoter (active ST2L) is responsible for transcriptional activation. Therefore, sST2, and not ST2L, is highly up-regulated in the first hour. sST2 is rapidly secreted into the circulation and functions as a “decoy” receptor for IL-33. It then inhibited IL-33/ST2L signaling which represents a crucial protective mechanism in case of mechanical overload. In this way, sST2 may extend the dissection and perhaps leads to further release [17]. These findings raise the possibility that the IL-33/ST2 system could be a potential pathophysiological mediator of dissection and includes the involvement of sST2 and ST2L in several different cellular processes, including cell alignment and differentiation, migration, survival or apoptosis, vascular remodeling, and cell-mediated inflammatory reactions in dissection progression. The role of sST2 and ST2L in these

specific pathologies remains to be addressed in future studies.

To our knowledge, this study provides the first evidence that sST2 is associated with aortic dissection and could be a novel biomarker for AD in the acute phase. A strength of this study is that we discovered as well as validated the diagnose performance of sST2 for AAD. We simultaneously measured D-dimer and sST2 for direct comparison to enhance the validity of our estimates of the diagnostic performance. The relatively large numbers of patients also allowed us to evaluate the corresponding results according to different clinically relevant subgroups (e.g. AMI or PE patients with or without suspected heart failure). This study had some limitations. Although this study had a relatively large number of patients to examine AAD, it was a single-center study. Though the prevalence of 1 in 3 suspected patients with AAD in our validation cohort was comparable to several published studies for D-dimer with similar study designs[14], it may still be enriched compared to Western population in general clinical practice (i.e. not large referral centers). Therefore, we firstly excluded patients transferred from other hospitals with confirmed disease outcomes (**Supplemental Figure 1**). Secondly, a comparable prevalence of disease, i.e. 25% used in other studies, was applied in the estimation of positive and negative predictive values to avoid inflation. However, the AUROC, positive and negative predictive values observed in our study are likely exaggerated by the design of our derivation and validation cohorts. In particular, our validation cohort is not an "all comers" cohort of patients with undifferentiated chest pain. Future validation studies evaluating sST2, and comparing sST2 with d-dimer, are thus needed. As a single-center study, the generalizability of our findings should also be verified by a large prospective multicenter study to confirm the diagnostic efficacy and accuracy of this novel assay. The absolute values for the concentrations of sST2 may be varied to the assay method used, which may affect the cutoff levels proposed. Calibration

across different assay methods could be applied where appropriate. Finally, other diseases, e.g. acute respiratory distress syndrome, can also cause the increment of sST2 levels[39], though they may not often be confused with AD according to the symptoms of the disease. Further medical image-based confirmatory diagnoses are still essential in the clinical practice.

Conclusion

sST2 showed superior overall diagnostic performance for acute AD over D-dimer or cTnI in patients with suspected AD in the emergency department. sST2 might be a useful “rule-out” marker for AAD but the utility of a positive prediction is less clear. Therefore, sST2 could be a sensitive candidate that may provide fast and cost-effective diagnostic testing to determine early AAD.

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Disclosures

None

References

1. Rogers AM, Hermann LK, Booher AM, Nienaber CA, Williams DM, Kazerooni EA, Froehlich JB, O'Gara PT, Montgomery DG, Cooper JV, Harris KM, Hutchison S, Evangelista A, Isselbacher EM, Eagle KA and Investigators I. Sensitivity of the aortic dissection detection risk score, a novel guideline-based tool for identification of acute aortic dissection at initial presentation: results from the international registry of acute aortic dissection. *Circulation*. 2011;123:2213-8.

2. Hagan PG, Nienaber CA, Isselbacher EM, Bruckman D, Karavite DJ, Russman PL, Evangelista A, Fattori R, Suzuki T, Oh JK, Moore AG, Malouf JF, Pape LA, Gaca C, Sechtem U, Lenferink S, Deutsch HJ, Diedrichs H, Marcos y Robles J, Llovet A, Gilon D, Das SK, Armstrong WF, Deeb GM and Eagle KA. The International Registry of Acute Aortic Dissection (IRAD): new insights into an old disease. *JAMA*. 2000;283:897-903.
3. Klompas M. Does this patient have an acute thoracic aortic dissection? *JAMA*. 2002;287:2262-72.
4. Blankenship JC and Almquist AK. Cardiovascular complications of thrombolytic therapy in patients with a mistaken diagnosis of acute myocardial infarction. *J Am Coll Cardiol*. 1989;14:1579-82.
5. Butler J, Davies AH and Westaby S. Streptokinase in acute aortic dissection. *BMJ*. 1990;300:517-9.
6. Wilcox RG, von der Lippe G, Olsson CG, Jensen G, Skene AM and Hampton JR. Trial of tissue plasminogen activator for mortality reduction in acute myocardial infarction. Anglo-Scandinavian Study of Early Thrombolysis (ASSET). *Lancet*. 1988;2:525-30.
7. Erbel R, Alfonso F, Boileau C, Dirsch O, Eber B, Haverich A, Rakowski H, Struyven J, Radegran K, Sechtem U, Taylor J, Zollikofer C, Klein WW, Mulder B, Providencia LA and Task Force on Aortic Dissection ESoC. Diagnosis and management of aortic dissection. *Eur Heart J*. 2001;22:1642-81.
8. Olin JW and Fuster V. Acute aortic dissection: the need for rapid, accurate, and readily available diagnostic strategies. *Arterioscler Thromb Vasc Biol*. 2003;23:1721-3.
9. Van Arsdell GS, David TE and Butany J. Autopsies in acute type A aortic dissection. Surgical implications. *Circulation*. 1998;98:II299-302; discussion II302-4.
10. Suzuki T, Katoh H, Watanabe M, Kurabayashi M, Hiramori K, Hori S, Nobuyoshi M, Tanaka H, Kodama K, Sato H, Suzuki S, Tsuchio Y, Yazaki Y and Nagai R. Novel biochemical diagnostic method for aortic dissection. Results of a prospective study using an immunoassay of smooth muscle myosin heavy chain. *Circulation*. 1996;93:1244-9.
11. Suzuki T, Distante A, Zizza A, Trimarchi S, Villani M, Salerno Uriarte JA, de Luca Tupputi Schinosa L, Renzulli A, Sabino F, Nowak R, Birkhahn R, Hollander JE, Counselman F, Bossone E, Eagle K and International Registry of Acute Aortic Dissection Substudy on Biomarkers I. Preliminary experience with the smooth muscle troponin-like protein, calponin, as a novel biomarker for diagnosing acute aortic dissection. *Eur Heart J*. 2008;29:1439-45.
12. Shinohara T, Suzuki K, Okada M, Shiigai M, Shimizu M, Maehara T and Ohsuzu F. Soluble elastin fragments in serum are elevated in acute aortic dissection. *Arterioscler Thromb Vasc Biol*. 2003;23:1839-44.
13. Vianello E, Dozio E, Rigolini R, Marrocco-Trischitta MM, Tacchini L, Trimarchi S and Corsi Romanelli MM. Acute phase of aortic dissection: a pilot study on CD40L, MPO, and MMP-1, -2, 9 and TIMP-1 circulating levels in elderly patients. *Immun Ageing*. 2016;13:9.
14. Suzuki T, Distante A, Zizza A, Trimarchi S, Villani M, Salerno Uriarte JA, De Luca Tupputi Schinosa L, Renzulli A, Sabino F, Nowak R, Birkhahn R, Hollander JE, Counselman F, Vijayendran R, Bossone E, Eagle K and Investigators IR-B. Diagnosis of acute aortic dissection by D-dimer: the International Registry of Acute Aortic Dissection Substudy on Biomarkers (IRAD-Bio) experience. *Circulation*. 2009;119:2702-7.
15. Cui JS, Jing ZP, Zhuang SJ, Qi SH, Li L, Zhou JW, Zhang W, Zhao Y, Qi N and Yin YJ. D-dimer as a biomarker for acute aortic dissection: a systematic review and meta-analysis. *Medicine (Baltimore)*. 2015;94:e471.

