

# Association between trimethylamine N-oxide and prognosis of patients with acute myocardial infarction and heart failure

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## Abstract

**Aims** This study aimed to investigate the association between trimethylamine N-oxide (TMAO) and the prognosis and association between high-sensitivity C-reactive protein (hsCRP) and TMAO-associated cardiovascular risk in patients with acute myocardial infarction (AMI) complicated by heart failure (HF).

**Methods and results** A total of 985 patients presenting with AMI and HF were consecutively enrolled at the Fuwai Hospital between March 2017 and January 2020. Patients were stratified into groups according to tertiles of TMAO levels and the median hsCRP levels. The primary endpoint was major adverse cardiac events (MACE), including all-cause death, recurrence of myocardial infarction, and rehospitalization due to HF. During a median follow-up of 716 days, 138 (14.0%) patients experienced MACE. Cox regression analyses showed that the adjusted hazard ratio (HR) for MACE was higher in patients in tertile 3 [TMAO > 9.52  $\mu\text{mol/L}$ , HR: 1.85, 95% confidence interval (CI): 1.18–2.89;  $P = 0.007$ ] than in tertile 1 (TMAO < 4.74  $\mu\text{mol/L}$ ), whereas no significant differences were detected between the patients in tertiles 1 and 2 (TMAO = 4.74–9.52  $\mu\text{mol/L}$ , HR: 0.96, 95% CI: 0.59–1.58;  $P = 0.874$ ). Restricted cubic spline regression depicted an S-shaped association between TMAO and MACE ( $P$  for nonlinearity = 0.012). In the setting of hsCRP above the median level (6.68 mg/L), per unit increase of TMAO was associated with a 20% increase of MACE risk (HR: 1.20, 95% CI: 1.05–1.37,  $P = 0.009$ ); increasing tertiles of TMAO were significantly associated with a higher risk of MACE (adjusted  $P = 0.007$  for interaction;  $P < 0.001$  for trend across tertiles). The Kaplan–Meier analysis indicated that patients in tertile 3 had a significantly lower event-free survival ( $P = 0.001$ ) when the hsCRP level was above the median level. No similar association between TMAO and MACE was observed when the hsCRP level was below the median level.

**Conclusions** High plasma TMAO levels were independently correlated with poor prognosis in patients with AMI complicated by HF, especially in those with higher hsCRP levels. There was an S-shaped relationship between TMAO and HR for MACE.

**Keywords** Trimethylamine N-oxide; Inflammation; Acute myocardial infarction; Heart failure

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## Introduction

Plasma trimethylamine N-oxide (TMAO) is one of the metabolites produced by gut microbiota. It has been proven to be involved in many pathophysiological processes and be related to the prognosis of patients with coronary artery disease and heart failure (HF).<sup>1,2</sup> A meta-analysis demon-

strated that plasma TMAO level was positively and dose-dependently associated with an increased cardiovascular risk in the general population.<sup>3</sup> The TMAO level is recognized as an independent risk factor for major adverse cardiovascular and cerebrovascular events in patients with acute coronary syndrome.<sup>4</sup> Our previous study found that TMAO might serve as a potential indicator of the character-

istics of coronary artery plaques.<sup>5</sup> Recent studies have displayed that elevated plasma levels of TMAO are associated with poor outcomes in a population with HF.<sup>6</sup> The combination of TMAO with B-type natriuretic peptide (BNP) could improve the prognostic value of mortality in patients with HF with preserved ejection fraction (HFpEF).<sup>7</sup> However, most previous studies have focused on patients with chronic HF,<sup>8,9</sup> and it is uncertain whether TMAO levels have the same effects on patients with acute HF.

High-sensitivity C-reactive protein (hsCRP), a systemic inflammatory response marker, is extensively used to evaluate inflammatory risk in patients with myocardial infarction (MI).<sup>10–12</sup> Some studies have suggested that TMAO may activate the inflammation cascade.<sup>13,14</sup> However, it remains unclear whether TMAO is related to a poorer prognosis in the population with acute myocardial infarction (AMI) and HF and whether it is associated with increased cardiovascular risk determined by hsCRP. Therefore, we intended to explore the association between TMAO and the prognosis and the association between hsCRP levels and TMAO-associated cardiovascular risk in patients with AMI complicated by HF.

## Methods

### Study population and design

The current study was conducted under the Declaration of Helsinki and obtained ethnic approval from the Ethics Committee of Fuwai Hospital. All patients signed a written informed consent form. Patients presenting with AMI and HF admitted to the emergency department of Fuwai Hospital between March 2017 and January 2020 were prospectively enrolled in this study cohort. The diagnostic criteria of AMI were based on the Fourth Universal Definition of Myocardial Infarction and guidelines,<sup>15–17</sup> including ST-segment elevation MI and non-ST-segment elevation MI. HF was diagnosed and classified based on typical symptoms and signs, laboratory tests, echocardiogram, and X-ray findings, following guidelines and statements by the European Society of Cardiology and Heart Failure Society of America.<sup>18,19</sup> We excluded patients who were in the acute phase of infectious or inflammatory diseases, missing hsCRP test results, or missing follow-up records. A flowchart of patient selection process is shown in Supporting Information, *Figure S1*. Relevant electronic medical records were retrieved using the hospital information system for subsequent analysis. Clinical data, including demographics, physical examination, medical history, blood test results, echocardiogram data, and medication at discharge, were obtained.

### Laboratory testing

Blood samples were gathered into tubes with ethylenediaminetetraacetic acid through radial or femoral artery before percutaneous coronary intervention (PCI). These were processed at 4°C within 3 h, and then stored at –80°C until further analysis. As mentioned previously, the stable isotope dilution high-performance liquid chromatography with online electrospray ionization tandem mass spectrometry was used to measure plasma TMAO levels, using an API 3200 triple quadrupole mass spectrometer (AB SCIEX, Framingham, Massachusetts) with a d9(trimethyl)-labelled internal standard.<sup>20</sup> HsCRP testing was routinely collected via cubital or basilic veins in an overnight fasting state on the day after the PCI procedure and measured using an immunoturbidimetric assay (Beckmann Assay, Brea, California). The other blood test indicators are routinely detected in the hospital central laboratory. The N-terminal pro-B-type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI) levels were measured several times during hospitalization, and the baseline and peak values were recorded.

