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Abstract

Background Trimethylamine *N*-oxide (TMAO) has been shown to promote the development of atherosclerosis. However, the relationship between plasma TMAO and neoatherosclerosis, an important underlying mechanism of very late stent thrombosis (VLST), is unknown.

Methods This post hoc study investigated the association between TMAO and neoatherosclerosis in two independent cohorts. These included a control group of 50 healthy volunteers and a study cohort of 50 patients with VLST who presented with ST-segment elevation myocardial infarction and underwent optical coherence tomography examination. Of the 50 patients with VLST, 23 had neoatherosclerosis and 27 did not have neoatherosclerosis. Patients with neoatherosclerosis were further divided into two subgroups, including 14 patients with plaque rupture and 9 without plaque rupture.

Results The plasma TMAO levels, detected using mass spectrometry, were significantly higher in patients with VLST than in healthy individuals (median [interquartile range]: 2.50 [1.67-3.84] vs. 1.32 [0.86-2.44] μM; P<0.001). Among the VLST patients, the plasma TMAO levels were significantly higher in patients with neoatherosclerosis than in those without neoatherosclerosis (3.69 [2.46-5.29] vs. 1.96 [1.39-2.80] μM; P<0.001). In addition, in patients with neoatherosclerosis, patients with plaque rupture had significantly higher plasma TMAO concentrations than those without plaque rupture (4.51 [3.41-5.85] vs. 2.46 [2.05-3.55] μM; P=0.005). Multivariate analysis indicated that TMAO was an independent predictor

of neoatherosclerosis (odds ratio 3.41; 95% confidence interval: 1.59-7.30; P=0.002). Moreover, the area under the receiver operating characteristic curve for TMAO, differentiated by neoatherosclerosis, was 0.85.

Conclusions Plasma TMAO was significantly correlated with neoatherosclerosis and plaque rupture in patients with VLST.

Keywords Trimethylamine *N*-oxide; Optical coherence tomography; Stent thrombosis; Neoatherosclerosis; Plaque rupture

Brief summary

The associations between plasma TMAO and neoatheroslcerosis and between plasma TMAO and plaque rupture, a complication of neoatherosclerosis, were investigated in patients with VLST. Plasma TMAO levels were significantly higher in patients with neoatherosclerosis than in those without neoatherosclerosis. Multivariable logistic regression analysis revealed that plasma TMAO was an independent predictor of neoatherosclerosis. In addition, in patients with neoatherosclerosis, patients with plaque rupture had significantly higher plasma TMAO concentration than those without plaque rupture.

Introduction

Very late stent thrombosis (VLST), defined as thrombus formation at the stented artery segment occurring >1 year after index stent implantation, is a catastrophic and life-threatening complication of percutaneous coronary intervention (PCI).[1] The incidence of VLST appears to be similar regardless of whether a bare metal stent (BMS) or drug eluting stent (DES) is used, with a cumulative rate of 1.1% for BMSs and 1.0% for DESs at 3-year follow-up.[2] Although the prevalence of VLST is low, it presents a severe health burden worldwide due to its high case fatality, reported to be as high as 23.3% at 1-year follow-up,[3] and the large number of patients with coronary artery disease (CAD) treated by stent implantation.[4]

In-stent neoatherosclerosis has recently been identified as an important mechanism of VLST formation using optical coherence tomography (OCT),[5, 6] an intravascular imaging modality with a high resolution, which enables *in vivo* visualization of the stented segment.[7] Despite the prominent contribution of neoatherosclerosis in the development of VLST, its mechanisms and contributing factors are currently unknown.

Trimethylamine *N*-oxide (TMAO), a gut-dependent metabolite generated from diets rich in phosphatidylcholine, has recently been shown to play a role in the pathogenesis and development of atherosclerosis.[8, 9] In addition, elevated plasma TMAO levels have been shown to play a predictive role in determining the burden of atherosclerosis[10, 11] and the risk of future cardiovascular events in patients with CAD.[12, 13] However, the relationship between plasma TMAO and neoatherosclerosis

in patients with VLST remains unknown. Therefore, we aimed to investigate the relationship between plasma TMAO and neoatherosclerosis in a prospective cohort consisting of patients with ST-segment elevation myocardial infarction (STEMI) who underwent OCT examination of the culprit lesion (Optical Coherence Tomography Examination in Acute Myocardial Infarction [OCTAMI], NCT03593928).