16. Ranasinghe AM and Bonser RS. Biomarkers in acute aortic dissection and other aortic syndromes. *J Am Coll Cardiol*. 2010;56:1535-41.
17. Kakkar R and Lee RT. The IL-33/ST2 pathway: therapeutic target and novel biomarker. *Nat Rev Drug Discov*. 2008;7:827-40.
18. Mueller T and Jaffe AS. Soluble ST2--analytical considerations. *Am J Cardiol*. 2015;115:8B-21B.
19. Weinberg EO, Shimpo M, Hurwitz S, Tominaga S, Rouleau JL and Lee RT. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. *Circulation*. 2003;107:721-6.
20. Rehman SU, Mueller T and Januzzi JL, Jr. Characteristics of the novel interleukin family biomarker ST2 in patients with acute heart failure. *J Am Coll Cardiol*. 2008;52:1458-65.
21. Manzano-Fernandez S, Mueller T, Pascual-Figal D, Truong QA and Januzzi JL. Usefulness of soluble concentrations of interleukin family member ST2 as predictor of mortality in patients with acutely decompensated heart failure relative to left ventricular ejection fraction. *Am J Cardiol*. 2011;107:259-67.
22. Lassus J, Gayat E, Mueller C, Peacock WF, Spinar J, Harjola VP, van Kimmenade R, Pathak A, Mueller T, Disomma S, Metra M, Pascual-Figal D, Laribi S, Logeart D, Nouira S, Sato N, Potocki M, Parenica J, Collet C, Cohen-Solal A, Januzzi JL, Jr., Mebazaa A and Network G. Incremental value of biomarkers to clinical variables for mortality prediction in acutely decompensated heart failure: the Multinational Observational Cohort on Acute Heart Failure (MOCA) study. *Int J Cardiol*. 2013;168:2186-94.
23. Gaggin HK, Szymonifka J, Bhardwaj A, Belcher A, De Berardinis B, Motiwala S, Wang TJ and Januzzi JL, Jr. Head-to-head comparison of serial soluble ST2, growth differentiation factor-15, and highly-sensitive troponin T measurements in patients with chronic heart failure. *JACC Heart Fail*. 2014;2:65-72.
24. Dieplinger B and Mueller T. Soluble ST2 in heart failure. *Clin Chim Acta*. 2015;443:57-70.
25. Erbel R, Aboyans V, Boileau C, Bossone E, Bartolomeo RD, Eggebrecht H, Evangelista A, Falk V, Frank H, Gaemperli O, Grabenwoger M, Haverich A, Iung B, Manolis AJ, Meijboom F, Nienaber CA, Roffi M, Rousseau H, Sechtem U, Sirnes PA, Allmen RS, Vrints CJ and Guidelines ESCCfP. 2014 ESC Guidelines on the diagnosis and treatment of aortic diseases: Document covering acute and chronic aortic diseases of the thoracic and abdominal aorta of the adult. The Task Force for the Diagnosis and Treatment of Aortic Diseases of the European Society of Cardiology (ESC). *Eur Heart J*. 2014;35:2873-926.
26. Hiratzka LF, Bakris GL, Beckman JA, Bersin RM, Carr VF, Casey DE, Jr., Eagle KA, Hermann LK, Isselbacher EM, Kazerooni EA, Kouchoukos NT, Lytle BW, Milewicz DM, Reich DL, Sen S, Shinn JA, Svensson LG, Williams DM, American College of Cardiology Foundation/American Heart Association Task Force on Practice G, American Association for Thoracic S, American College of R, American Stroke A, Society of Cardiovascular A, Society for Cardiovascular A, Interventions, Society of Interventional R, Society of Thoracic S and Society for Vascular M. 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with Thoracic Aortic Disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. *Circulation*. 2010;121:e266-369.

27. Editorial Board of Chinese Journal of C, Consensus Panel of Standardized A, Diagnosis of Chest P, Editorial Board of Chinese Journal of C, Consensus Panel of Standardized A and Diagnosis of Chest P. [Chinese expert consensus on standardized assessment and diagnosis of chest pain]. *Zhonghua Xin Xue Guan Bing Za Zhi*. 2014;42:627-32.
28. Weir RA, Miller AM, Murphy GE, Clements S, Steedman T, Connell JM, McInnes IB, Dargie HJ and McMurray JJ. Serum soluble ST2: a potential novel mediator in left ventricular and infarct remodeling after acute myocardial infarction. *J Am Coll Cardiol*. 2010;55:243-50.
29. Dworakowski R, Wendler O, Bhan A, Smith L, Pearson P, Alcock E, Rajagopal K, Brickham B, Dew T, Byrne J, Monaghan MJ, Sherwood R, Shah AM and MacCarthy PA. Successful transcatheter aortic valve implantation (TAVI) is associated with transient left ventricular dysfunction. *Heart*. 2012;98:1641-6.
30. Chida A, Sato H, Shintani M, Nakayama T, Kawamura Y, Furutani Y, Inai K, Saji T, Matsuoka R, Nonoyama S and Nakanishi T. Soluble ST2 and N-terminal pro-brain natriuretic peptide combination. Useful biomarker for predicting outcome of childhoodpulmonary arterial hypertension. *Circ J*. 2014;78:436-42.
31. Lu DP, Zhou XY, Yao LT, Liu CG, Ma W, Jin F and Wu YF. Serum soluble ST2 is associated with ER-positive breast cancer. *BMC Cancer*. 2014;14:198.
32. Demyanets S, Speidl WS, Tentzeris I, Jarai R, Katsaros KM, Farhan S, Krychtiuk KA, Wonnerth A, Weiss TW, Huber K and Wojta J. Soluble ST2 and interleukin-33 levels in coronary artery disease: relation to disease activity and adverse outcome. *PLoS One*. 2014;9:e95055.
33. Choi BC. Slopes of a receiver operating characteristic curve and likelihood ratios for a diagnostic test. *Am J Epidemiol*. 1998;148:1127-32.
34. Zhen Chen AL, Yongming Qu, Larry Tang, Naitee Ting, Yi Tsong. *Applied Statistics in Biomedicine and Clinical Trials Design*: Springer; 2015.
35. Konstantinides SV. 2014 ESC Guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J*. 2014;35:3145-6.
36. El-Hamamsy I and Yacoub MH. Cellular and molecular mechanisms of thoracic aortic aneurysms. *Nat Rev Cardiol*. 2009;6:771-86.
37. Iwahana H, Yanagisawa K, Ito-Kosaka A, Kuroiwa K, Tago K, Komatsu N, Katashima R, Itakura M and Tominaga S. Different promoter usage and multiple transcription initiation sites of the interleukin-1 receptor-related human ST2 gene in UT-7 and TM12 cells. *Eur J Biochem*. 1999;264:397-406.
38. Miller AM, Xu D, Asquith DL, Denby L, Li Y, Sattar N, Baker AH, McInnes IB and Liew FY. IL-33 reduces the development of atherosclerosis. *J Exp Med*. 2008;205:339-46.
39. Alladina JW, Levy SD, Hibbert KA, Januzzi JL, Harris RS, Matthay MA, Thompson BT, Bajwa EK, National Heart L and Blood Institute Acute Respiratory Distress Syndrome N. Plasma Concentrations of Soluble Suppression of Tumorigenicity-2 and Interleukin-6 Are Predictive of Successful Liberation From Mechanical Ventilation in Patients With the Acute Respiratory Distress Syndrome. *Crit Care Med*. 2016;44:1735-43.

Table 1. Diagnostic performance of patients with AAD *vs.* others using sST2 compared with D-Dimer in the validation cohort

	Threshold	Sensitivity	Specificity	Accuracy	PLR	NLR	PPV‡	NPV‡
333 patients (114 patients with AAD)								
sST2	34.6*	99.1%	84.9%	89.8%	6.6	0.01	68.7%	99.7%
	36	93.0%	88.1%	89.8%	7.8	0.08	72.3%	97.4%
	40	87.7%	91.3%	90.1%	10.1	0.13	77.1%	95.7%
	50	74.6%	95.0%	88.0%	16.3	0.27	83.2%	91.8%
D-Dimer	323*	93.9%	78.5%	83.8%	4.4	0.08	59.3%	97.5%
	500 (Recommended)†	87.7%	82.2%	84.1%	4.9	0.15	62.2%	95.3%

Abbreviations: PLR indicates positive likelihood ratio; NLR, negative likelihood ratio; PPV, positive predictive value; and NPV, negative predictive value.

* Optimal threshold value obtained from the data, which was the threshold leading to the maximum summation of sensitivity and specificity, i.e. the Youden index.

† Pre-defined threshold values based on information from previous literature.

‡ Because the prevalence of AD in patients presenting with suspicion of AD is poorly understood, to ease the generalization of our estimations, we used 25% (i.e. 1 in 4 patients) as suggested in previous literatures.

Figure Legends

Figure 1. Overall study design.

& Detailed population information of the validation cohort was shown in Supplemental Figure 1.

*Detailed population information and the corresponding objectives in the discovery set was shown in eAppendix 1.

Figure 2. Distribution of sST2 according to disease status.

(a) AAD *vs.* AMI in the discovery set. * Including 189 patients with type A AAD, 53 patients with type B AAD, and three patients with acute abdominal AAD. Analysis included all patients within 24-h from time of onset.

(b) AAD *vs.* PE in the discovery set. * Including 327 patients with type A AAD, 112 patients with type B AAD, and four patients with acute abdominal AAD. Analysis included all patients within 14-day from time of onset.

(c) Distribution of sST2 according to disease status in the validation cohort. Medians of sST2 concentrations were 25.0 ng/mL(IQR: 15.5-37.2) in all AMI patients and 14.9 ng/mL (IQR: 10.2-30.1) in all PE patients.

Figure 3. Levels of sST2 according to the time after onset in patients with dissection in the discovery set.