### Endpoints and follow-up

The primary endpoint was a composite of all-cause death, recurrence of MI, and rehospitalization due to HF, called major adverse cardiac events (MACE). Endpoint data were collected by professionals in charge of follow-up via outpatient visits and telephone interviews. Routine follow-up of patients was performed at 6 and 12 months after PCI and annually thereafter. The protocol for follow-up was ratified by the Institutional Review Board of Fuwai Hospital.

### Statistical analysis

Mean  $\pm$  standard deviation or median with interquartile range (IQR) for continuous variables and number (percentage) for categorical variables were reported. For continuous variables with normal distribution, one-way analysis of variance or the Kruskal–Wallis *H* test was used for comparison among groups, while for categorical variables, the chi-square test or Fisher's exact test was conducted. To explore the impact of TMAO and hsCRP on cardiovascular risk in patients with AMI and HF, we divided the patients into three groups according to plasma TMAO levels and further grouped them into two categories based on the median hsCRP levels. The correlation of variables and TMAO were ln-transformed and subsequently analysed using Spearman correlation tests with coefficients reported for 1000 bootstrapped samples. The event-free survival distribution of groups was evaluated using Kaplan–Meier analysis and log-rank test. The relationship between variables (including TMAO) and the primary endpoints was first

Table 1 Baseline characteristics according to plasma TMAO levels

Variables	Total (n = 985)	Tertile 1 (n = 328)	Tertile 2 (n = 329)	Tertile 3 (n = 328)	P-value
Male	766 (77.8)	249 (75.9)	264 (80.2)	253 (77.1)	0.388
Age (years)	63.0 (54.0, 70.0)	61.0 (52.0, 67.0)	62.0 (54.0, 68.0)	66.0 (57.0, 73.0)	<0.001
BMI (kg/m <sup>2</sup> )	25.7 (23.3, 27.8)	25.0 (22.9, 27.7)	25.7 (23.5, 27.8)	25.9 (23.5, 27.8)	0.053
Killip (II–IV)	142 (14.4)	31 (9.5)	39 (11.9)	72 (22.0)	<0.001
LVEF (%)	55.0 (48.0, 58.0)	55.0 (50.0, 58.0)	55.0 (48.0, 58.0)	55.0 (46.0, 59.0)	0.132
MI-type					0.746
STEMI	917 (93.1)	305 (93.0)	304 (92.4)	308 (93.9)	
NSTEMI	68 (6.9)	23 (7.0)	25 (7.6)	20 (6.1)	
HF type					0.012
HFpEF	713 (72.4)	253 (77.1)	239 (72.6)	221 (67.4)	
HFmrEF	207 (21.0)	57 (17.4)	75 (22.8)	75 (22.9)	
HFrEF	65 (6.6)	18 (5.5)	15 (4.6)	32 (9.8)	
Medical history					
Current smoker	697 (70.8)	219 (66.8)	246 (74.8)	232 (70.7)	0.079
Hypertension	654 (66.4)	218 (66.5)	213 (64.7)	223 (68.0)	0.678
Hyperlipaemia	914 (92.8)	303 (92.4)	301 (91.5)	310 (94.5)	0.306
Diabetes mellitus	358 (36.3)	89 (27.1)	111 (33.7)	158 (48.2)	<0.001
Previous stroke	164 (16.6)	38 (11.6)	54 (16.4)	72 (22.0)	0.002
CKD	92 (9.3)	21 (6.4)	19 (5.8)	52 (15.9)	<0.001
PAD	62 (6.3)	16 (4.9)	18 (5.5)	28 (8.5)	0.117
Previous MI	186 (18.9)	59 (18.0)	63 (19.1)	64 (19.5)	0.873
Previous PCI	175 (17.8)	55 (16.8)	62 (18.8)	58 (17.7)	0.784
Previous CABG	24 (2.4)	8 (2.4)	7 (2.1)	9 (2.7)	0.877
Laboratory indexes					
TMAO (μmol/L)	6.7 (4.0, 11.7)	3.2 (2.2, 4.0)	6.7 (5.6, 8.0)	15.2 (11.7, 22.8)	<0.001
eGFR (mL/min/1.732 m <sup>2.6a</sup> )	77.0 (59.4, 97.2)	81.7 (64.2, 103.2)	78.9 (62.6, 98.3)	68.8 (48.9, 89.1)	<0.001
cTnl-baseline (ng/mL)	1.3 (0.2, 7.1)	2.1 (0.3, 9.9)	1.2 (0.2, 6.3)	0.9 (0.1, 5.5)	0.009
cTnl-peak (ng/mL)	13.5 (3.8, 32.6)	15.7 (5.7, 34.2)	10.5 (2.8, 29.7)	11.4 (3.0, 34.1)	0.007
NT-proBNP-baseline (ng/mL)	439.3 (141.9, 1290.0)	401.0 (147.2, 1093.0)	420.6 (106.6, 1203.0)	495.2 (168.1, 1531.0)	0.090
NT-proBNP-peak (ng/mL)	1614.0 (783.9, 3174.0)	1480.0 (762.6, 2890.0)	1424.0 (686.1, 2890.0)	1891.0 (878.8, 3806.0)	0.001
hsCRP (mg/L)	6.7 (2.1, 11.3)	7.9 (2.6, 11.4)	6.2 (2.0, 11.3)	5.8 (2.1, 11.2)	0.241
LDL-C (mmol/L)	2.6 (2.0, 3.2)	2.8 (2.1, 3.4)	2.6 (2.0, 3.2)	2.4 (1.8, 3.0)	<0.001
Angiography finding					
MVD, n (%)	745 (75.6)	239 (72.9)	244 (74.2)	262 (79.9)	0.084
Initial TIMI flow 0–1, n (%)	561 (57.0)	192 (58.5)	197 (59.9)	172 (52.4)	0.122
Aspiration, n (%)	249 (25.3)	88 (26.8)	86 (26.1)	75 (22.9)	0.459
PTCA, n (%)	2 (0.2)	2 (0.6)	0 (0.0)	0 (0.0)	0.213
Stenting, n (%)	694 (70.5)	231 (70.4)	239 (72.6)	224 (68.3)	0.474
Minimal diameter	3.0 (2.8, 3.5)	3.0 (2.8, 3.5)	3.0 (2.8, 3.5)	3.0 (2.8, 3.5)	0.576
Total length (mm)	25.0 (18.0, 35.0)	25.0 (18.0, 35.0)	25.0 (18.0, 33.5)	25.0 (18.0, 37.0)	0.779
Final TIMI Flow 3	945 (95.9)	321 (97.9)	315 (95.7)	309 (94.2)	0.058

