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ORIGINAL ARTICLE

# Prognostic role of tissue plasminogen activator in coronary artery disease with or without aortic valve sclerosis

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## Abstract

**Aims** We sought to investigate the relationship between circulating tissue plasminogen activator (t-PA) level and long-term outcomes in stable coronary artery disease patients with or without aortic valve sclerosis (AVSc).

**Methods and results** Serum levels of t-PA were determined in 347 consecutive stable angina patients with ($n$ = 183) or without ($n$ = 164) AVSc. Outcomes were prospectively recorded as planned clinic evaluations every 6 months up to 7 years. The primary endpoint was a composite of cardiovascular death and rehospitalization due to heart failure. The secondary endpoint included all-cause mortality, cardiovascular death, and rehospitalization due to heart failure. Serum t-PA was significantly higher in AVSc than in non-AVSc patients (2131.22 pg/mL vs. 1495.85 pg/mL, $P < 0.001$). For patients with AVSc, those with t-PA level above the median (>1840.68 pg/mL) were more likely to meet the primary and secondary endpoints (all $P < 0.001$). After adjusting for potential confounding factors, serum t-PA level remained significantly predictive for each endpoint in the Cox proportional hazard models. The prognostic value of t-PA was good, with an AUC-ROC of 0.753 ($P < 0.001$). The combination of t-PA with traditional risk factors improved the risk reclassification of AVSc patients, with a net reclassification index of 0.857 and an integrated discrimination improvement of 0.217 (all $P < 0.001$). However, for patients without AVSc, both primary and secondary endpoints were similar, irrespective of t-PA levels.

**Conclusions** Elevated circulating t-PA confers an increased risk for poor long-term clinical outcomes in stable coronary artery disease patients with AVSc.

**Keywords** Aortic valve sclerosis; Coronary artery disease; Prognosis; Tissue plasminogen activator

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## Introduction

Aortic valve sclerosis (AVSc), non-uniform thickening and calcification of aortic valve leaflet in the absence of ventricular outflow obstruction,[1] is increasingly prevalent and usually considered an age-related degenerative process followed by progressive valve motion abnormality.[2] Robust evidence has demonstrated that AVSc shares strikingly similar pathophysiology with atherosclerosis.[3,4] Several traditional risk factors for coronary artery disease (CAD), such as dyslipidemia and hypertension, also affect the incidence of AVSc.[5,6] Besides, the development of AVSc involves chronic inflammatory reactions characterized by endothelial damage, deposition of lipoproteins, infiltration of inflammatory cells, and oxidative stress, which directly results in extracellular matrix degradation and calcification, akin to atherosclerosis as well.[7] Additionally, AVSc predicts all-cause and cardiovascular mortality.[8]

Tissue plasminogen activator (t-PA), a glycoprotein produced by vascular endothelial cells, induces clot dissolution by converting plasminogen to plasmin,[9] and acts as an *in vivo* marker of endothelial cell injury. Circulating t-PA has been shown to correlate with the presence and severity of CAD[10] and plays an important role in plaque instability through destabilizing the fibrous caps of atheromatous plaques[11] and initiating a proteolytic cascade of matrix degradation of the plaque.[4,12] Previously, we observed that circulating t-PA provided good diagnostic performance as a biomarker of AVCs.[4] However, the prognostic value of t-PA in CAD patients, especially for those with AVSc, remains not well understood. In this study, we sought to examine the relationship between serum t-PA and long-term clinical outcomes in stable CAD patients with and without AVSc.

# Methods

## Patient population

A total of 805 patients diagnosed as CAD by coronary angiography in the Department of Cardiovascular Medicine, Shanghai Ruijin Hospital, from September 2011 to June 2012 due to typical anginal symptoms and/or electrocardiographic ST-T wave changes were consecutively recruited. Among them, 571 patients underwent standard transthoracic echocardiography and Doppler flow imaging, according to the recommendations of the American Society of Echocardiography during hospitalization, and were included in the screening procedure for the current study. Patients with acute coronary syndrome or stroke ($n$ = 62), cancer ($n$ = 10), infectious disease ($n$ = 5), severe renal impairment [estimated glomerular filtration rate (eGFR) $<$ 15 mL/min/1.73 $m^2$ or requiring haemodialysis] ($n$ = 11), anticoagulation treatment ($n$ = 26), hypertrophic or dilated cardiomyopathy ($n$ = 17), atrial fibrillation ($n$ = 30), pulmonary heart disease ($n$ = 6), haematological ($n$ = 3), or rheumatic disease ($n$ = 3) were excluded. According to the echo result, we also excluded patients with valve stenosis ($n$ = 12), congenital aortic valve malformation ($n$ = 9), previous aortic valve replacement ($n$ = 8), and congenital heart disease ($n$ = 11). Furthermore, 11 patients refused to participate in the study. The remaining 347 patients were included in the final analysis. AVSc and non-AVCs were identified in 183 and 164 patients, respectively (*Figure 1*).