Methods

Study population and design

This study performed a post hoc analysis of the OCTAMI registry and comprised of two independent cohorts. The first study cohort consisted of consecutive patients (age ≥18 years) who presented with STEMI and underwent emergent procedures at Fuwai Hospital, the largest PCI centre in China. The culprit lesions of the enrolled patients were evaluated by OCT. STEMI was defined according to an established criteria.[14] The exclusion criteria of the study included the following: cardiac shock, congestive heart failure, and a history of coronary artery bypass graft. Additionally, patients with left main coronary artery diseases, extremely tortuous or heavily calcified vessels, or chronic total occlusion were also excluded due to the challenge of performing OCT in these patients. Between March 2017 and April 2019, a series of 470 patients with STEMI who underwent OCT examination were enrolled in the OCTAMI registry. Among these patients, 53 patients had stent thrombosis, which was defined according to the Academic Research Consortium criteria.[15] The study flow chart is shown in Figure 1. The second cohort included an independent set of 50 prospectively recruited, age- and sex-matched, individuals (age ≥18 years) without

any known cardiovascular diseases, detected by health screens. This cohort provided normal TMAO reference values. The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Fuwai Hospital. All patients provided written informed consent.

Classification of stents by type

The underlying stents were classified as BMS (n=5), first-generation DES (n=16), second-generation DES (n=27), and unknown (n=2). The underlying stents classified as either first-generation DES or second-generation DES types are described in further detail in the supplemental materials.

Acquisition of OCT imaging

Patients were administered aspirin 300 mg, ticagrelor 180 mg or clopidogrel 600 mg, and heparin 100 IU/kg prior to the interventional procedure. PCI was performed via radial or femoral access. Thrombus aspiration was performed in order to reduce the thrombus burden and restore antegrade coronary flow. Small-sized balloon dilation (≤2.0 mm in diameter) at low pressures was performed in order to acquire adequate images in patients with poor quality imaging following the thrombectomy and in patients where the aspiration catheter was not able to pass the culprit lesion into the distal vessel. OCT images of the culprit lesions were acquired using the frequency domain ILUMIEN OPTIS or C7-XR OCT system and a dragonfly catheter (St. Jude Medical, Westford, MA, USA).

OCT qualitative analysis

All OCT images were anonymously analysed on the St Jude OCT Offline Review

Workstation (St. Jude Medical, Westford, MA, USA) by three independent experienced observers (XL, SZ, and LY), who were blinded to the other data, in a core laboratory. Inconsistent results were resolved by consensus of the investigators. Neoatherosclerosis was defined as the presence of a fibroatheroma or fibrocalcific plaque within the stented coronary segment. Fibroatheromas were characterized by a signal-poor region displaying high attenuation with diffuse borders and a lateral extension of at least one quadrant. Fibrocalcific plaques were characterised by a signal-poor region with low attenuation and clearly visible borders. Plaque rupture was identified by the presence of a disrupted fibrous cap of neoatherosclerosis, resulting in the communication of blood with the necrotic core (Figure 2). The culprit lesions, in patients without neoatherosclerosis, were categorised into uncovered struts, stent malapposition, restenosis, stent underexpansion, and edge dissection, according to an established criteria.[16] Intra-observer and inter-observer variability were assessed by the evaluation of 20 randomly selected patients by the same observer and the three independent observers, respectively. The intra-observer Kappa coefficients were 0.90 for XL, 0.90 for SZ, and 0.79 for LY. The inter-observer Kappa coefficients were 0.90 for XL and SZ, 0.79 for XL and LY, and 0.89 for SZ and LY.

Definitions of quantitative variables

Definitions of the quantitative variables are described in the supplemental materials.

Laboratory tests

Blood samples were collected before heparinisation using vacutainer tubes

containing ethylenediaminetetraacetic acid. Samples were maintained at 4°C, processed within 3 hours, and then stored at -80°C until further analysis. The TMAO plasma levels were quantified using stable isotope dilution high-performance liquid chromatography with online electrospray ionization tandem mass spectrometry according to a previously described technique using an API 3200 triple quadrupole mass spectrometer (AB SCIEX, Framingham, MA, USA) with a d9-(trimethyl)–labelled internal standard.[17] The estimated glomerular filtration rate (mL/min/1.73 m^2) was calculated using the Modification of Diet in Renal Disease study equation.[18]

Statistical analysis

Continuous data were presented as mean ± standard deviation or median [interquartile range]. Student's *t*-test or nonparametric test was used for statistical comparisons, where appropriate. Categorical variables were presented as numbers (percentage); the chi-squared test or Fisher's exact test was used to compare variables between groups. Cox proportional hazards and logistic regression analysis were performed to identify factors associated with neoatherosclerosis. The area under the receiver operating characteristic curves (AUC) was calculated to evaluate ability of plasma TMAO to predict neoatherosclerosis. A two-tailed P-value <0.05 was considered statistically significant. The statistical analyses were performed using SPSS version 22 (IBM, Armonk, NY, USA).