(Continues)

Table 1 (continued)

Variables	Total (n = 985)	Tertile 1 (n = 328)	Tertile 2 (n = 329)	Tertile 3 (n = 328)	P-value
Medication					
Aspirin	933 (94.7)	315 (96.0)	313 (95.1)	305 (93.0)	0.253
Ticagrelor	451 (45.8)	169 (51.5)	151 (45.9)	131 (39.9)	0.013
Clopidogrel	511 (51.9)	153 (46.6)	173 (52.6)	185 (56.4)	0.013
ACE/ARB	685 (69.5)	228 (69.5)	238 (72.3)	219 (66.8)	0.145
Beta-blocker	840 (85.3)	286 (87.2)	287 (87.2)	267 (81.4)	0.075
Statins	933 (94.7)	317 (96.6)	309 (93.9)	307 (93.6)	0.174

Continuous variables are presented as medians (25 – 75th percentiles), and categorical variables are reported as counts (%). ACEIs/ARBs, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; BMI, body mass index; CABG, coronary artery bypass graft (ing); CKD, chronic kidney disease; cTnI, cardiac troponin I; HF, heart failure; HFmrEF, heart failure with mildly reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MVD, multiple vessels disease; NSTMI, Non-ST-segment elevation myocardial infarction; NT-proBNP, N-terminal pro B-type natriuretic peptide; STMI, ST-segment elevation myocardial infarction; PAD, peripheral artery disease; PCI, percutaneous coronary intervention; PTCA, percutaneous transluminal coronary angioplasty; TIMI flow, Thrombolysis in Myocardial Infarction flow; TMAO, trimethylamine-N-oxide.  
<sup>a</sup>Estimated glomerular filtration rate (eGFR) was calculated according to the Modification of Diet in Renal Disease formula.

assessed using univariate Cox proportional hazards regression. Adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) were then computed using multivariate Cox regression analysis, adjusting for the following factors: age, sex, body mass index, estimated glomerular filtration rate (eGFR), current smoking, HF type, hypertension, diabetes mellitus (DM), peripheral arterial disease, and history of stroke and MI. A forest plot was graphically displayed to summarize the results of the various subgroup analyses of the HR of MACE comparing the first and third tertiles of plasma TMAO levels. Possible non-linear associations were evaluated using restricted cubic spline (RCS) regression with TMAO as a continuous variable with four knots. Finally, we tested the effect of TMAO and its interaction with hsCRP on the HRs for MACE. All data were analysed using the SPSS software (version 26.0; IBM Corp., Armonk, New York, USA) and R (<http://www.r-project.org/>) statistical packages. A P-value <0.05 was considered as indicative of statistical significance.

## Results

### Baseline characteristics and the association between trimethylamine N-oxide and laboratory variables

Between March 2017 and January 2020, 1006 patients were admitted for AMI complicated by HF, of which 985 patients with complete clinical data were ultimately included in this study (Supporting Information, Figure S1). Table 1 presents the detailed baseline characteristics of the included patients. The median age of total patients was 63 (IQR 54–70) years, and 766 (77.8%) patients were male. Overall, the median plasma levels of TMAO were 6.7 (IQR 4.0–11.7) μmol/L. When grouped by the Killip class, the plasma levels of TMAO in patients with Killip I–IV were 6.4 (IQR 3.8–11.3), 9.2 (IQR 4.6–13.9), 10.5 (IQR 5.5–18.1), and 13.4 (IQR 6.1–23.3) μmol/L, respectively, showing an increasing trend (P < 0.001). Besides, the plasma levels of TMAO for patients with HF with mildly reduced ejection fraction (HFmrEF), HF with preserved ejection fraction (HFpEF), and HF with reduced ejection fraction (HFrEF) were 6.4 (IQR 3.8–11.2), 7.0 (IQR 4.4–12.6), and 8.0 (IQR 4.6–17.9) μmol/L, respectively, also showing an increasing trend (P = 0.007). Patients in tertile 3 of TMAO levels were inclined to be older and presented with a higher prevalence of previous strokes, Killip II–IV, HF with reduced ejection fraction (HFrEF), and chronic kidney disease (CKD). Besides, TMAO levels were positively associated with peak NT-proBNP levels (r = 0.087, P = 0.002), and inversely correlated with hsCRP levels (r = –0.062, P = 0.016), baseline and peak cTnI levels (r = –0.028 and –0.038, P = 0.013 and 0.019, respectively), eGFR (r = –0.495, P < 0.001), and low-density lipoprotein-

cholesterol levels ( $r = -0.281$ ,  $P < 0.001$ ), as shown in Supporting Information, Table S1.