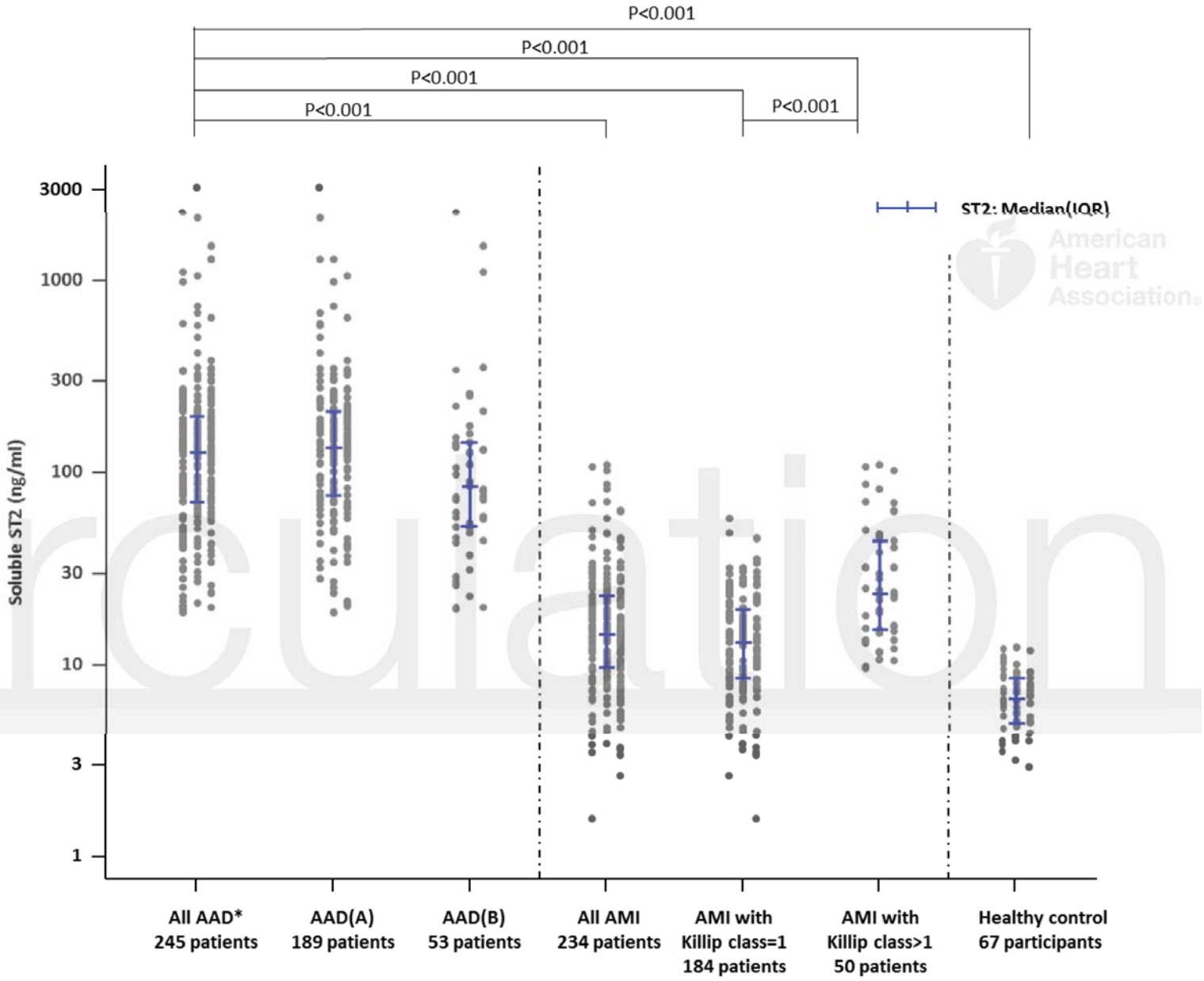
Figure 4. Receiver-operating characteristics curves of sST2 in the validation cohort.

(a) AAD *vs.* all control patients comparing with D-Dimer and cTnI

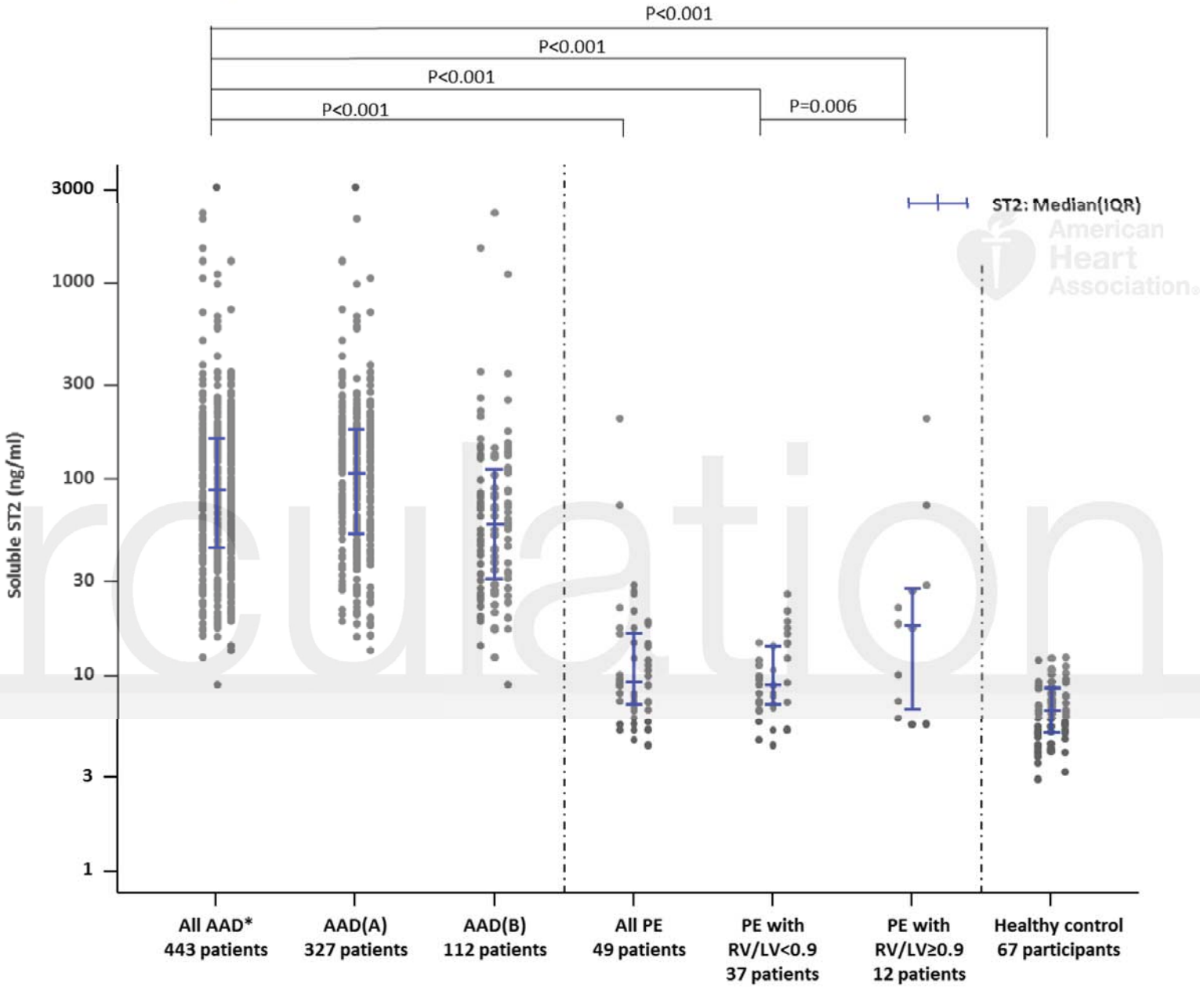
(b) AAD *vs.* each of control diseases

Study set	Discovery Set* (n=1027: 677 AD + 234 AMI + 49 PE + 67 Healthy control)		Validation cohort& (n=333)	
Study population		AD cases	Non-AD controls	
	AAD vs AMI (Time onset ≤ 24 hours)			
	AAD	n=245		AAD n=114
	AMI		n=234	AMI n=72
	AAD vs PE (Time onset ≤ 14 days)			
	AAD	n=443		PE n=24
	PE		n=49	Angina n=54
	Additional subjects for sST2 distribution			
	AD (time onset > 14 days)	n=234		Others n=69
	Healthy control		n=67	
Study design	Retrospective		Prospective	