Results

Patient characteristics

Of the 470 patients with STEMI enrolled in the OCTAMI registry, 50 patients presented with VLST. In the present study, only patients with VLST were analysed and split into two strata, according to the presence or absence of neoatherosclerosis (stratum 1), and patients with neoatherosclerosis were further divided into subgroups, with or without plaque rupture as identified by OCT (stratum 2). Among them, 23 patients had neoatherosclerosis, and 27 patients did not have neoatherosclerosis. Patients without neoatherosclerosis were classified as having either stent malapposition (n=11), uncovered stents (n=10), severe restenosis (n=3), stent underexpansion (n=2), or edge dissection (n=1). The 23 patients with neoatherosclerosis were further divided into two groups, including 14 patients with plaque rupture and 9 without plaque rupture (Figure 1).

The patients' baseline and clinical characteristics, according to the presence or absence of neoatherosclerosis, are shown in Table 1, and angiographic and procedural characteristics were shown in Table 2. The median stent duration from index stenting to presentation with VLST was 7.0 years. The stent duration in 5 BMS-treated patients (median [interquartile range]: 14.0 [13.0-16.0] years) was significantly longer than that in the 43 DES-treated patients (7.0 [5.0-9.0] years) ($P<0.001$). The proportion of patients adhering to statin therapy was significantly lower in those with neoatherosclerosis than that in those without neoatherosclerosis (56.5% vs. 85.2%; $P=0.031$). The baseline and clinical characteristics, according to the presence or absence of plaque rupture in patients with neoatherosclerosis, are shown in Table 3.

Optical coherence tomographic morphometric analysis

The OCT morphometric data, according to the presence of neoatherosclerosis, are reported in Table 4. The minimum and mean luminal area and diameter were significantly smaller in patients with neoatherosclerosis than in those without. A stent expansion index <0.8 was observed in 13.0% of patients with neoatherosclerosis and in 51.9% of patients without neoatherosclerosis (P=0.006).

Comparison of plasma TMAO levels between groups

We first performed a cross-sectional comparison of TMAO levels between patients with VLST and the independent set of normal healthy controls (baseline characteristics in Supplemental Table S1). The plasma TMAO levels were significantly higher in patients with VLST compared to that in normal healthy individuals (2.50 [1.67-3.84] vs. 1.32 [0.86-2.44] μM; P<0.001) (Figure 3A).

In our study cohort of 50 patients with VLST, plasma TMAO levels were significantly higher in patients with neoatherosclerosis compared to that patients without neoatherosclerosis (3.69 [2.46-5.29] vs. 1.96 [1.39-2.80] μM; P<0.001) (Figure 3B). Moreover, we found significantly higher plasma TMAO levels in patients with neoatherosclerosis compared to that in normal healthy controls (P<0.001), whereas no significant difference in the plasma TMAO levels was observed between patients without neoatherosclerosis and normal healthy controls (P=0.125) (Figure 3B).

We then performed a further subgroup analysis of patients with neoatherosclerosis according to the presence or absence of plaque rupture. A significantly higher plasma TMAO level was observed in patients with plaque rupture compared to that in patients

without plaque rupture (4.51 [3.41-5.85] vs. 2.46 [2.05-3.55] μM; P=0.005) (Figure 3C).

Predictors of in-stent neoatherosclerosis

Univariate logistic regression analysis showed that TMAO levels (OR 3.43; 95% CI: 1.68-6.99; P=0.001) and poor adherence to statin therapy (OR 4.42; 95% CI: 1.15-16.97; P=0.030) were significantly associated with the presence of neoatherosclerosis (Supplemental Table S2*)*. However, after adjusting for age, sex, history of hypertension and diabetes mellitus, and smoking status, plasma TMAO was found to be a unique independent predictor of neoatherosclerosis (OR 3.41; 95% CI: 1.59-7.30; P=0.002) (Supplemental Table S2*)*.

Additionally, based on the significantly longer duration from index stent implantation to presentation with VLST in patients implanted with BMSs compared to that in patients with DESs, we performed a Cox regression analysis to identify whether the implantation of a DES was a predictor of neoatherosclerosis. The result indicated that DES was marginally associated with the formation of neoatherosclerosis (HR 6.73; 95% CI: 0.87-51.90; P=0.068).

Diagnostic value of TMAO in neoatherosclerosis

We performed a receiver operating characteristic curve analysis to evaluate the diagnostic value of TMAO in discriminating patients with neoatherosclerosis from those without neoatherosclerosis. The AUC was 0.85 (95% CI: 0.74-0.95) (Figure 4). A TMAO cut-off level of 3.23 μM was shown to be the optimal point at which the maximum summation of sensitivity and specificity, in distinguishing the presence of

neoatherosclerosis from the absence of neoatherosclerosis, is achieved. The corresponding sensitivity and specificity were 73.9% and 85.2%, respectively.