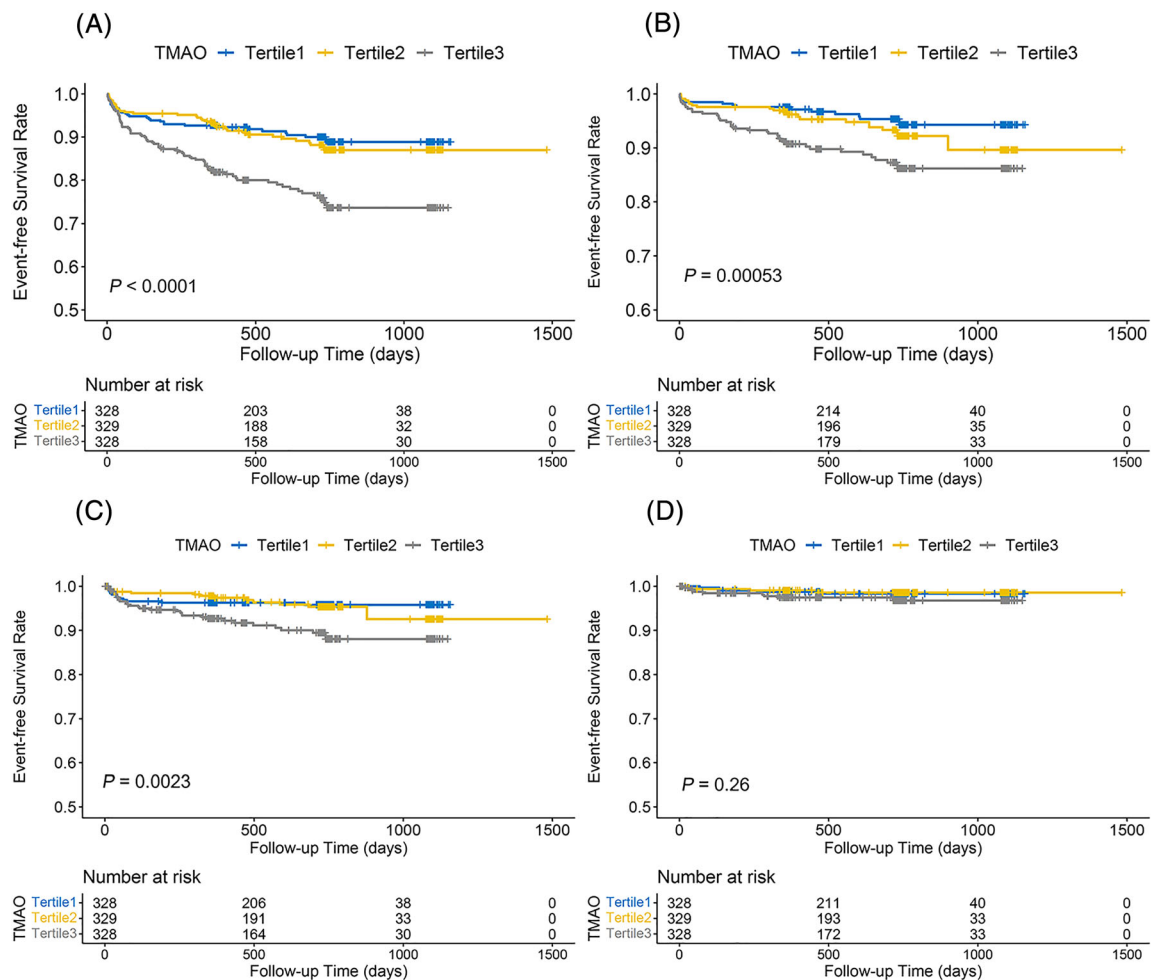
### Trimethylamine N-oxide and study endpoints

There were 138 (14.0%) patients who experienced MACE during the follow-up, and the median follow-up time was 716 days. The Kaplan–Meier analysis of TMAO stratified by tertiles is shown in Figure 1. The results showed a significant increase in MACE risk in the tertile 3 ( $P < 0.001$ ), similar to the risk for all-cause death and recurrence of MI ( $P < 0.001$ ), whereas the difference for rehospitalization due to HF was not statistically significant ( $P = 0.260$ ). The forest plot in Figure 2 compares the HR for MACE between the patients in tertile 3 and tertile 1 according to different subgroups. The results showed that patients with higher TMAO

levels had a higher risk of MACE regardless of age, sex, concomitant hypertension and DM, and history of stroke. In the tertile 3, women, younger patients (<65 years old), and patients with worse renal function had a relatively higher risk. However, when it comes to hsCRP level, only in the setting of hsCRP more than the median level, patients with higher TMAO levels had a significantly higher risk.

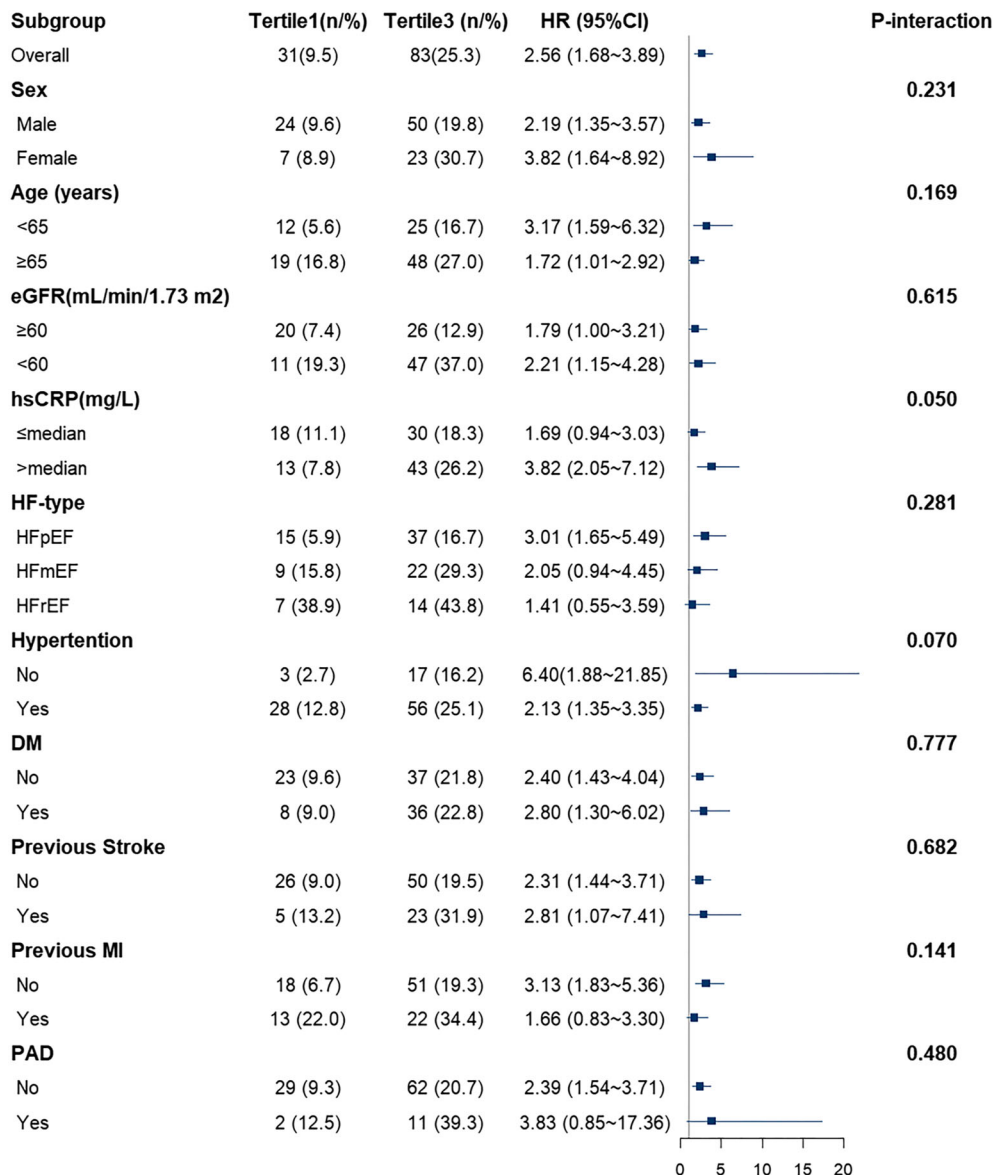
Table 2 describes the univariable and multivariable relationships between all endpoints and TMAO levels. The results revealed that TMAO was an independent risk factor for MACE ( $P = 0.003$ ) and recurrent MI ( $P = 0.011$ ). Meanwhile, there were no statistical significances for the all-cause death and rehospitalization due to HF ( $P = 0.125$  and  $P = 0.607$ , respectively). The adjusted HR for MACE was higher in tertile 3 ( $>9.52 \mu\text{mol/L}$ , HR: 1.85, 95% CI: 1.18–2.89;  $P = 0.007$ ) than in tertile 1 ( $<4.74 \mu\text{mol/L}$ ), while no significant difference was detected between tertile 2 and tertile 1