The study protocol was approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, and written informed consent was obtained from all patients.

## Definitions and measurements

AVSc was defined as non-transparent aortic valve leaflets with focal areas of mild thickening or increased echogenicity without motive restriction and a peak flow velocity across the aortic valve $<$2.0 m/s (*Figure 2*).[13] CAD was diagnosed when there was $\geq$50% luminal diameter stenosis in at least one major epicardial coronary artery, according to the lesion classification scheme of the American College of Cardiology/American Heart Association.[14] Renal function was evaluated by eGFR, which was calculated using the Modification of Diet in Renal Disease equation.[15] Serum level of t-PA was measured in the fasting venous blood samples using an ELISA (Tissue Plasminogen Activator (t-PA) Human SimpleStep ELISA Kit, Abcam, Cat No. ab190812) according to the product instructions.

## Outcomes

Patients were followed until death or the last visit. Outcomes were prospectively recorded as planned clinic evaluations every 6 months up to 7 years after enrollment. The primary endpoint was a composite of cardiovascular death or rehospitalization due to heart failure, and the second endpoint included cardiovascular death, rehospitalization due to heart failure, and all-cause mortality. Heart failure was diagnosed based on clinical symptoms with an elevation of plasma N-terminal pro-brain natriuretic peptide (NT-proBNP). Death was identified primarily by the review of medical records and direct contact with the patient's family. Cardiovascular death included sudden cardiac death or death caused by acute myocardial infarction. Rehospitalization due to heart failure was confirmed.

## Statistical analysis

Continuous variables are expressed as the mean and standard deviation when normally distributed and the median with interquartile interval when not normally distributed. Categorical data are summarized as proportions and frequencies. Continuous and categorical variables were compared by independent $t$-test and nonparametric or $\chi^2$ test, respectively. Kaplan–Meier (KM) curves were constructed to predict the outcome for each endpoint (primary endpoint, cardiovascular death, rehospitalization, and all-cause mortality). Log-rank test was used to compare survival situations among groups. Cox proportional hazard analysis was used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) and assess whether t-PA could predict the prognosis of patients using log-transformed t-PA levels. Multivariate analyses were performed to evaluate the association of t-PA with the composite primary outcome, cardiovascular death, rehospi-