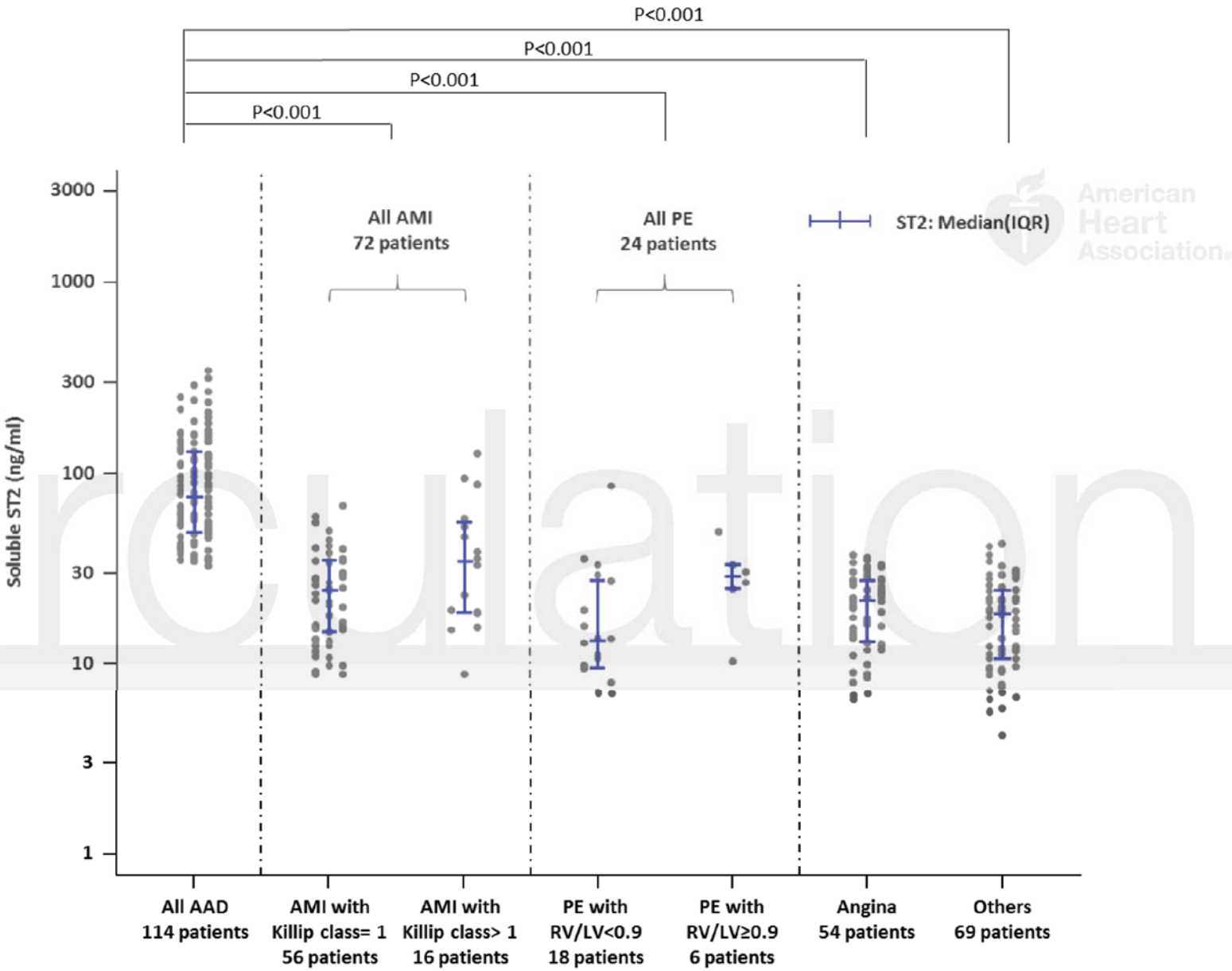
(a) AAD *vs.* AMI in the discovery set

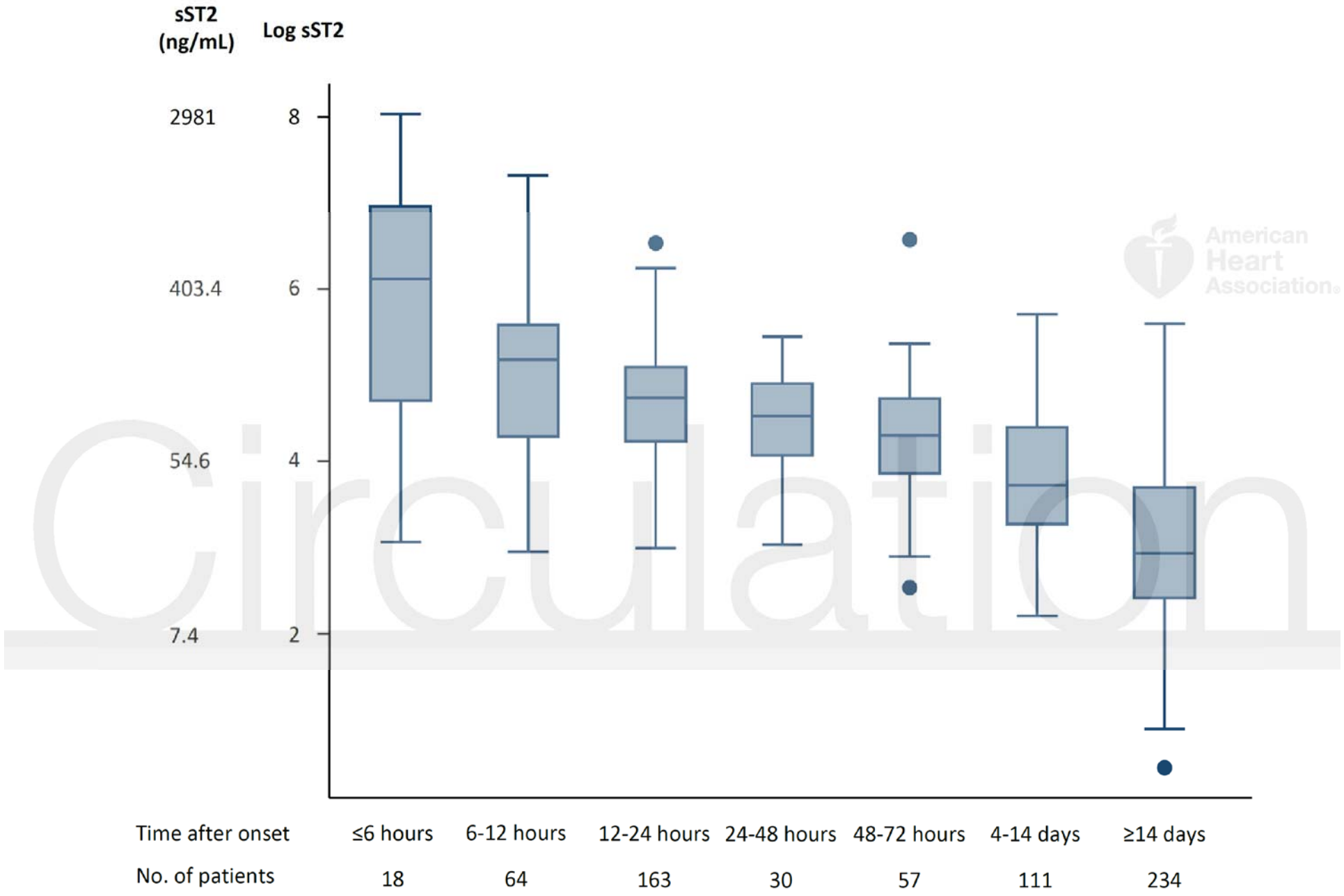


(b) AAD *vs.* PE in the discovery set

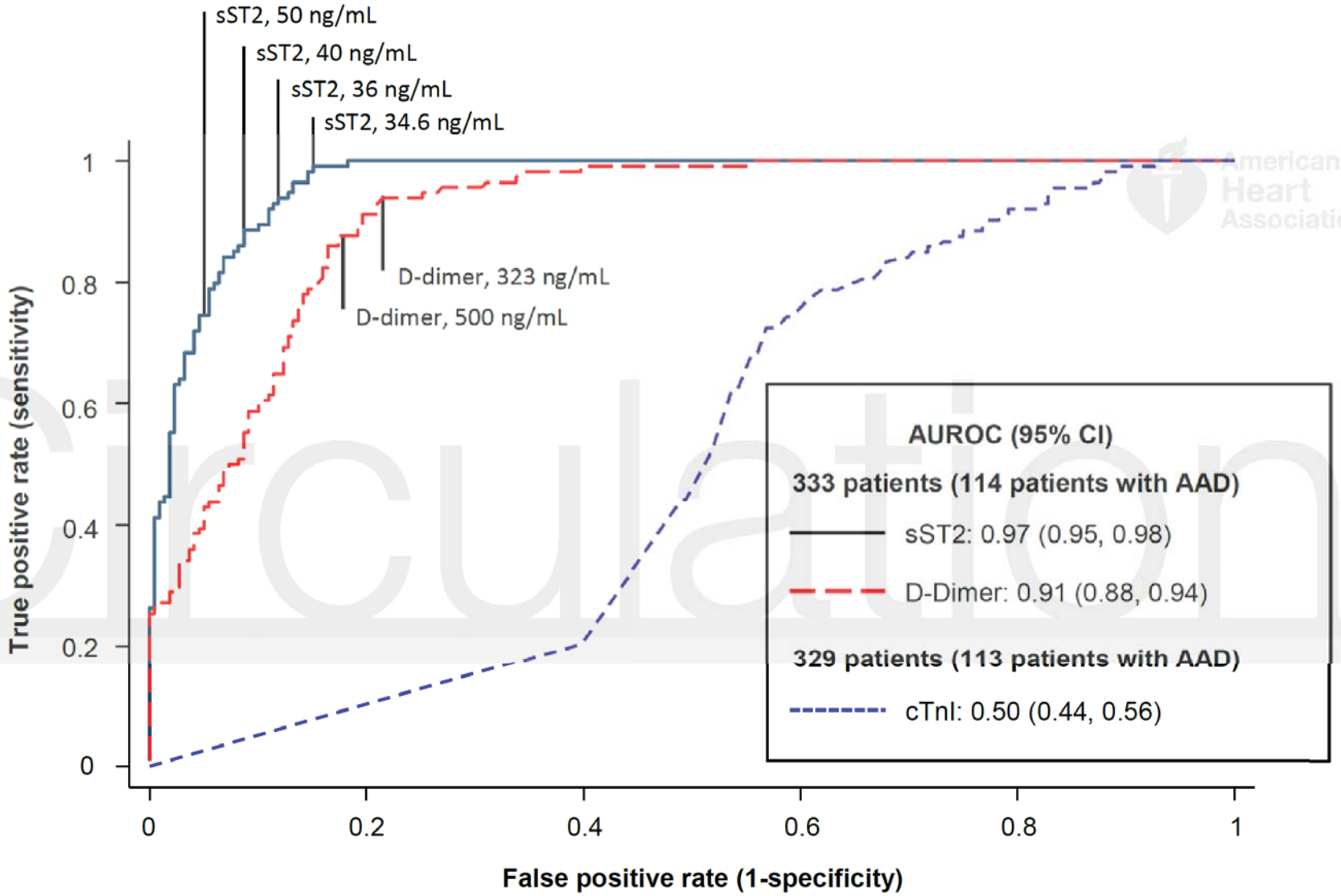


(c) Distribution of sST2 according to disease status in the validation cohort

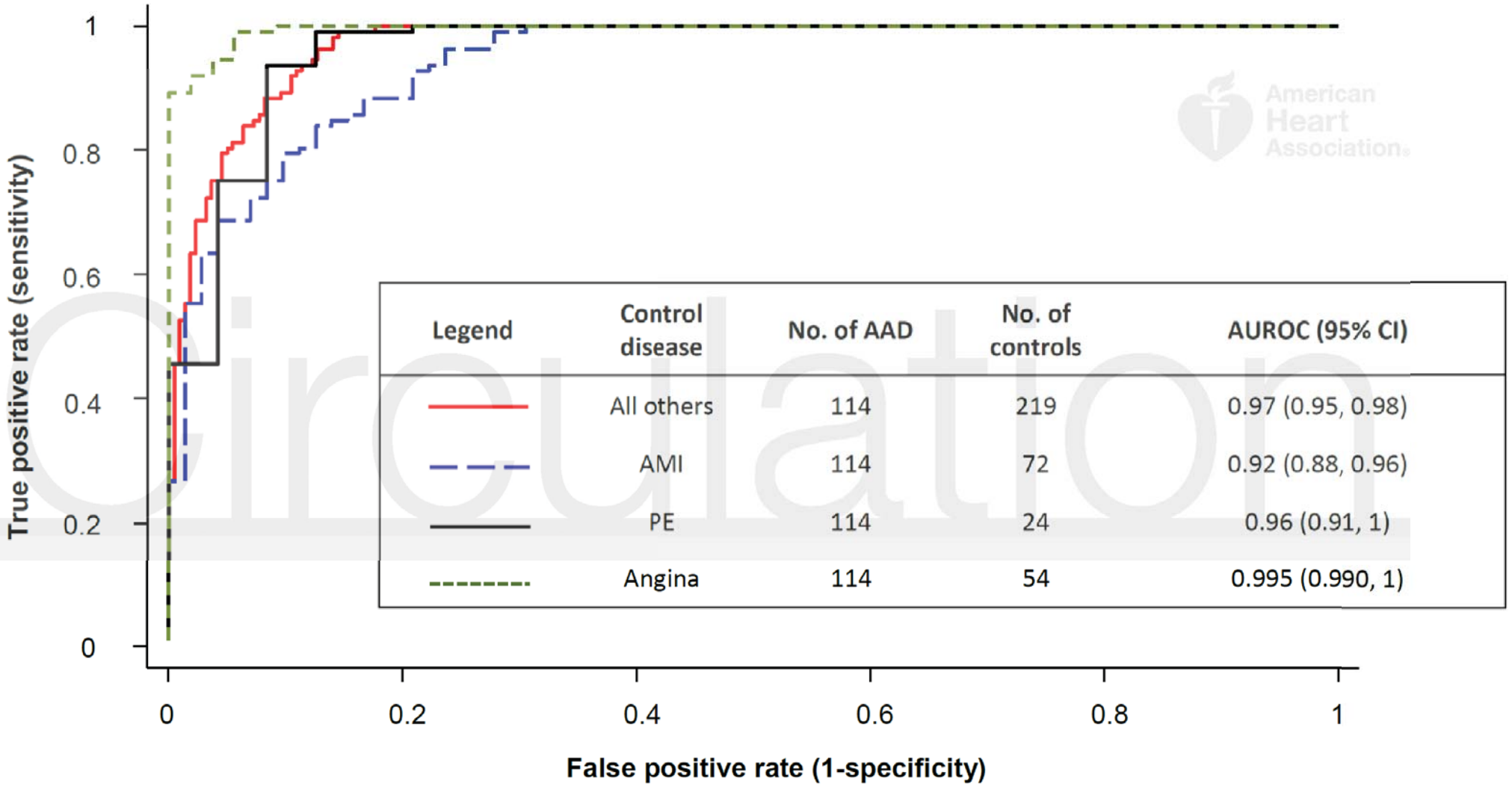




(a) AAD *vs.* all control patients comparing with D-Dimer and cTnI