**Figure 1** Kaplan–Meier curve for cumulative event-free survival in different patient groups stratified by TMAO levels. (A) major adverse cardiac event, (B) all-cause death, (C) myocardial infarction, (D) rehospitalization due to heart failure. TMAO, trimethylamine-N-oxide.





**Figure 2** Forest plot of hazard ratios for major adverse cardiac events comparing first and third tertiles of plasma TMAO levels and stratified by baseline characteristics. DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; HF, heart failure; HFmrEF, heart failure with mildly reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; MI, myocardial infarction; PAD, peripheral artery disease; TMAO, trimethylamine-N-oxide.



(4.74–9.52 μmol/L, HR: 0.96, 95% CI: 0.59–1.58; *P* = 0.874). Similarly, patients in tertile 3 had a higher HR for recurrent MI than patients in tertile 1 (HR: 2.19, 95% CI: 1.13–4.26; *P* = 0.020).

As a continuous variable, the RCS regression analysis displayed an S-shaped relationship between TMAO levels and HR for MACE (*P* for nonlinearity = 0.012) after adjusting for the confounding factors (Figure 3). The cutoff level of TMAO for the predicted HR was 10.0 μmol/L. However, there was no statistical evidence to support a nonlinear association between TMAO and all-cause death (*P* for nonlinearity = 0.237), recurrent MI (*P* for nonlinearity = 0.088), or re-hospitalization due to HF (*P* for nonlinearity = 0.372).

ity = 0.237), recurrent MI (*P* for nonlinearity = 0.088), or re-hospitalization due to HF (*P* for nonlinearity = 0.372).

### Associations between major adverse cardiac events stratified by high-sensitivity C-reactive protein and trimethylamine-N-oxide

The Kaplan–Meier curve of cumulative event-free probability for the tertiles stratified by median hsCRP levels is shown in Figure 4. The results demonstrated a significant difference

**Table 2** Association between TMAO levels and all endpoints

Endpoint	Group	Event (n, %)	Crude HR (95% CI)	P-value	Adjusted HR (95% CI) <sup>a</sup>	P-value
<b>MACE</b>	Tertile 1	31 (9.5)	1 (Ref)		1 (Ref)	
	Tertile 2	34 (10.3)	1.11 (0.68–1.81)	0.673	0.96 (0.59–1.58)	0.874
	Tertile 3	73 (22.3)	2.57 (1.69–3.92)	<0.001	1.85 (1.18–2.89)	0.007
	Trend test	138 (14.0)	1.69 (1.36–2.10)	<0.001	1.42 (1.12–1.79)	0.003
<b>All-cause death</b>	Tertile 1	14 (4.3)	1 (Ref)		1 (Ref)	
	Tertile 2	20 (6.1)	1.48 (0.75–2.92)	0.264	1.14 (0.57–2.31)	0.709
	Tertile 3	38 (11.6)	2.92 (1.58–5.40)	0.001	1.63 (0.84–3.18)	0.151
	Trend test	72 (7.3)	1.75 (1.29–2.37)	<0.001	1.30 (0.93–1.80)	0.125
<b>reMI</b>	Tertile 1	13 (4.0)	1 (Ref)		1 (Ref)	
	Tertile 2	13 (4.0)	1.01 (0.47–2.18)	0.975	0.92 (0.42–1.99)	0.831
	Tertile 3	30 (9.1)	2.48 (1.29–4.76)	0.006	2.19 (1.13–4.26)	0.020
	Trend test	56 (5.7)	1.67 (1.19–2.35)	0.003	1.58 (1.11–2.23)	0.011
<b>reHF</b>	Tertile 1	5 (1.5)	1 (Ref)		1 (Ref)	
	Tertile 2	4 (1.2)	0.82 (0.22–3.04)	0.762	0.69 (0.18–2.61)	0.584
	Tertile 3	9 (2.7)	1.92 (0.64–5.73)	0.242	1.28 (0.40–4.05)	0.679
	Trend test	18 (1.8)	1.45 (0.81–2.60)	0.210	1.17 (0.64–2.16)	0.607

HR, hazard ratio; MACE, major adverse cardiac event; reMI, recurrent myocardial infarction; reHF, rehospitalization due to heart failure; TMAO, trimethylamine-N-oxide.

<sup>a</sup>Adjusted for age, sex, body mass index, estimated glomerular filtration rate, current smoking, HF-type, hypertension, diabetes mellitus, peripheral artery disease, and history of stroke and MI.

among tertiles ( $P < 0.0001$ ) in the setting of hsCRP above the median level (6.68 mg/L). However, when hsCRP level was below the median level, the difference among the tertiles was not statistically significant ( $P = 0.056$ ).