**Figure 1** Flowchart of patient enrollment.

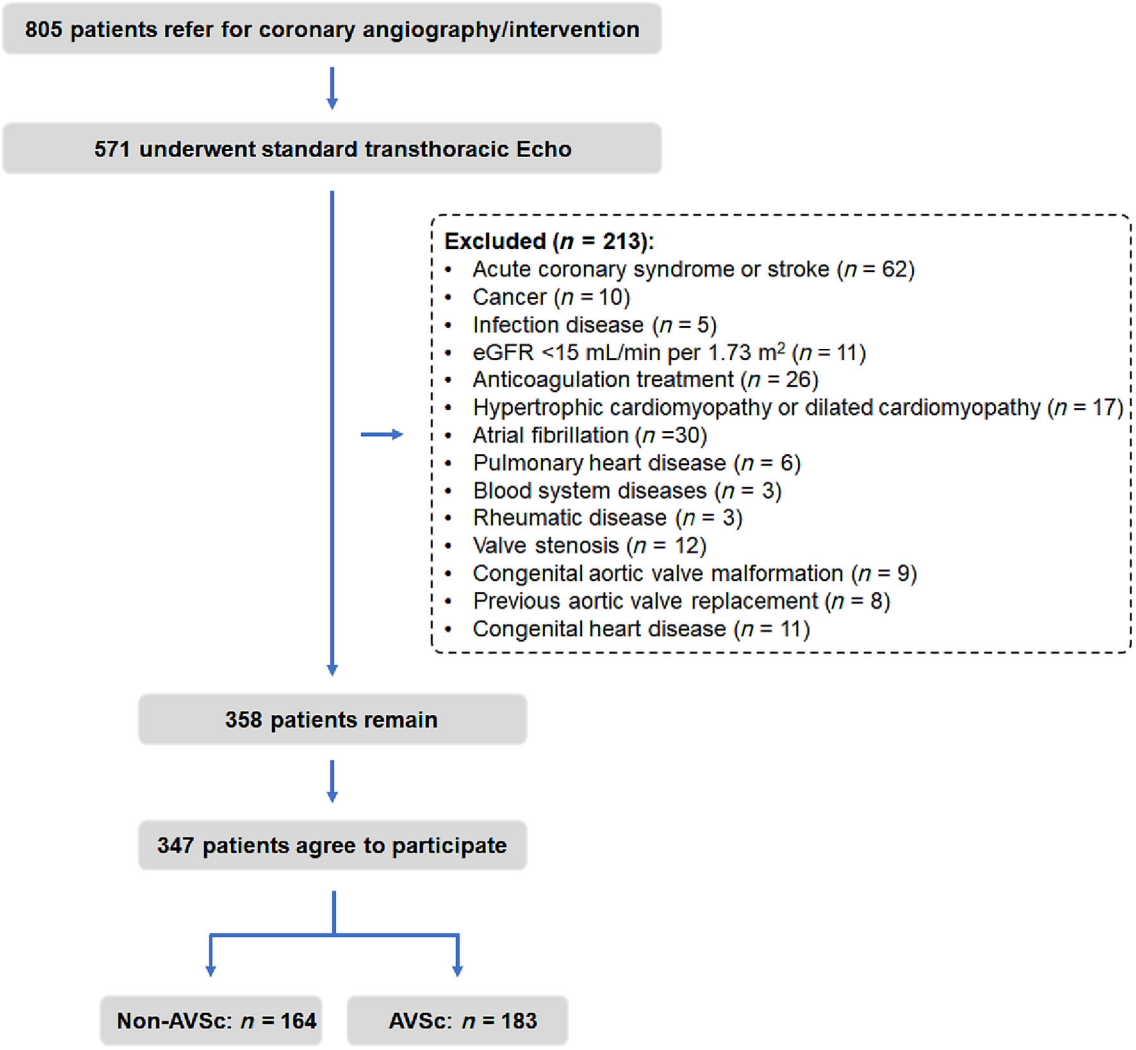
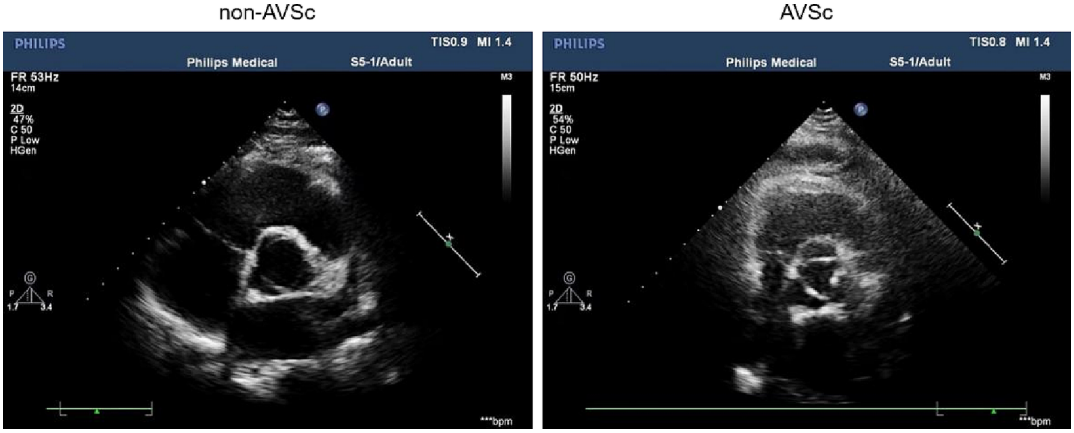



**Figure 2** Typical echo images of non-AVSc (left) and AVSc (right).





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talization, and all-cause mortality. The results were first adjusted for age and sex and then for the full model, including age, sex, risk factors for CAD, alcohol consumption, glycosylated haemoglobin (HbA1c), lipid profiles including triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein (HDL-C), r-glutamine transaminase (r-GT), eGFR, and drug usage. Moreover, each of the analyses was performed separately in CAD patients with and without AVSc.