(b) AAD *vs.* each of control diseases



Legend	Control disease	No. of AAD	No. of controls	AUROC (95% CI)
	All others	114	219	0.97 (0.95, 0.98)
	AMI	114	72	0.92 (0.88, 0.96)
	PE	114	24	0.96 (0.91, 1)
	Angina	114	54	0.995 (0.990, 1)

Magnitude of Soluble ST2 as a Novel Biomarker for Acute Aortic Dissection

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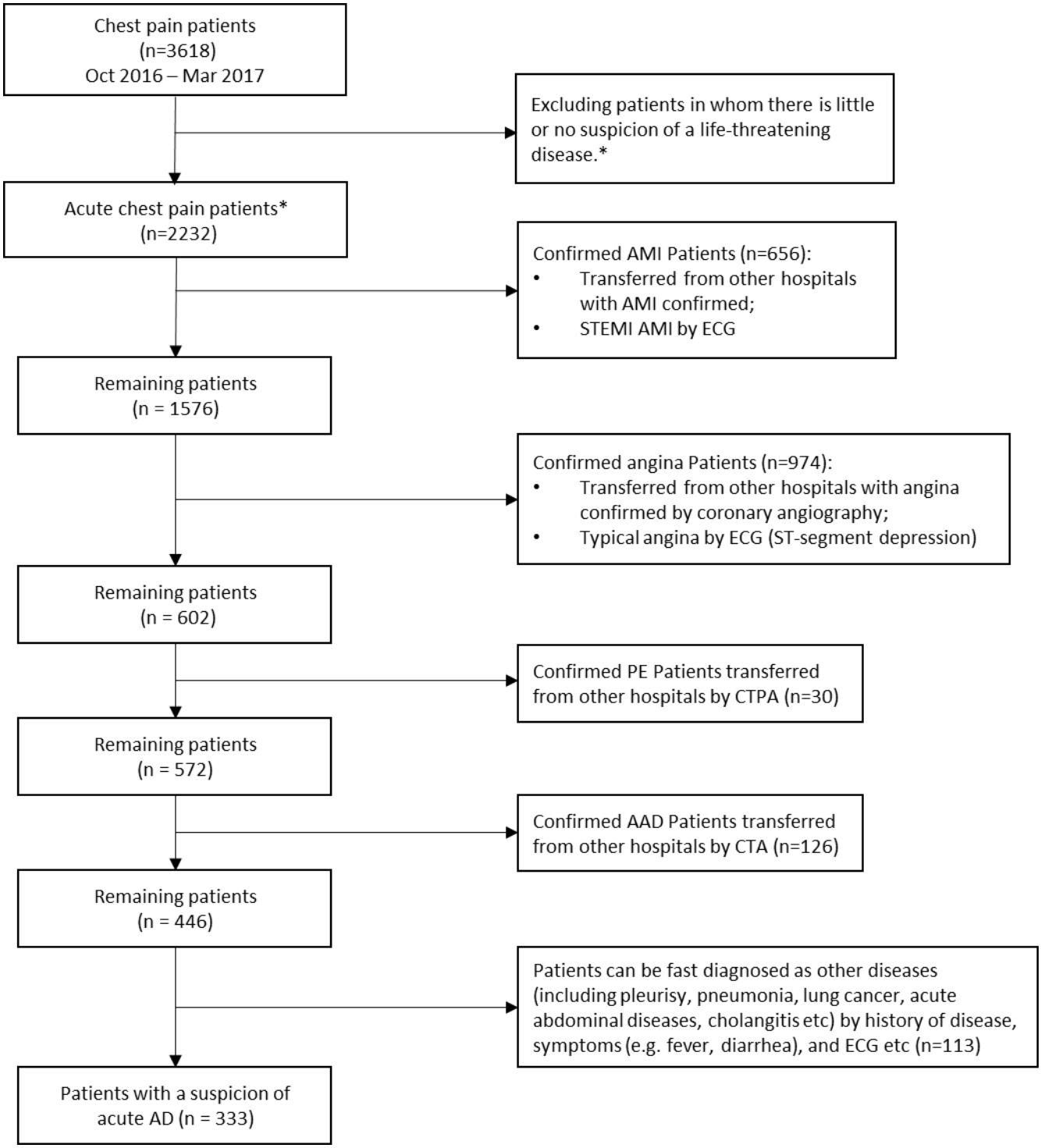
SUPPLEMENTAL MATERIAL