As presented in *Table 3*, in the setting of hsCRP above the median level, per unit increase of TMAO was associated with a 20% increase of MACE risk (HR: 1.20, 95% CI: 1.05–1.37,  $P = 0.009$ ); patients in tertile 3 had a significantly higher risk for MACE than those in tertile 1 (HR: 2.91, 95% CI: 1.52–5.56;  $P = 0.001$ ), whereas no significant differences were obtained between the patients in tertile 2 and tertile 1 (HR: 1.27, 95% CI: 0.62–2.61;  $P = 0.520$ ). Additionally, there was a significant interaction for MACE between TMAO tertiles and hsCRP dichotomy ( $P$  for interaction = 0.007). Meanwhile, there were no similar findings when hsCRP levels were below the median level.

## Discussion

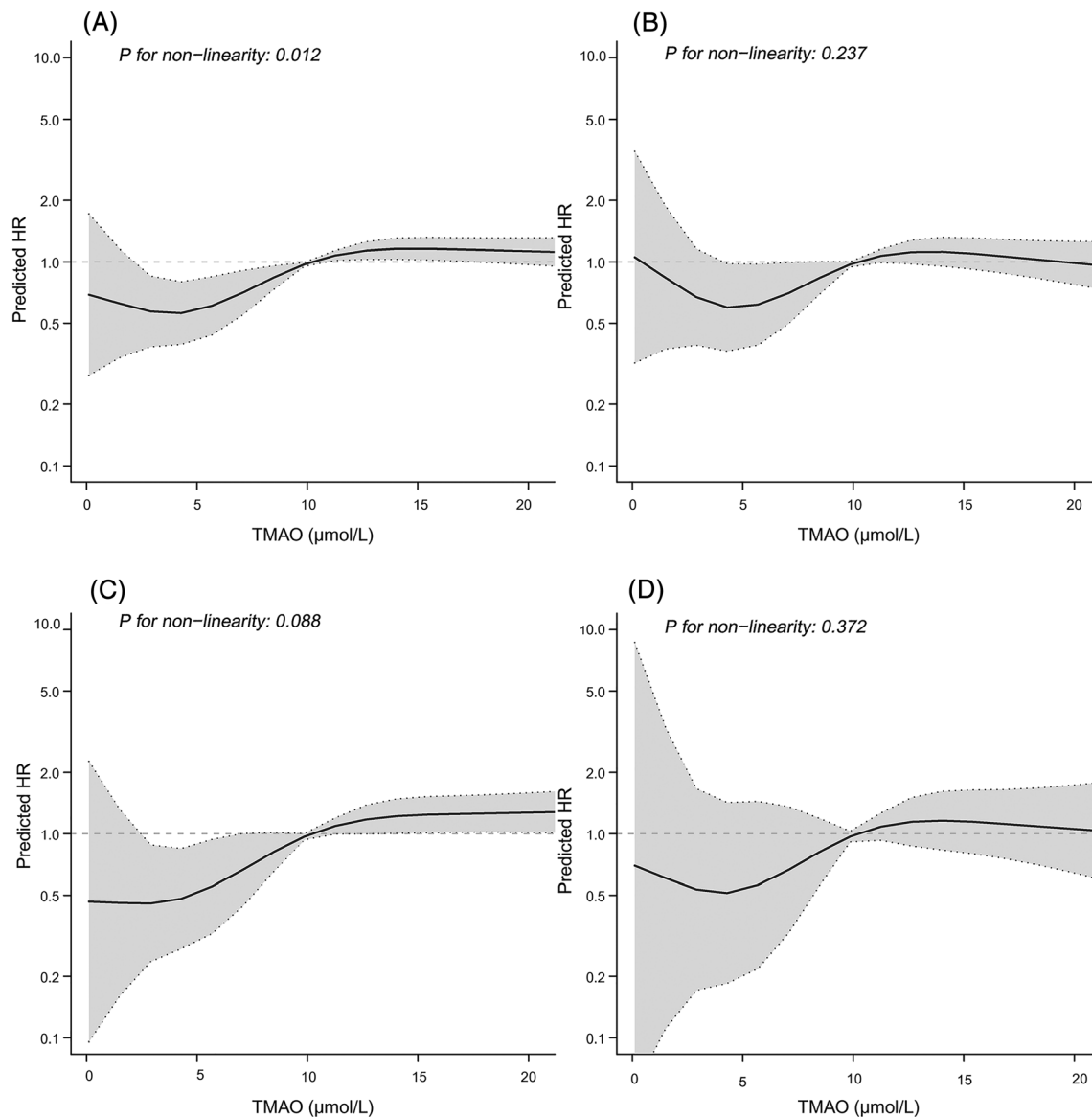
This prospective cohort study explored the association between plasma TMAO and hsCRP levels and the prognosis of patients with AMI and HF. The main finding was that high plasma TMAO levels were independently associated with poor outcomes, particularly in patients with higher hsCRP levels. There may be a potential congenerous effect of TMAO and inflammation on cardiovascular risk. Besides, an S-shaped curve relationship was recorded between TMAO and HR for MACE.

TMAO is a small organic compound that is formed by the oxidation of trimethylamine in the host liver by flavin

monooxygenases.<sup>21</sup> Alkaloids such as choline, carnitine, and betaine are converted to trimethylamine by intestinal flora.<sup>22,23</sup> Robust evidence suggests that TMAO is involved in immunity, inflammation, cholesterol metabolism, and atherothrombosis. The median (IQR) plasma levels of TMAO were 6.7 (4.0–11.7)  $\mu\text{mol/L}$  in this study, which is similar to the range reported in previous studies related to HF.<sup>8</sup> The concentration of TMAO may vary according to the disease state, course, and severity. In a systematic review, the median levels of TMAO ranged from 2.87 to 88  $\mu\text{mol/L}$  in the general population, whereas patients with CKD had higher TMAO levels.<sup>3</sup> The plasma TMAO levels in the groups classified as New York Heart Association II, III, and IV groups were  $3.5 \pm 0.9$ ,  $6.0 \pm 0.8$ , and  $8.1 \pm 1.0$   $\mu\text{mol/L}$ , respectively, showing a significantly increasing trend ( $P < 0.01$ ) in the chronic HF population.<sup>24</sup> Similarly, levels of TMAO also showed an increasing trend according to the Killip class in this study. Thus, different levels of TMAO reflect various states of pathophysiological processes.