All statistical analyses were performed by SPSS software (version 19.0) or R software (version 4.0.2). Statistical significance was set at two-tailed, and $P$ values $<0.05$ were considered statistically significant.

# Results

## Baseline clinical characteristics

Compared with non-AVSc patients, those with AVSc were older and had a higher proportion of males and hypertension (*Table 1*, all $P < 0.05$). In addition, serum levels of TG, HDL-C and eGFR were lower, but the Hb1Ac level was higher in patients with AVSc (all $P < 0.05$). However, the two groups did not differ with respect to the percentage of diabetes, smokers, body mass index (BMI), and fast blood glucose. The proportion of medications with statins, beta-blocker, and antiplatelet agents was higher in the AVSc group (*Table 1*). Moreover, seven patients developed aortic valve stenosis, and their t-PA level was higher compared with those who did not develop valve stenosis (3085.68 pg/mL vs. 2133.01 pg/mL, $P = 0.006$). Further analysis showed that in non-AVSc group, patients with high t-PA level were older and had greater BMI compared with those with low t-PA level. In AVSc group, patients with high t-PA level had higher rGT and lower HDL and eGFR (*Table S1*).

## Serum tissue plasminogen activator (t-PA) and outcome in aortic valve sclerosis (AVSc) and non-AVSc patients

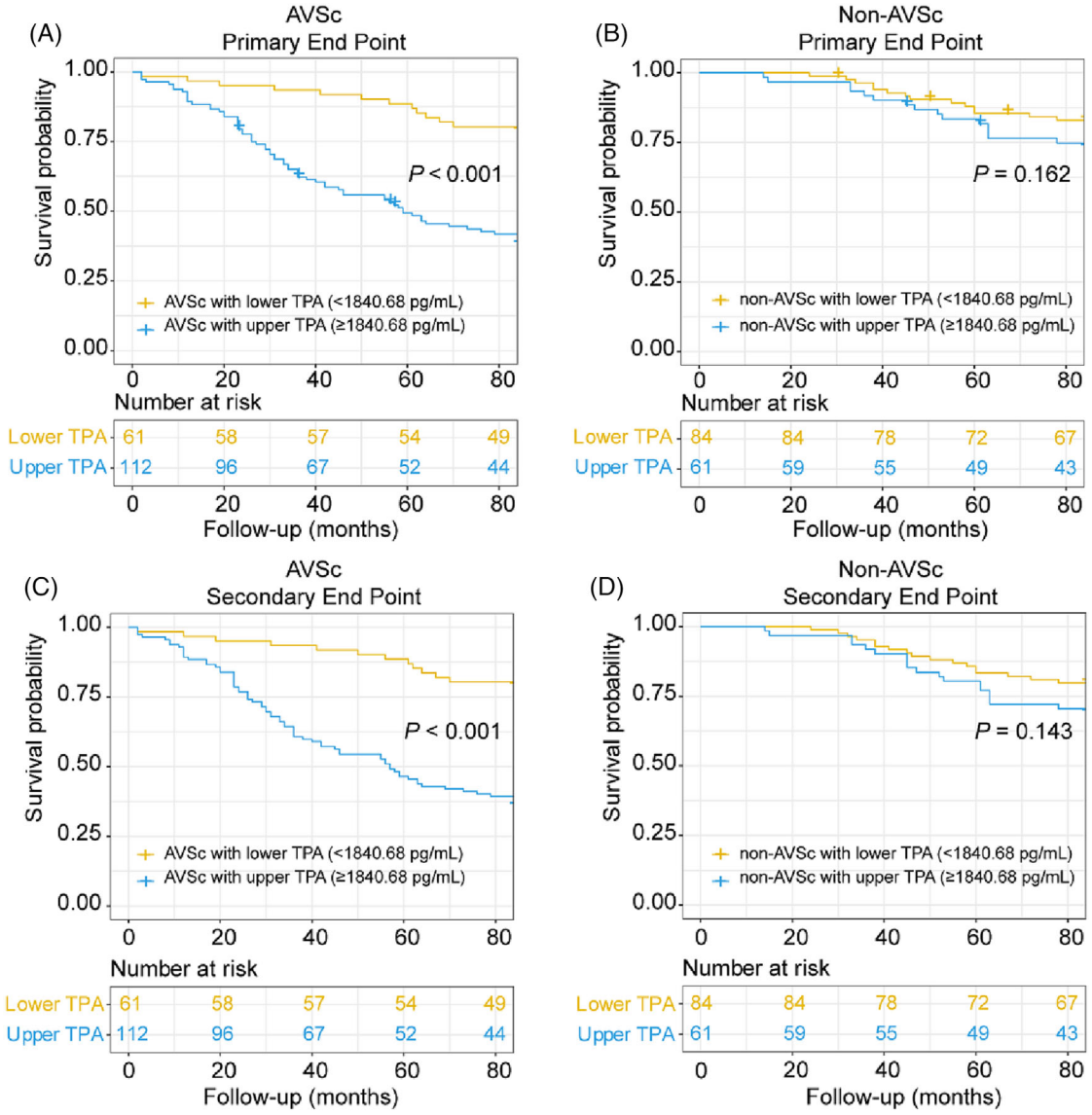
For patients with AVSc, those with higher t-PA levels in serum (above the median value 1840.68 pg/mL) were more likely to meet primary ($P < 0.001$, *Figure 3A,B*) and secondary endpoints ($P < 0.001$, *Figure 3C,D*). Higher t-PA levels conferred an increased risk of the primary endpoint (HR = 2.934, $P < 0.001$), cardiovascular death (HR = 3.712, $P < 0.001$), re-hospitalization due to heart failure (HR = 3.220, $P < 0.001$), and all-cause mortality (HR = 3.561, $P < 0.001$) (*Figure 4*). After adjusting for traditional confounding factors, including age, sex, BMI, smoking, alcohol consumption, history of diabetes or hypertension, levels of HbA1c, TG, TC, HDL, LDL, rGT, eGFR, and use of aspirin, angiotensin-converting enzyme inhibitor (ACEI) /angiotensin receptor blocker (ARB), beta-blocker or statins, serum t-PA still remained significantly predictive for the primary endpoint (HR = 2.920, $P < 0.001$), cardiovascular death (HR = 3.237, $P < 0.001$), rehospitalization due to heart failure (HR = 3.284, $P < 0.001$), and all-cause mortality (HR = 3.111, $P < 0.001$) (*Figure 4*). The C-statistic analyses revealed an increased predictive value for the primary endpoint when considering t-PA levels (AUC-ROC = 0.846, $P < 0.001$) (*Table 2*). The addition of serum