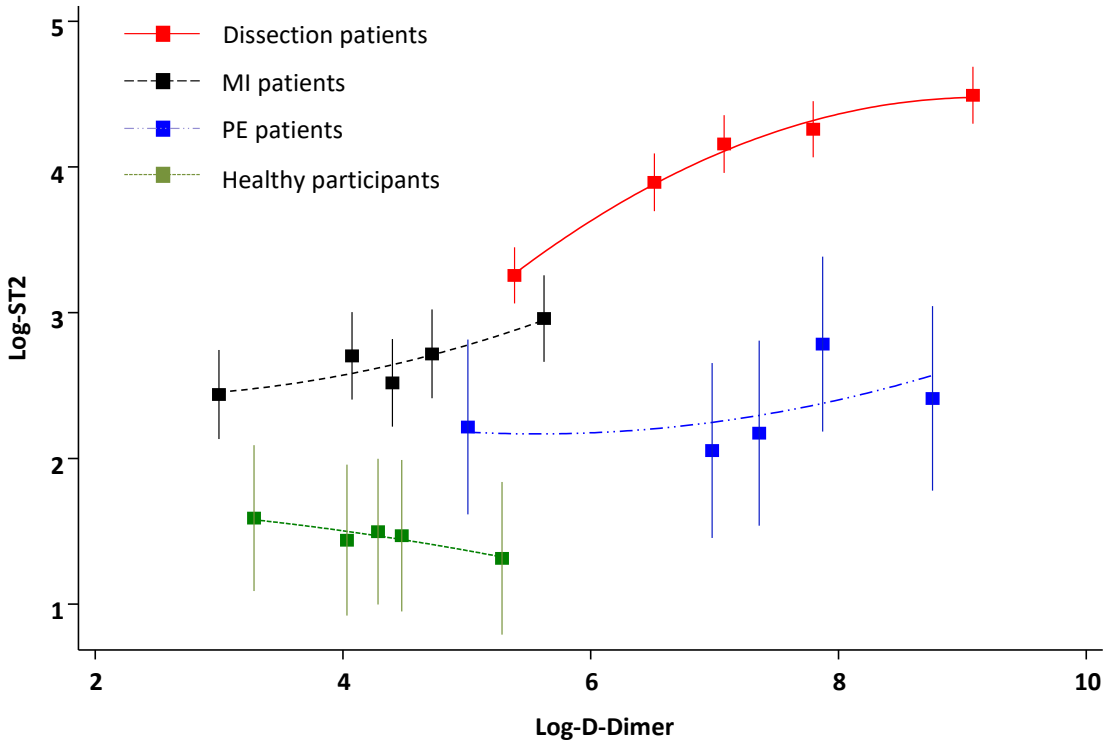
Magnitude of Soluble ST2 as a Novel Biomarker for Acute Aortic Dissection

Supplemental Figure 1 Study population in the validation cohort



* Acute chest pain patients refer to high risk patients quickly for the fast track, excluding patients in whom there is little or no suspicion of a life-threatening disease. AD= aortic dissection; AMI= acute myocardial infarction; PE= pulmonary embolism; STEMI= ST-segment elevation myocardial infarction; ECG=electrocardiograph; CTPA= computed tomography pulmonary arteriography; CTA= computed tomographic angiography.

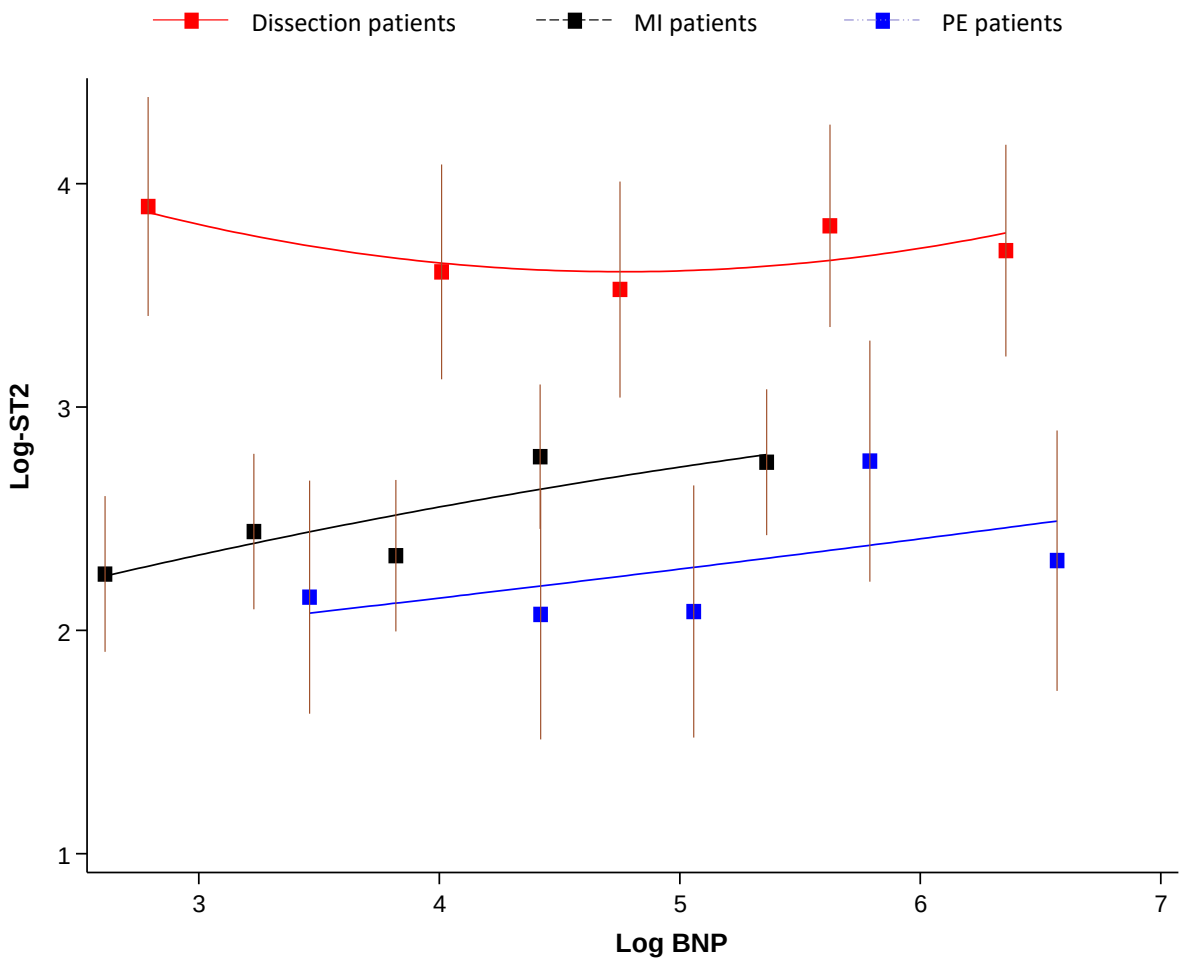
Supplemental Figure 2 Correlation between log sST2 with log D-Dimer by disease status in the discovery set



Analyses involved 1027 participants.
Pearson's correlation coefficients were calculated based on the individual measurements of log-sST2 and log-D-dimer: Pearson's correlation coefficients were 0.39 in patients with dissection, 0.27 in patients with AMI, 0.14 in patients with PE, and −0.06 in healthy participants.

Shapes of association was assessed using linear regression model. To avoid any a priori assumptions regarding shapes, continuous log-D-dimer were entered into the models as fifths of the overall distribution in both men and women combined in each of the disease category. The regression model was adjusted for age and sex. The mean values and corresponding 95% confidence intervals for log-ST2 were estimated by different outcomes, within fifth of log-D-dimer, with age fixed at 50 years. The mean values for log-ST2 so obtained were then plotted against the mean values within fifth of log-D-dimer to assess the shapes of association. Additionally, an inverse-variance weighted polynomial was superimposed on the means to aid interpretation of shapes.

Supplemental Figure 3 Correlation between log sST2 with log BNP by disease status in the discovery set



Analyses involved 269 participants.
Pearson's correlation coefficients were calculated based on the individual measurements of log-sST2 and log-BNP:
Pearson's correlation coefficients were -0.04 in patients with dissection, 0.30 in patients with AMI, 0.21 in patients with PE.

Shapes of association was assessed using linear regression model. To avoid any a priori assumptions regarding shapes, continuous log-BNP were entered into the models as fifths of the overall distribution in both men and women combined in each of the disease category. The regression model was adjusted for age and sex. The mean values and corresponding 95% confidence intervals for log-ST2 were estimated by different outcomes, within fifth of log-BNP, with age fixed at 50 years. The mean values for log-ST2 so obtained were then plotted against the mean values within fifth of log-BNP to assess the shapes of association. Additionally, an inverse-variance weighted polynomial was superimposed on the means to aid interpretation of shapes.