Discussion

The main finding of this post hoc study was the strong association between plasma TMAO level and in-stent neoatherosclerosis and the association between plasma TMAO level and plaque rupture, a complication of neoatherosclerosis, in patients with VLST. High plasma TMAO levels were found to be an independent predictor of neoatherosclerosis. Furthermore, the receiver operating characteristic curve analysis indicated that TMAO may serve as a peripheral biomarker of neoatherosclerosis.

As an important underlying phenotype of VLST,[5, 6] the potential mechanisms and contributing factors of neoatherosclerosis remain unknown. The gut microbiota-related metabolite TMAO has been shown to play a role in the pathogenesis and development of atherosclerotic disease.[8, 10, 11] Our study contributes to these prior findings by demonstrating the significant association between plasma TMAO and neoatherosclerosis in patients with VLST. Recent mechanistic studies have suggested several potential pathways through which TMAO exerts a pro-atherosclerotic effect, including changes in macrophage phenotype, lipid metabolism, and endothelial cell activation.[8, 9, 19-22]

An interesting finding of the present analysis was the observed similar plasma TMAO levels between patients with VLST without neoatherosclerosis and healthy controls. This indicates that the difference between patients with VLST and healthy controls

were mainly resulted from the high plasma TMAO levels in patients with neoatherosclerosis.

Plaque rupture, and subsequent thrombus formation, are the main mechanisms resulting in AMI.[23] Moreover, recent studies further demonstrated that plaque rupture is also the major phenotype of neoatherosclerosis leading to VLST.[6] We recently reported significantly higher plasma TMAO levels in patients with plaque rupture compared to that in patients without plaque rupture who have experienced STEMI, caused by *de novo* lesions in native arteries as assessed by OCT.[24] However, whether plasma TMAO concentration is associated with plaque rupture in patients with VLST presenting with neoatherosclerosis remains unknown. Our study extends the previous observations by demonstrating the association between elevated plasma TMAO levels and ruptured neoatherosclerotic plaques.

Our study showed that plasma TMAO had an AUC of 0.85 to differentiate between patients with neoatherosclerosis and those without neoatherosclerosis, indicating that plasma TMAO may serve as a diagnostic peripheral biomarker used to predict neoatherosclerosis and provide clinical utility to improve risk stratification and the clinical management of VLST. Furthermore, as a metabolite of gut microbes from specific dietary nutrients, plasma TMAO level was demonstrated to be modifiable by dietary intervention [8, 25] or small molecular inhibitors.[26, 27] Therefore, plasma TMAO has the potential of becoming a therapeutic target, used to reduce the risk of neoatherosclerosis and plaque rupture.

Previous studies demonstrated that neoatherosclerosis occurred more frequently and

earlier in patients with DESs compared to that in those with BMSs.[28] In our study, the Cox regression analysis showed a marginal significant association between the implantation of DESs and the formation of neoatherosclerosis in patients with VLST, which further corroborated the previous study findings.

In the present analysis, patients with neoatherosclerosis had significantly smaller minimum luminal areas and diameters compared to that in patients without neoatherosclerosis, which could explain the higher rate of balloon predilation used in patients with neoatherosclerosis in this study.

Limitations

Several limitations exist in this study. An important limitation is the study's lack of a control group consisting of patients with a similar duration of stent implantation without VLST. Further study is needed to identify the association between plasma TMAO and neoatherosclerosis in patients without VLST. Secondly, the plasma samples, taken from the subjects with VLST who had presented with STEMI, were not necessarily taken during a fasting state; therefore, the effects of patients' diets on plasma TMAO levels could not be excluded. Thirdly, thrombus aspiration catheters and small-sized predilation balloons were used to acquire clear OCT images in some cases, which may affect the assessment of the morphology of the culprit lesions in patients with VLST. Lastly, this was a single-centre study with a relatively small sample size; thus, selection bias may exist. An independent study with a larger sample size is warranted to verify our study results.

Conclusions

To the best of our knowledge, we are the first to investigate the association between plasma TMAO and culprit lesion morphology as assessed by OCT in patients with VLST presenting with STEMI. Our study demonstrated a significant association between plasma TMAO levels, neoatherosclerosis, and plaque rupture in patients with VLST. Further studies are warranted to explore the possibility of using plasma TMAO as a potential peripheral biomarker of neoatherosclerosis in order to improve risk stratification and the clinical management of patients with VLST. Furthermore, studies are required in order to confirm the use of plasma TMAO as an interventional target to reduce the risk of neoatherosclerosis and plaque rupture.

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Disclosures

None.