Moreover, increasing numbers of studies have exhibited that TMAO may be a promising cardiovascular risk marker. A prospective multicentre cohort study by Lee *et al.* reported that for persistently higher levels of plasma TMAO in the patients aged  $>65$  years were associated with more incident atherosclerotic cardiovascular disease (the extreme quintile vs. the lowest quintile, HR: 1.21, 95% CI, 1.02–1.42;  $P$ -trend = 0.029).<sup>25</sup> Higher TMAO levels were an independent risk factor for short- and long-term composite outcome of MI, stroke, need for revascularization, or all-cause death in patients with acute coronary syndrome.<sup>4</sup> A nested case–control study indicated that the risk for cardiovascular death, MI, or stroke in patients with higher TMAO levels was ele-

**Figure 3** Continuous hazard ratios across TMAO for major adverse cardiac events (A), all-cause death (B), myocardial infarction (C), and rehospitalization due to heart failure (D). HR, hazard ratio; TMAO, trimethylamine-N-oxide.



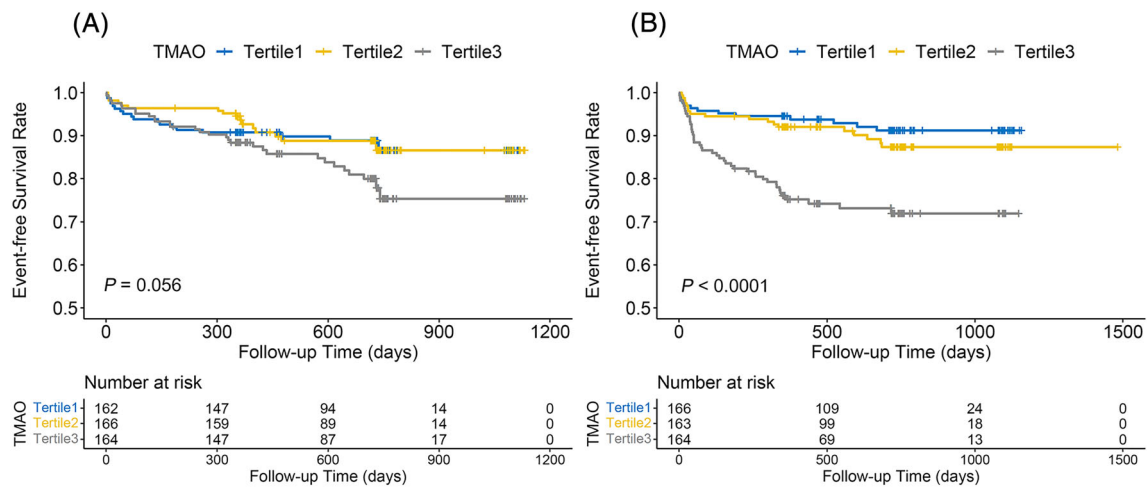
vated by approximately 50% (quartile 4 vs. quartile 1, odds ratio = 1.43, 95% CI, 1.06–1.93,  $P$ -trend = 0.015) in patients with stable coronary artery diseases.<sup>26</sup> For patients with HF, a meta-analysis reported that elevated TMAO levels predicted a higher risk of composite outcomes (including all-cause death, hospitalization with HF, and heart transplantation) with a 1.68-fold (95% CI: 1.44–1.96) increase in the HR of the highest tertile compared with that of the lowest tertile.<sup>8</sup> Our current study observed that, the relative risk for MACE was increased by 1.85 (95% CI: 1.18–2.89) times in patients with AMI and HF who had the highest levels of TMAO than that in the lowest tertile. In addition, this is the first study to show the S-shaped association between TMAO

and HR for MACE ( $P$  for nonlinearity = 0.012), implying that the HR of MACE may no longer increase after TMAO exceeds a certain level. However, the specific mechanism of this association requires further investigation.

Remarkably, our study reported that the higher risk for MACE was mainly driven by all-cause death and recurrence of MI, and not rehospitalization due to HF. As reported in previous studies, the results for rehospitalization due to HF were inconsistent in different HF subtypes, especially in patients with acute HF or HFpEF.<sup>27,28</sup> For patients with acute HF, a study by Suzuki *et al.* reported that TMAO was not associated with the risk of death or rehospitalization due to HF, after adjusting for cofounders especially eGFR.<sup>29</sup> In con-



**Figure 4** Kaplan–Meier curve for cumulative MACE-free survival rate among TMAO tertiles stratified by hsCRP below median levels (A) and above median levels (B). hsCRP, high-sensitivity C-reactive protein; MACE, major adverse cardiac event; TMAO, trimethylamine-N-oxide.



**Table 3** Association between MACE and TMAO levels stratified by hsCRP median levels

Variable	n, total	n, event (%)	Crude HR (95% CI)	P-value	Adjusted HR (95% CI) <sup>a</sup>	P-value
<b>hsCRP<sup>b</sup></b>						
>median vs. ≤median			1.17 (0.84–1.64)	0.356	1.20 (0.85–1.69)	0.292
<b>TMAO</b>						
>median vs. ≤median			2.14 (1.50–3.05)	<0.001	1.56 (1.08–2.26)	0.019
<b>hsCRP ≤ 6.68 mg/L</b>						
TMAO per SD <sup>c</sup>	-	-	1.08 (0.88–1.32)	0.482	0.89 (0.71–1.11)	0.297
<b>TMAO by median</b>						
≤median	249	27 (10.8)	1 (Ref)		1 (Ref)	
>median	243	37 (15.2)	1.46 (0.89–2.40)	0.134	1.14 (0.66–1.95)	0.646
<b>TMAO by tertile</b>						
Tertile 1	162	5 (6.6)	1 (Ref)		1 (Ref)	
Tertile 2	82	10 (12.2)	1.91 (0.65–5.59)	0.238	1.78 (0.57–5.54)	0.317
Tertile 3	79	11 (13.9)	2.14 (0.74–6.16)	0.159	2.27 (0.73–7.03)	0.155
<b>hsCRP &gt; 6.68 mg/L</b>						
TMAO per SD <sup>c</sup>	-	-	1.27 (1.14–1.41)	<0.001	1.20 (1.05–1.37)	0.009
<b>TMAO by median</b>						
≤median	243	19 (7.8)	1 (Ref)		1 (Ref)	
>median	250	55 (22.0)	3.12 (1.85–5.25)	<0.001	2.35 (1.36–4.07)	0.002
<b>TMAO by tertile</b>						
Tertile 1	166	13 (7.8)	1 (Ref)		1 (Ref)	
Tertile 2	163	18 (11.0)	1.44 (0.71–2.94)	0.315	1.27 (0.62–2.61)	0.520
Tertile 3	164	43 (26.2)	3.86 (2.07–7.19)	<0.001	2.91 (1.52–5.56)	0.001

HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; MACE, major adverse cardiac event; TMAO, trimethylamine-N-oxide.