**Table 1** Baseline characteristics of CAD patients with or without AVSc

| | AVSc ($n = 183$) | Non-AVSc ($n = 164$) | $P$ value |
|---|---|---|---|
| Age, years | 74.00 (66.00, 80.00) | 61.00 (56.00, 68.00) | <0.001 |
| Male, sex | 117 (63.93%) | 85 (51.83%) | 0.022 |
| Smoking | 57 (31.15%) | 41(25.00%) | 0.204 |
| Alcohol use | 21 (11.5%) | 17 (10.37%) | 0.741 |
| Hypertension | 144 (78.69%) | 109 (66.46%) | 0.011 |
| DM | 73 (39.89%) | 51 (31.10%) | 0.088 |
| Fasting blood-glucose | 5.02 (4.56, 5.95) | 4.92 (4.50, 5.68) | 0.451 |
| Hb1Ac% | 6.00 (5.70, 6.80) | 5.90 (5.60, 6.40) | 0.029 |
| TG mmol/L | 1.30 (0.98, 2.01) | 1.47 (1.17, 2.07) | 0.034 |
| TC | 3.85 (3.13, 4.53) | 3.95 (3.36, 4.62) | 0.086 |
| HDL | 1.05 (0.91, 1.23) | 1.10 (0.96, 1.29) | 0.045 |
| LDL mmol/L | 2.25 (1.74, 2.99) | 2.32 (1.72, 2.89) | 0.956 |
| rGT mmol/L | 21.00 (15.00, 29.00) | 19.00 (12.25, 30.00) | 0.295 |
| eGFR mL/min/1.73 $m^2$ | 72.51 ± 19.24 | 87.06 ± 17.46 | <0.001 |
| T-PA pg/mL | 2131.22 (1415.44, 2690.03) | 1495.85 (1043.11, 2145.82) | <0.001 |
| Aspirin usage | 156 (85.25%) | 108 (65.85%) | <0.001 |
| ACEI/ARB | 121 (66.12%) | 106 (64.63%) | 0.771 |
| Beta-blocker | 139 (75.96%) | 104 (63.41%) | 0.011 |
| Statin | 156 (85.25%) | 121 (73.78%) | 0.008 |

ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BMI, body mass index; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; rGT, γ-glutamyl transferase; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglyceride; TC, total cholesterol; T-PA, tissue plasminogen activator.