Supplemental Table 1 Baseline characteristics of patients with AAD *vs.* AMI in the discovery set

	Dissection		MI		P value for AAD vs AMI	Healthy Control	
	No. of participants	Mean (SD), Median(IQR) or %	No. of participants	Mean (SD), Median(IQR) or %		No. of participants	Mean (SD), Median(IQR) or %
sST2 (ng/mL)	245	129.18 (71.29, 197.48)	234	14.66 (9.85, 23.32)		67	6.72 (5.1, 8.69)
log-sST2	245	4.82 (0.89)	234	2.71 (0.7)	<0.001	67	1.47 (0.51)
D-Dimer (ng/mL)	245	1877 (920, 3339)	234	80 (55, 126)		67	72 (52, 92)
Log-D-Dimer	245	7.62 (1.28)	234	4.36 (1.19)	<0.001	67	4.26 (0.8)
cTnI	218	0.03 (0.01, 0.16)	233	0.85 (0.14, 6.47)			
Log-cTnI	218	-3.37 (2.73)	223	-0.14 (2.64)	<0.001		
Age (years)	245	50.01 (11.3)	234	53.09 (11.63)	0.003	67	49.36 (8.52)
Sex (male)	245	184 (75.1%)	234	199 (85%)	0.007	67	18 (26.9%)
Smoking status (Current)	245	98 (40%)	229	134 (58.5%)	<0.001	67	23 (34.8%)
Diabetes (Yes)	244	7 (2.9%)	234	62 (26.5%)	<0.001	67	2 (3%)
Hypertension (Yes)	245	163 (66.5%)	234	125 (53.4%)	0.003	67	25 (37.3%)
Hyperlipidemia (Yes)	245	41 (16.7%)	234	152 (65%)	<0.001	67	20 (29.9%)
Marfan (Yes)	240	2 (0.8%)					
History of CAD (Yes)	244	11 (4.5%)	234	8 (3.4)%	0.542		
MI type (STEMI)			234	204 (87.2%)			
Treatment	245		234		<0.001		
Nonoperative		78 (31.8%)		37 (15.8%)			
Surgery		137 (55.9%)		3 (1.3%)			
Endovascular		30 (12.3%)		194 (82.9%)			
BNP>100 pg/mL	18	6 (33.3%)	168	38 (22.6%)	0.309		
Log-BNP (pg/mL)	18	4.22 (1.22)	168	3.87 (1.02)	0.176		
(E wave/A wave)<1	206	121 (58.7%)	198	119 (60.1%)	0.780		
LVEF<50%	240	6 (2.5%)	222	57 (25.7%)	<0.001		

CAD= coronary artery disease; LVEF = left ventricular ejection fraction

Supplemental Table 2 **Baseline characteristics of patients with AAD *vs.* PE in the discovery set**

	Dissection		PE		P value
	No. of participants	Mean (SD), Median(IQR) or %	No. of participants	Mean (SD), Median(IQR) or %	
sST2 (ng/mL)	443	88.64 (45.29, 162.02)	49	9.31 (7.22, 16.43)	
log-sST2	443	4.49 (0.92)	49	2.42 (0.72)	<0.001
D-Dimer (ng/mL)	443	1431 (759, 3019)	49	1594 (964, 2822)	
Log-D-Dimer	443	7.43 (1.23)	49	7.17 (1.94)	0.190
Age (years)	443	49.33 (11.15)	49	63.92 (14.9)	<0.001
Sex (male)	443	335 (75.6%)	49	23 (46.9%)	<0.001
Smoking status (Current)	443	173 (39.1%)	49	14 (28.6%)	0.152
Diabetes (Yes)	442	11 (2.5%)	49	7 (14.3%)	<0.001
Hypertension (Yes)	443	303 (68.4%)	49	24 (49%)	0.006
Hyperlipidemia (Yes)	443	104 (23.5%)	49	22 (44.9%)	0.001
History of CAD (Yes)	442	18 (4.1%)	49	4 (8.2%)	0.189
Marfan (Yes)	436	3 (0.7%)			
BNP>100 pg/mL	37	17 (45.9%)	41	24 (58.5%)	0.266
Log-BNP (pg/mL)	37	4.41 (1.25)	41	5 (1.13)	0.032
(E wave/A wave)<1	368	200 (54.3%)	37	27 (73%)	0.030

Supplemental Table 3 **Comparison of the baseline characteristics of dissection patients in the different cohorts in the discovery set**

	Dissection patients within 24h time from symptoms onset (AAD vs AMI set)		Additional dissection patients for AD vs PE (time from symptoms onset was >24h – 14days)		P value
	No. of participants	Mean (SD), Median(IQR) or %	No. of participants	Mean (SD), Median(IQR) or %	
log-sST2	245	4.82 (0.89)	198	4.09 (0.77)	<0.001
Log-D-Dimer	245	7.62 (1.28)	198	7.2 (1.11)	<0.001
Age (years)	245	50.01 (11.3)	198	48.49 (10.92)	0.154
Sex (male)	245	184 (75.1%)	198	151 (76.3%)	0.777
Smoking status (Current)	245	98 (40%)	198	75 (37.9%)	0.649
Diabetes (Yes)	244	7 (2.9%)	198	4 (2%)	0.569
Hypertension (Yes)	245	163 (66.5%)	198	140 (70.7%)	0.347
Hyperlipidemia (Yes)	245	41 (16.7%)	198	63 (31.8%)	<0.001
BMI (kg/m2)	243	26.18 (7.03)	197	25.82 (3.63)	0.515
Marfan (Yes)	240	2 (0.8%)	196	1 (0.5%)	1.000
History of CAD (Yes)	244	11 (4.5%)	198	7 (3.5%)	0.607
LVEF<50%	240	6 (2.5%)	193	9 (4.7%)	0.221

Supplemental Table 4 Baseline characteristics of patients in the validation cohort

Diagnose	No. of patients	Age (SD)	Male (%)	sST2 (ng/mL)*	D-Dimer (ng/mL)*
AAD	114	51.0 (11.5)	79 (69.3%)	76.4(49.6, 130.3)	2107.5(960, 6373)
Non-AD controls	219	54.2 (11.8)	150 (68.5%)	19.9 (12.5, 29.4)	79 (48, 228)
P values for AAD vs all non-AD controls		0.018	0.880	<0.001	<0.001
AMI	72	54.0 (11.6)	61 (84.7%)	25.0(15.5, 35.0)	71.5(52.5, 126)
PE	24	62.4 (9.6)	12 (50%)	14.9(10.2, 30.1)	2393.5(1373, 2990.5)
Angina	54	55.8 (11.2)	36 (66.7%)	21.5(13.1, 27.6)	75.5(46, 120)
Others	69	50.4 (12)	41 (59.4%)	18.6(10.7, 24.4)	75(42, 254)

Mean age (SD) of all 333 patients in the validation cohort was 53.1 (11.9). 229 (68.8%) were male.
* Median (IQR) were present for skewed variables.

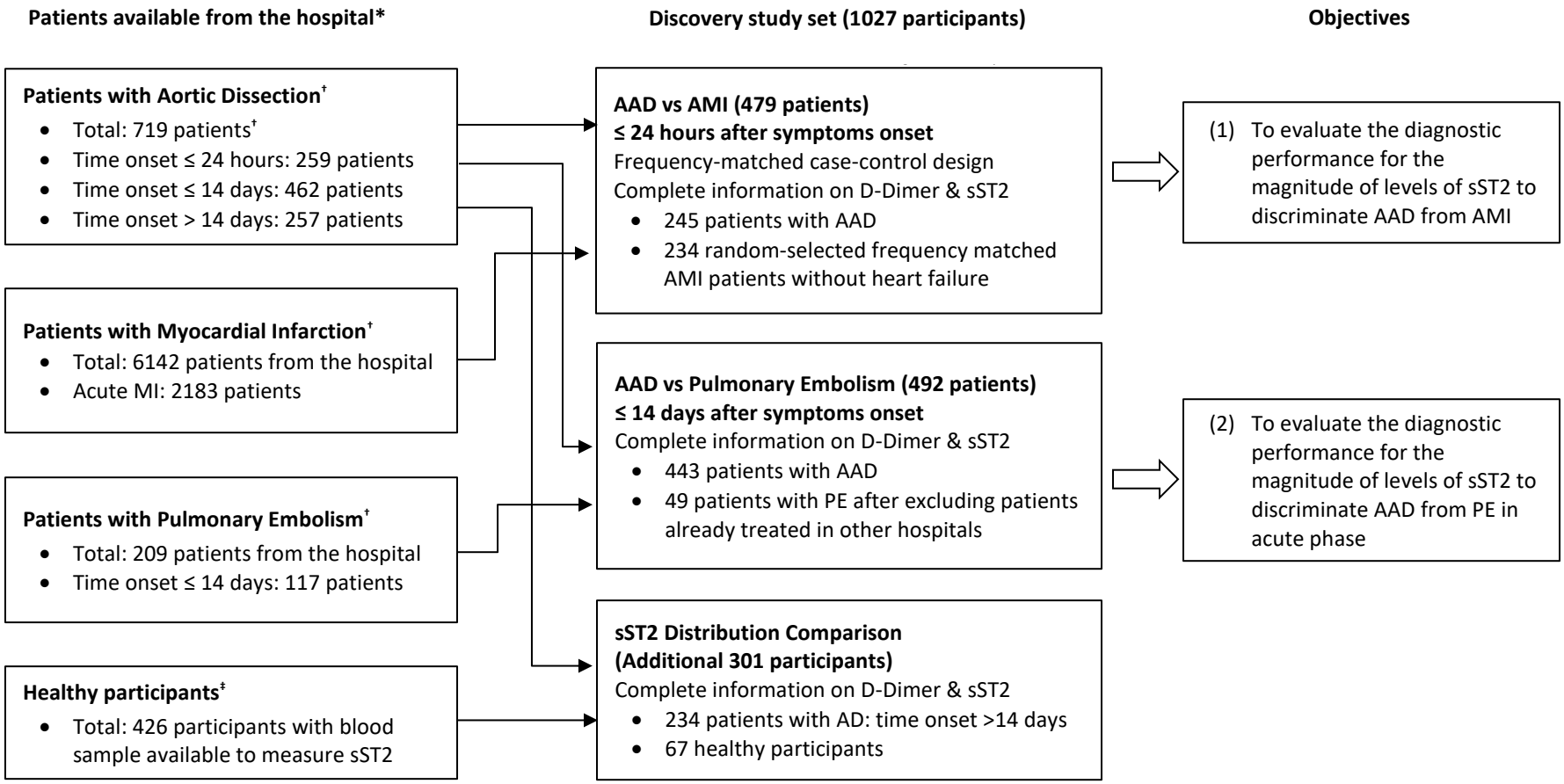
eAppendix 1 Study design and population sample of the discovery set