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Table 1. Baseline characteristics of patients with and those without neoatherosclerosis

	Patients with neoatherosclerosis (n=23)	**Patients without neoatherosclerosis (n=27)**	**P value**
Age, yrs	63.7±7.2	63.2±10.2	0.816
Male	19 (82.6)	25 (92.6)	0.395
Body mass index, kg/m^2	25.6±3.6	26.4±3.5	0.455
Diabetes mellitus	10 (43.5)	11 (40.7)	0.845
Insulin dependent	7 (30.4)	5 (18.5)	0.325
Hypertension	13 (56.5)	19 (70.4)	0.309
Smoker	15 (65.2)	21 (77.8)	0.324
Current smoker	8 (34.8)	13 (48.1)	0.340
Prior myocardial infarction	11 (47.8)	17 (63.0)	0.283
Left ventricular ejection fraction, %	53.0 (45.0-59.0)	52.0 (48.0-55.0)	1.000
Estimated glomerular filtration rate, mL/min/1.73 m^2	85.6 (75.9-101.9)	83.2 (70.0-105.7)	0.553
Triglyceride, mg/dL	121.3 (87.6-158.4)	103.6 (84.1-135.4)	0.448
Low-density lipoprotein-cholesterol, mg/dL	84.0 (62.7-120.0)	76.2 (58.1-89.0)	0.243
High-density lipoprotein-cholesterol, mg/dL	40.6 (33.7-44.9)	45.7 (36.0-51.5)	0.108
High-sensitivity C-reactive protein, mg/L	5.6 (1.5-8.5)	6.5 (3.0-9.8)	0.448
Peak troponin I, ng/mL	22.6 (9.9-66.1)	16.6 (8.1-43.7)	0.785
D-dimer, mg/L	0.15 (0.13-0.47)	0.17 (0.13-0.37)	0.823

Trimethylamine *N*-oxide, μM	3.69 (2.46-5.29)	1.96 (1.39-2.80)	<0.001
Pre-hospital medications			
Statin	13 (56.5)	23 (85.2)	0.031
Aspirin	15 (65.2)	18 (66.7)	0.914
$P2Y_{12}$ inhibitor	7 (30.4)	7 (25.9)	0.723

1 Continuous data are presented as mean ± standard deviation or median (interquartile
2 range), categorical variables are presented as count (%).
3

Table 2. Angiographic and procedural characteristics of patients with and those without neoatherosclerosis

	Patients with neoatherosclerosis (n=23)	Patients without neoatherosclerosis (n=27)	P value
Culprit artery			0.535
LAD	15 (65.2)	14 (51.9)	
LCX	1 (4.3)	3 (11.1)	
RCA	7 (30.4)	10 (37.0)	
Multi-vessel disease	21 (91.3)	19 (70.4)	0.085
Stent type at index procedure			0.285
BMS	4 (17.4)	1 (3.7)	0.167
First-generation DES	5 (21.7)	11 (40.7)	0.151
Second-generation DES	13 (56.5)	14 (51.9)	0.741
Unknown	1 (4.3)	1 (3.7)	1.000
Stent duration, yrs	7.0 (6.0-12.0)	7.0 (3.0-10.0)	0.218
Initial TIMI flow grade			0.769
0	17 (73.9)	17 (63.0)	
1	1 (4.3)	2 (7.4)	
2	1 (4.3)	3 (11.1)	
3	4 (17.4)	5 (18.5)	
Thrombus aspiration	14 (60.9)	19 (70.4)	0.480

Balloon predilation	16 (69.6)	11 (40.7)	0.042

Continuous data are presented as mean ± standard deviation or median (interquartile range), categorical variables are presented as count (%). BMS=bare metal stent; DES=drug-eluting stent; LAD=left anterior descending coronary artery; LCX=left circumflex coronary artery; RCA=right coronary artery; TIMI=thrombolysis in myocardial infarction

Table 3. Baseline characteristics of patients with neoatherosclerosis with and those without plaque rupture

	Patients with plaque rupture (n=14)	Patients without plaque rupture (n=9)	P value
Age, yrs	63.2±7.9	64.6±6.4	0.671
Male	13 (92.9)	6 (66.7)	0.260
Body mass index, kg/m^2	25.0±3.5	26.5±3.7	0.323
Diabetes mellitus	5 (35.7)	5 (55.6)	0.417
Insulin dependent	3 (21.4)	4 (44.4)	0.363
Hypertension	9 (64.3)	4 (44.4)	0.417
Smoker	8 (57.1)	7 (77.8)	0.500
Current smoker	5 (35.7)	3 (33.3)	1.000
Prior myocardial infarction	9 (64.3)	2 (22.2)	0.089
Left ventricular ejection fraction, %	51.5 (44.5-56.8)	57.0 (45.0-59.5)	0.394
Estimated glomerular filtration rate, mL/min/1.73 m^2	89.5 (76.2-108.2)	80.6 (75.6-96.0)	0.395
Triglyceride, mg/dL	129.2 (98.2-170.4)	98.2 (75.2-149.6)	0.270
Low-density lipoprotein-cholesterol, mg/dL	81.3 (61.7-120.2)	91.3 (75.7-118.2)	0.284
High-density lipoprotein-cholesterol, mg/dL	40.2 (33.2-48.4)	41.0 (34.8-43.2)	0.753
High-sensitivity C-reactive protein, mg/L	5.7 (2.7-10.6)	3.8 (1.2-8.4)	0.508
Peak troponin I, ng/mL	26.7 (4.7-51.6)	18.6 (10.6-68.4)	0.950
D-dimer, mg/L	0.14 (0.13-0.42)	0.25 (0.13-0.52)	0.311