**Figure 3** Prediction of primary and secondary outcomes according to median t-PA level. Primary outcomes of AVSc (A) and non-AVSc (B), and secondary outcomes of AVSc (C) and non-AVSc (D).




t-PA also improved the risk reclassification of AVSc patients, with a net reclassification index (NRI) of 0.857 and an integrated discrimination improvement (IDI) of 0.217 (both $P < 0.001$, *Table 2*). In fact, this value of t-PA level was associated with a six-fold increased risk for the primary endpoint (HR = 6.218 [3.168–12.204], $P < 0.001$) and five- to seven-fold increased risk for secondary endpoints (cardiovascular death, HR = 6.973 [2.460–19.764], $P < 0.001$; rehospitalization due to heart failure, HR = 5.650 [2.841–11.235], $P < 0.001$; all-cause mortality, HR = 7.626 [2.705–21.498], $P < 0.001$) (*Table 3*). The AUC-ROC analysis showed that an optimal cutoff of t-PA 1818.71 pg/mL best predicted the primary end point in these patients. The number of patients with t-PA levels above and below this value was 170 and 177, respectively. Furthermore, there was a significant difference in event rates stratified according to the t-PA cut-off value (*Figure 5A,B*).

In contrast, both primary and secondary endpoints were similar for non-AVSc patients, irrespective of t-PA levels (*Figures 3C,D* and *4*). Moreover, serum t-PA did not provide additive value in risk prediction for these patients (*Tables 2* and *3*).

## Discussion

Our results indicated that elevated circulating t-PA level was significantly associated with long-term adverse outcomes in stable CAD patients with AVSc.

**Figure 4** Forest plot of t-PA as a predictor for endpoints in CAD patients with or without AVSc. T-PA level was analysed as a log-transformed continuous variable. Model 1: adjusted for age and sex, Model 2: adjusted for age and sex, and then for the whole model, including age, sex, risk factors for coronary artery disease, alcohol consumption, HbA1c, lipid profiles, r-GT, eGFR, and drug usage.