The discovery set comprised patients who had visited Anzhen Hospital, Beijing, China between March 2014 and September 2015. The study design and the corresponding objectives are shown in **Supplemental Figure A1**. All patients who were referred to the surgical service for evaluation and management of aortic dissection were included. Exclusion criteria of the discovery set were: (a) patients who received packed red blood cells, whole blood, or platelets less than 10 days before the blood sample was taken; (b) patients with aortic trauma, pseudo aneurysm, history of heart failure, renal dysfunction, severe pulmonary diseases, or active cancer; (c) patients who entered the hospital for checkups after surgery. Additionally, for direct comparison between sST2 with D-dimer, we also restricted the study to participants with complete information on both biomarkers.

To evaluate the diagnostic performance of sST2 at discriminating AAD from AMI, we first established a retrospective, frequency-matched, case-control study set. Considering the urgency of the treatment required, we selected all patients with AAD with 24-hour symptom onset. AMI patients within the same time frame were randomly selected from the hospital. Second, because of the limited number of PE cases with 24-hour symptom onset, we established a retrospective, case-control study set including all patients with AAD or PE available with 14-day symptom onset to evaluate the diagnostic performance of sST2 at discriminating AAD from PE. All patients with AMI or PE were subject to the same exclusion criteria described above. The reason to set up two separate case-control groups was mainly due to the consideration of time from symptoms onset and numbers of patients available. Generally speaking, fast diagnosis in the less than 24-hour group would be terribly important for

emergency room. In addition, for AMI patients with time from symptoms onset over 24 hours, treatments would be already applied to majority of patients. Numbers of eligible AMI patients within 24-hour time from symptoms onset were large enough for us to investigate the performance of sST2. However, patients with PE within 24-hour time from symptoms onset were very limited. We also measured sST2 in patients with AD but who visited the hospital after 14 days of symptom onset as well as healthy participants to compare the distribution of sST2 by different time frames and disease status.

Supplemental Figure A1 Study design and objectives in the discovery set



* Patients visited the hospital between Mar 2014 and Sep 2015

† After exclusion criteria were applied. Exclusion criteria were: (a) patients who received packed red blood cells, whole blood, or platelets less than 10 days before the blood sample was taken; (b) patients who had aortic trauma, pseudo aneurysm, diagnosed heart failure, renal dysfunction, severe pulmonary diseases, or active cancer; (c) patients who entered the hospital for checkups after surgery.

‡ Healthy participants were participants without aortic diseases who underwent routine annual health checks and who had a blood sample saved at the hospital.

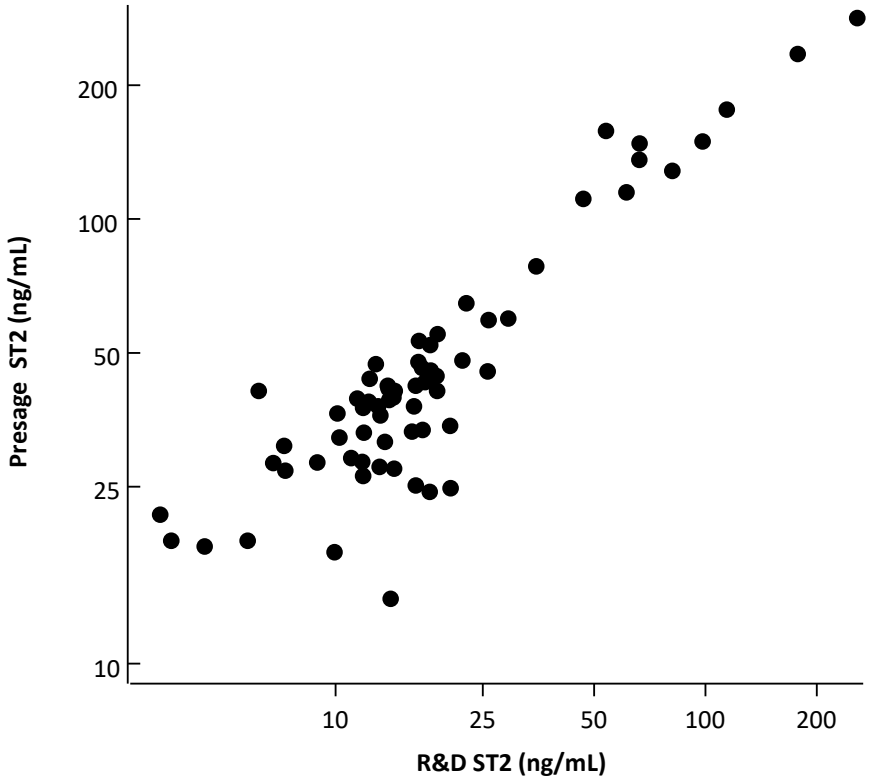
eAppendix 2 Measurement of sST2

For plasma samples, whole blood was drawn into sodium citrate tubes, processed immediately into plasma, and stored at −80°C. Circulating sST2 was measured using a duoset ELISA kit (DY523B-05; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Briefly, a 96-well microtiter plate was coated and incubated overnight at 4°C with 100 µL of capture antibody at a concentration of 1.0 µg/mL. Phosphate-buffered saline Tween-20 (PBST) was used as the washing buffer, comprising phosphate-buffered saline (PBS) containing 0.1% Tween-20. All washing steps were carried out three times between steps. Wells were blocked with 300 µL of 1% bovine serum albumin (BSA) in PBST for 2 h at 37°C. Either 100 µL of a diluted standard (ranging between 62.5 and 2000 pg/mL, seven dilutions) or 100 µL of a plasma sample (in 40-fold dilutions) was added and incubated for 2 h at room temperature. The plate was treated with a second biotinylated antibody (200 ng/mL) for 2 h and a solution of streptavidin-HRP was added, before 100 µL of substrate solution and 50 µL of stop solution were added. The absorbance at 450 nm was determined for each well using a spectra reader (Multiskan MK3 microplate reader; Thermo Scientific). The standard curve was fitted with the 4-parameter logistic method by Origin Lab 2016. The limit of detection for sST2 is 0.019 ng/mL, with mean intra-assay coefficient of variation of <6.0% and mean inter-assay coefficient of variation of <9.5%.

The Presage assay, currently recommended by the US Food and Drug Administration, was not used because it is not validated with our retrospective citrated plasma. We therefore evaluated DY523B-05 by R&D Systems based on the measurement range, recovery ration, limit of detection and required blood sample type before applied in our study. In addition, to verify and calibrate the different assay

methods to measure sST2, we compared sST2 plasma concentrations measured with the R&D Systems assay *vs.* the Presage assay in the evaluation set with 67 patients available. Pearson's correlation coefficient of log-transformed ST2 levels with the two essays was 0.9048 **(Supplemental Figure B1**). Regression equitation was log-Presage (ng/mL) = 1.82 + 0.69* log-R&D (ng/mL). 35 ng/mL using R&D assay was equivalent to 71 ng/mL using Presage assay in our evaluation set. Meanwhile, we assessed the values of standards in R&D kit with Presage assay and the values of standards in Presage kit with R&D assay (ranging between 0.0625 and 2 ng/mL). The measured values were consistent. The corresponding Pearson's correlation was 0.9999. The difference of the absolute values between these assay methods may be caused by the different standards used(1).

Supplemental Figure B1 Correlations between R&D ST2 and Presage ST2



1. Mueller T, Jaffe AS. Soluble ST2--analytical considerations. The American journal of cardiology 2015;115:8B-21B.