Trimethylamine *N*-oxide, μM	4.51 (3.41-5.85)	2.46 (2.05-3.55)	0.005
Pre-hospital medications			
Statin	6 (42.9)	7 (77.8)	0.197
Aspirin	8 (57.1)	7 (77.8)	0.400
$P2Y_{12}$ inhibitor	5 (35.7)	2 (22.2)	0.657

1 Continuous data are presented as mean ± standard deviation or median (interquartile
2 range), categorical variables are presented as count (%).

Table 4. Optical coherence tomographic morphometric analysis in patients with and those without neoatherosclerosis

	Patients with neoatherosclerosis (n=23)	Patients without neoatherosclerosis (n=27)	P value
Minimum stent area, mm^2	6.32±2.01	6.22±1.61	0.846
Mean stent area, mm^2	7.33±2.07	7.72±1.87	0.485
Minimum stent diameter, mm	2.80±0.44	2.77±0.36	0.811
Mean stent diameter, mm	3.02±0.41	3.09±0.38	0.574
Minimum luminal area, mm^2	1.63±0.57	3.67±1.63	<0.001
Mean luminal area, mm^2	3.52±0.86	5.49±1.65	<0.001
Minimum luminal diameter, mm	1.41±0.24	2.10±0.47	<0.001
Mean luminal diameter, mm	2.01±0.25	2.55±0.40	<0.001
Proximal luminal area, mm^2	7.99±2.41	9.38±2.56	0.055
Proximal luminal diameter, mm	3.15±0.47	3.42±0.49	0.053
Distal luminal area, mm^2	5.73±2.04	6.21±2.15	0.417
Distal luminal diameter, mm	2.65±0.46	2.76±0.50	0.398
Reference area, mm^2	6.86±2.09	7.80±2.18	0.127
Reference diameter, mm	2.90±0.44	3.10±0.46	0.136
Expansion index	0.93±0.13	0.81±0.12	0.003
Stent expansion <80%	3 (13.0)	14 (51.9)	0.006

Continuous data are presented as mean ± standard deviation, categorical variables are presented as count (%).

Figure legends

Figure 1. Study flowchart

A study flow of patients who are stratified according to the presence or absence of neoatherosclerosis (stratum 1) and plaque rupture (stratum 2). OCTAMI=Optical Coherence Tomography Examination in Acute Myocardial Infarction, STEMI=ST-segment elevation myocardial infarction.

Figure 2. Representative images of OCT findings in patients presenting with very late stent thrombosis

(A) Neoatherosclerosis with a lipid-rich plaque (white arrows) and plaque rupture, accompanied by a cavity (asterisk) and thrombus formation (thr). **(B)** Neoatherosclerosis with a fibrocalcific plaque (yellow arrows), without plaque rupture, accompanied by thrombus formation (thr). **(C)** Malapposed struts with thrombus formation (thr). **(D)** Uncovered struts with thrombus formation (thr).