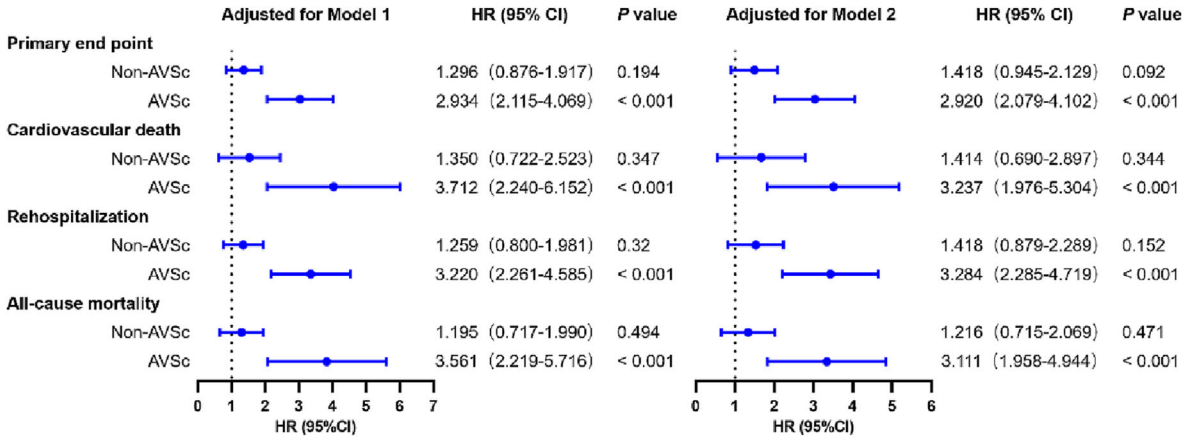



**Table 2** C-statistic analysis of t-PA as predictors for primary endpoint

| | Total cohort ($n$ = 347) | AVSc ($n$ = 183) | Non-AVSc ($n$ = 164) |
|---|---|---|---|
| AUC-ROC (T-PA only) | 0.709 (0.650–0.768) | 0.753 (0.682–0.823) | 0.561 (0.442–0.681) |
| | $P < 0.001$ | $P < 0.001$ | 0.301 |
| AUC-ROC (Traditional factors only) | 0.657 (0.595–0.719) | 0.679 (0.599–0.759) | 0.581 (0.466–0.697) |
| | $P < 0.001$ | $P < 0.001$ | 0.171 |
| AUC-ROC (Traditional factors + t-PA) | 0.750 (0.693–0.806) | 0.846 (0.789–0.904) | 0.581 (0.466–0.697) |
| | $P < 0.001$ | $P < 0.001$ | 0.171 |
| NRI | 0.493 (0.272–0.714) | 0.857 (0.590–1.124) | 0.165 (−0.236–0.566) |
| | $P < 0.001$ | $P < 0.001$ | 0.419 |
| IDI | 0.096 (0.064–0.128) | 0.217 (0.156–0.277) | 0.027 (−0.006–0.061) |
| | $P < 0.001$ | $P < 0.001$ | 0.106 |

Traditional factors included age, sex, BMI, smoking, alcohol use, history of DM, history of hypertension, HbA1c, TG, TC, HDL, LDL, rGT, eGFR, aspirin use, ACEI/ARB use, beta-blocker use, and statin use.

**Table 3** Multivariate Cox proportional hazard models for t-PA as a predictor of endpoints in CAD patients with or without AVSc

| | AVSc | | Non-AVSc | |
|---|---|---|---|---|
| t-PA level | HR (CI) | *P* value | HR (CI) | *P* value |
| Primary endpoint | | | | |
| <1818.71 pg/mL | 1 | 1 | 1 | 1 |
| ≥1818.71 pg/mL | 6.218 [3.168–12.204] | <0.001 | 2.073 [0.972–4.423] | 0.059 |
| Cardiovascular death | | | | |
| <1818.71 pg/mL | 1 | 1 | 1 | 1 |
| ≥1818.71 pg/mL | 6.973 [2.460–19.764] | <0.001 | 2.734 [0.823–9.081] | 0.101 |
| Rehospitalization due to heart failure | | | | |
| <1818.71 pg/mL | 1 | 1 | 1 | 1 |
| ≥1818.71 pg/mL | 5.650 [2.841–11.235] | <0.001 | 1.833 [0.719–4.676] | 0.205 |
| All-cause mortality | | | | |
| <1818.71 pg/mL | 1 | 1 | 1 | 1 |
| ≥1818.71 pg/mL | 7.626 [2.705–21.498] | <0.001 | 2.096 [0.751–5.847] | 0.157 |

In Model 2, traditional factors as in *Table 2* were adjusted.

Previous studies showed that t-PA secretion was positively related to the severity of myocardial ischaemia[16–18] and coronary stenosis.[19,20] Besides, circulating t-PA served as a reliable predictor of subsequent myocardial infarction[21,22] and a risk factor for cardiac arrhythmia after thrombolytic therapy.[23–25] The present study is the first to show that t-PA level in serum was associated with adverse outcomes for CAD patients with AVSc. Interestingly, a significant association between t-PA