<sup>a</sup>Adjusted for age, sex, body mass index, estimated glomerular filtration rate, current smoking, HF-type, hypertension, diabetes mellitus, peripheral artery disease, and history of stroke and MI.

<sup>b</sup>The median level of hsCRP was 6.68 mg/L.

<sup>c</sup>P = 0.041 for interaction between TMAO tertiles and hsCRP dichotomy for MACE, and adjusted P = 0.007.

trast, Israr *et al.* indicated that TMAO was independently related to the short- and long-term outcomes of a composite of all-cause mortality or rehospitalization caused by HF.<sup>30</sup> Schuett *et al.* revealed that elevated TMAO levels could not predict all-cause mortality and cardiovascular mortality in patients with HFpEF.<sup>28</sup> However, Kinugasa *et al.* reported that elevated TMAO levels at discharge were associated with an increased risk of post-discharge cardiac events in patients with HFpEF.<sup>31</sup> Given the relatively mild symptoms and signs,

and different pathophysiological processes of HFpEF, the results might be inverse. However, clinical data on acute HF or HFpEF are limited, and further studies are necessary to examine the association between TMAO levels and the prognosis in patients with acute HF or HFpEF. In addition, the precursors of trimethylamine, such as choline and carnitine, were proved to be associated with poor outcomes in patients with HF. A study by Israr *et al.* extensively suggested that circulating levels of multiple metabolites of the choline/carnitine-

TMAO pathway were graded associations with the severity and adverse prognosis of chronic HF.<sup>32</sup> It is worth expecting that combining TMAO with these biomarkers might play a better role in the risk stratification of different types of HF.

Currently, substantial evidence reveals that systemic inflammation is the predominant driver of atherosclerosis and the underlying pathology of cardiovascular diseases.<sup>33</sup> HsCRP is regarded as a marker for measuring the intensity of systemic inflammation.<sup>34</sup> In this study, we found that hsCRP levels were not associated with MACE risk ( $P = 0.077$ ), which may be because we selected the median level of hsCRP as the cutoff while the cutoff was 2 or 3 mg/L to assess residual cardiovascular risk in most previous studies.<sup>11,34</sup> Besides, our previous study found that both low and high hsCRP levels were associated with increased risk of death in AMI patients,<sup>12</sup> which may partially explain that no significance in the median levels of hsCRP and MACE risk in this study. In addition, we observed that TMAO levels were negatively correlated with hsCRP levels ( $P = 0.009$ ), which is consistent with the result of the previous study.<sup>35,36</sup> Whereas a meta-analysis<sup>37</sup> reported that various sample sources and diseases would cause different results, and there was a non-linear association between TMAO and CRP levels. More importantly, we observed that the difference in the risk for MACE between tertiles 3 and 1 was only statistically significant if the hsCRP level was above the median level. Furthermore, increasing hsCRP might enhance the effect of TMAO levels on the HR of MACE. These may be because TMAO and inflammation may have potential synergistic effects on cardiovascular risk. In the past few years, some experimental studies have suggested that TMAO activates different signalling pathways, resulting in the release of inflammatory cytokines, such as interleukin-1 $\beta$ , which induces systemic inflammation.<sup>13,14,38</sup> Seldin *et al.* reported that TMAO could activate the nuclear factor- $\kappa$ B signalling pathway, which is essential for the inflammatory effects of TMAO. Another study by Chen *et al.* revealed that TMAO caused vascular inflammation through activating the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome. Notably, NLRP3 inflammasome is required for TMAO to induce inflammatory cascades and can cause the release of interleukin-1 $\beta$  and interleukin-18, which extensively triggers systemic inflammation. These results may explain why the TMAO-associated HR for MACE was significantly elevated only when hsCRP was elevated. However, it is unclear why there is a negative correlation between TMAO and hsCRP levels. Even so, it is worth noting that elevated hsCRP levels would strengthen the impact of TMAO on adverse outcomes. The potential mechanism of interaction between body inflammatory status and TMAO levels deserves further attention and research.

The present study had a few limitations that should be acknowledged. First, the study measured the TMAO concentrations before PCI, and changes in TMAO levels during the

course were unavailable. Besides, dietary changes may influence the TMAO levels. Second, given that this study included patients with AMI and HF in the emergency department, there may have been bias of patient selection, resulting in a sample with relatively severe condition. Third, although this is a prospective cohort study, the causality between TMAO levels and its interaction with hsCRP and cardiovascular outcomes in patients with AMI and HF remains elusive. Furthermore, race and region may also affect TMAO levels.<sup>27,39</sup> Therefore, large-scale, multicentre studies are needed to verify these findings.

## Conclusions

In summary, increased TMAO levels before PCI were independently related to an increased risk of MACE in patients with AMI and HF, especially in those with elevated hsCRP levels. It is worth paying attention to the potential synergistic effects of TMAO and inflammation in the future. Notably, there is no linear relationship between the risk of MACE and increasing TMAO levels.

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## Conflict of interest

All authors declare no conflicts of interest.

## Supporting information

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

**Table S1.** Spearman's correlation coefficients between variables and ln-transformed TMAO levels.

**Table S2.** Association between variables and major adverse cardiac event.

**Figure S1.** Subject selection flow chart. AMI, acute myocardial infarction; HF, heart failure; hsCRP, high-sensitivity C-reactive protein.

**Figure S2.** Kaplan–Meier curve for cumulative event-free survival rate among groups stratified by TMAO and hsCRP median levels. A: major adverse cardiac event, B: all-cause death, C: myocardial infarction, D: rehospitalization due to

heart failure; hsCRP, high-sensitivity C-reactive protein; TMAO, trimethylamine-N-oxide.

**Figure S3.** Kaplan–Meier curve for cumulative event-free survival rate among TMAO tertiles in patients with STEMI. A: ma-

ior adverse cardiac event, B: all-cause death, C: myocardial infarction, D: rehospitalization due to heart failure; STEMI, ST-segment elevation myocardial infarction; TMAO, trimethylamine-N-oxide.

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