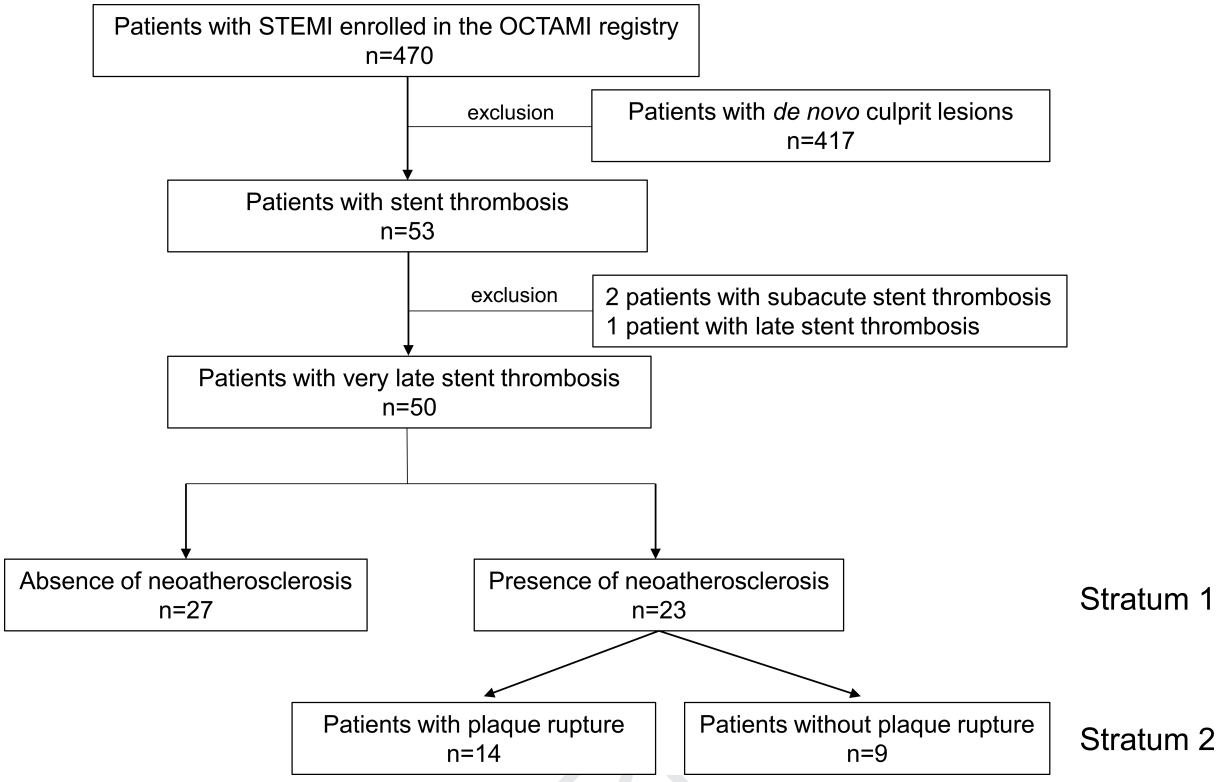
Figure 3. Comparison of plasma trimethylamine *N*-oxide (TMAO) levels between the different groups

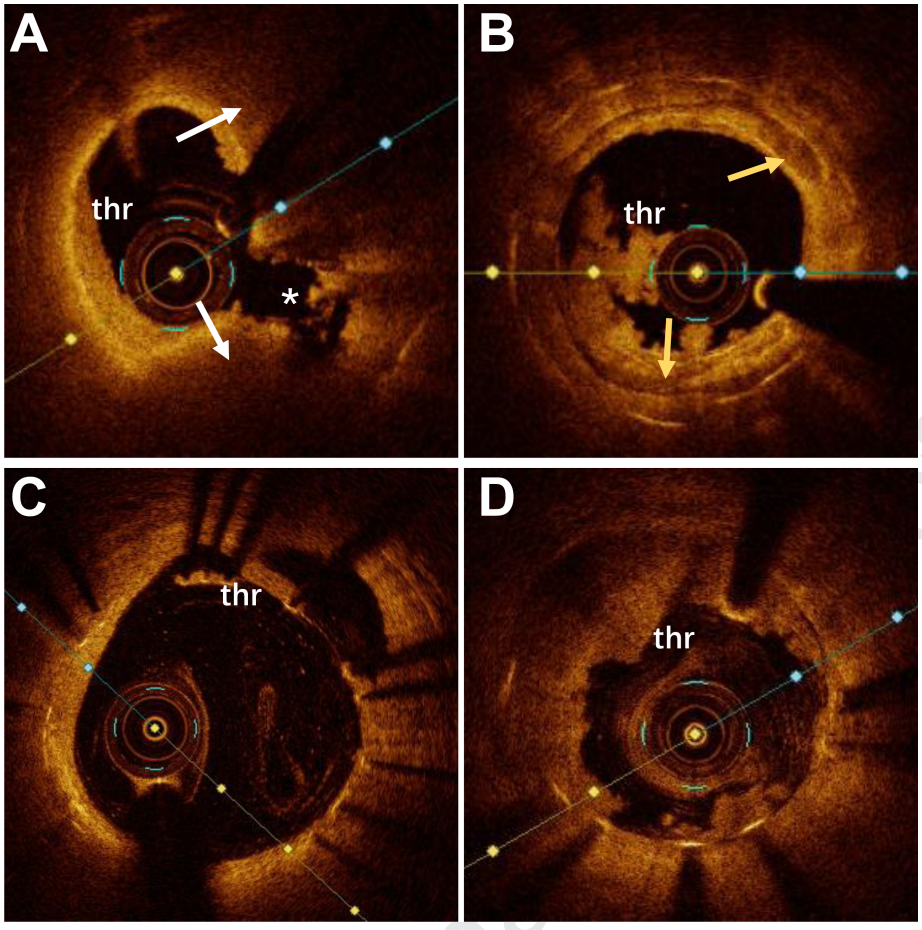
(A) Plasma TMAO level in healthy controls compared to that in patients with very late stent thrombosis. **(B)** Plasma TMAO level in patients with neoatherosclerosis compared to that in patients without neoatherosclerosis and that in healthy controls. **(C)** Plasma TMAO level in patients with plaque rupture compared to that in patients without plaque rupture. NE=neoatherosclerosis.