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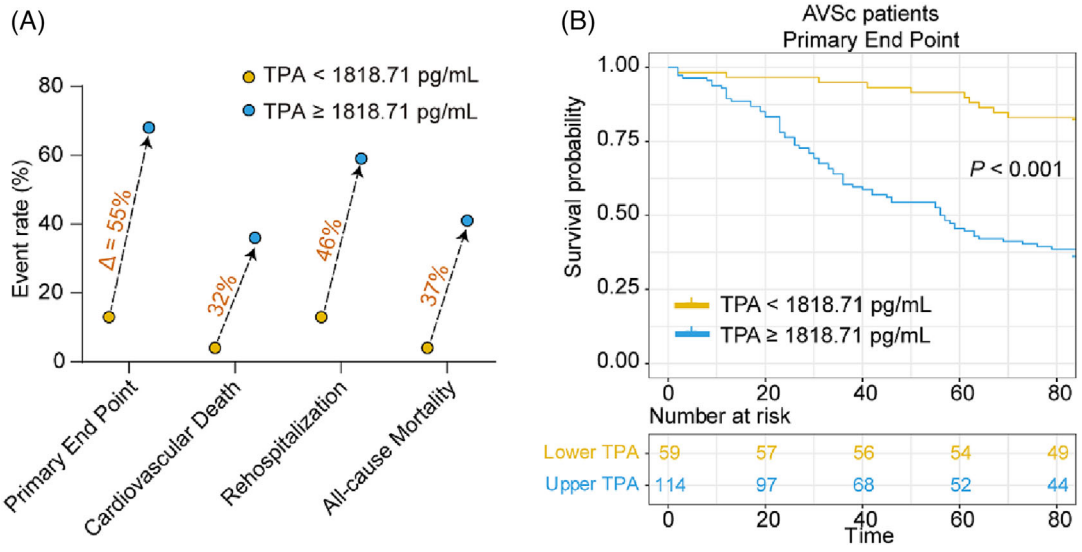
**Figure 5** Event rates and Kaplan–Meier curve analysis according to estimated t-PA cut-off value in patients with AVSc. (A) Rates of the primary endpoint, cardiovascular death, rehospitalization, and all-cause mortality for patients below and above t-PA cut-off value (1818.71 pg/mL) in AUC-ROC analysis. (B) Kaplan–Meier curves for primary endpoint for all patients according to the cut-off value of t-PA level.




level and adverse outcomes was not observed in CAD patients without AVSc, suggesting that t-PA may have potential relationships with the pathological manifestation of AVSc.

T-PA has been reported to directly regulate cytokine signalling through non-enzymatic mechanisms, thus promoting inflammatory responses in fibrinolytic systems.[26] Several studies have shown that t-PA could damage the endothelial cell barrier by inhibiting the Sonic Hedgehog (Shh) pathway[27] or induction of C-C motif chemokine ligand 2 (CCL2).[28] Although thrombolytic therapy for acute ischaemic stroke is applicable, t-PA-induced damages to human microvascular endothelial cells still occur.[29] As endothelial-to-mesenchymal transition was influential in the pathological process of AVSc,[30,31] t-PA-mediated endothelial dysfunction might be a possible trigger of AVSc. Overall, these observations support a notion that elevated t-PA might be responsible for the deterioration of endothelial cells, leading to poor prognosis of CAD patients with AVSc.

It is well recognized that t-PA activates matrix metalloproteinases (MMPs),[32] including MMP-2,[33] MMP-8,[34] and MMP-9,[35,36] which are associated with the progression of ventricular remodelling,[37,38] valvular disease,[39] and heart failure.[40] The expression of MMP-9 was significantly decreased after t-PA knockout with focal cerebral ischaemia. In human microvascular endothelial cells, the expression of MMP-9 was up-regulated when adding exogenous recombinant t-PA. Moreover, the development of AVSc involves phenotypic changes of aortic valve interstitial cells through the osteogenic pathway, substantiating the role of interstitial components in the pathological progression of AVSc.[41,42] Therefore, it was postulated that high circulating t-PA might induce up-regulation of MMPs expression, leading to remodelling of the local extracellular matrix in the aortic valve and accelerating subsequent aortic valve fibrosis. Nevertheless, the detailed mechanisms of how t-PA influences the development of AVSc still require more investigations.

Our study showed that in patients without AVSc, both primary and secondary endpoints were similar, irrespective of t-PA levels. The reason for this might be multifactorial. AVSc was reported to predict all-cause and cardiovascular mortality.[8] Moreover, the development of AVSc shares certain similar mechanism of atherosclerosis, such as endothelial damage, deposition of lipoproteins, and calcification,[7] which suggests that AVSc itself might be a marker of more widespread arterial disease.

There are some limitations in our study. First, we only enrolled patients with CAD in our study, and the sample size was relatively small. Further studies with a large cohort of patients with versatile backgrounds, including those without CAD, are warranted to prove our results. Second, the diagnosis of AVSc was mainly based on transthoracic echocardiography. Cardiac computed tomography could provide a quantitative assessment. Last, despite careful adjustment for major known confounding factors, unspecified elements may also interfere with our results.

Taken together, this study demonstrates circulating t-PA level as an independent factor for the prognosis of stable CAD patients with AVSc. The findings may provide insight to physicians who are involved in decision-making for these patients, and further large-scale, prospective studies are warranted to confirm our results.

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## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Baseline characteristics between patients with high or low t-PA levels in non-AVSc and non-AVSc groups.

## Conflict of interest

None.

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