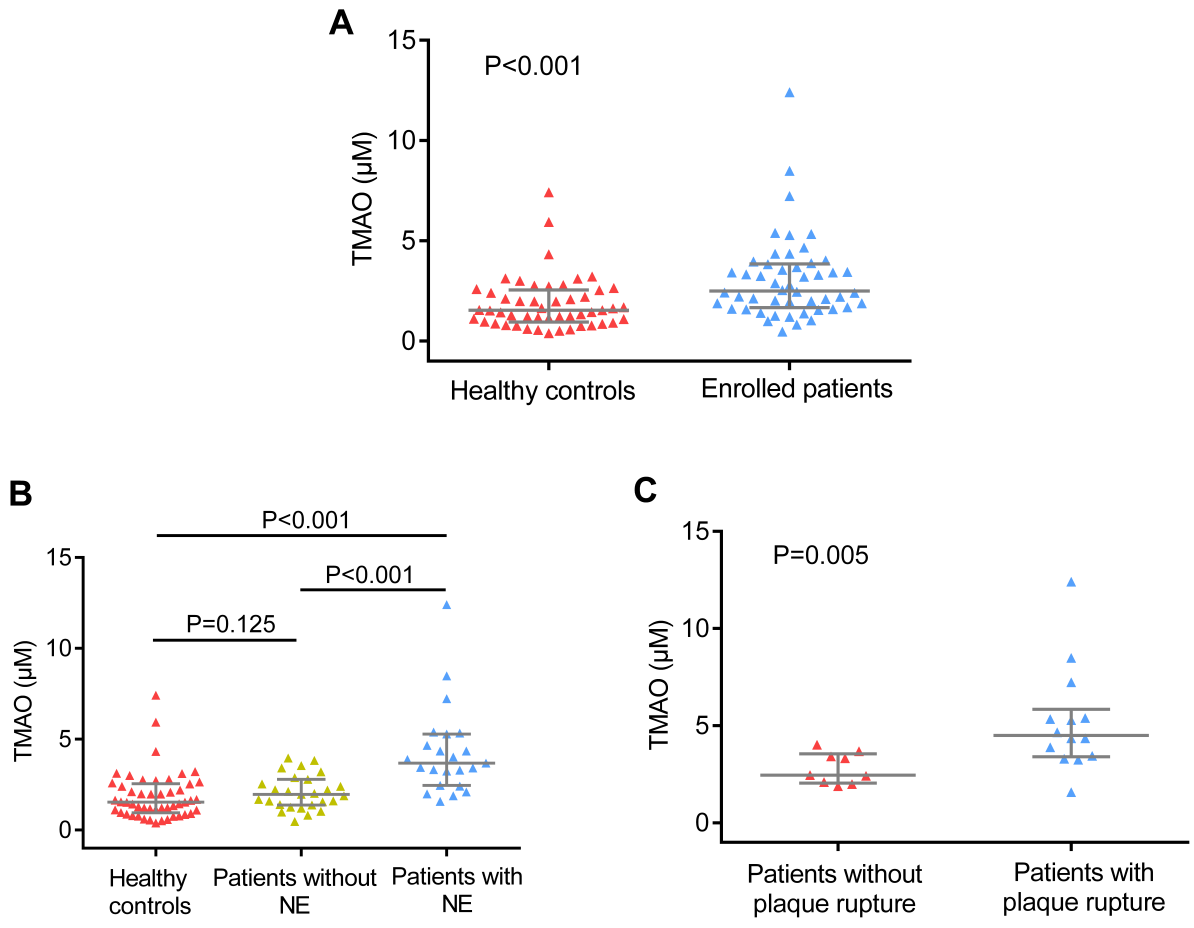
Figure 4. Receiver operating characteristic curve of trimethylamine *N*-oxide for predicting neoatherosclerosis in patients with very late stent thrombosis. AUC, area

1 under the receiver operating characteristic curve.

2






A
P<0.001
TMAO (μM)
15
10
5
0
Healthy controls
Enrolled patients
B
P<0.001
P<0.001
P=0.125
TMAO (μM)
15
10
5
0
Healthy controls
Patients without NE
Patients with NE
C
P=0.005
TMAO (μM)
15
10
5
0
Patients without plaque rupture
Patients with plaque rupture