

# Trimethylamine N-oxide and cardiovascular outcomes in patients with chronic heart failure after myocardial infarction

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## Abstract

**Aim** Accumulating evidence has demonstrated that intestinal microbiota-dependent trimethylamine N-oxide (TMAO) is involved in the pathogenesis of various cardiovascular diseases. The present study was designed to investigate the prognostic value of TMAO in patients with chronic heart failure (CHF) after myocardial infarction (MI).

**Methods and results** We included 1208 CHF patients after MI in a prospective cohort study and determined the association between plasma TMAO and cardiovascular outcomes using Cox regression analysis. Patients with elevated TMAO levels were more likely to be older and have histories of atrial fibrillation and diabetes. Cox regression analysis indicated that TMAO was a significant predictor of major adverse cardiac events (MACE) (hazard ratio = 2.31, 95% confidence interval 1.42–3.59,  $P < 0.01$ ) following adjustment for conventional risk factors. Integrated discrimination and net reclassification improvements for MACE were markedly improved by addition of TMAO to the model of traditional risk factors. The Kaplan–Meier survival analysis showed that MACE risk increased with the elevation in TMAO levels and this positive correlation became more significant when TMAO levels were higher than the median. TMAO was also found to be an independent predictor of all-cause mortality (hazard ratio = 2.15, 95% confidence interval 1.37–3.24,  $P < 0.01$ ) after adjusting for traditional risk factors.

**Conclusions** Our study suggests that TMAO is a valuable prognostic indicator of MACE in patients with CHF after MI.

**Keywords** Cardiovascular outcomes; Chronic heart failure; Myocardial infarction; Trimethylamine N-oxide

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## Introduction

In recent years, growing evidence has demonstrated that intestinal microbiota play critical roles in cardiovascular health and disease.<sup>1</sup> Dietary choline and L-carnitine are metabolized by gut bacteria to generate trimethylamine, which is reabsorbed into the bloodstream and oxidized to trimethylamine N-oxide (TMAO) in the liver. Emerging experimental and clinical research have suggested that TMAO is involved in the pathogenesis and prognosis of cardiovascular disease, metabolic disease, and chronic kidney disease.<sup>2–5</sup>

A prospective study by Tang *et al.* showed that increased TMAO levels portended a higher risk of long-term mortality independent of conventional risk factors in patients with stable heart failure (HF).<sup>6</sup> Senthong *et al.* reported that plasma TMAO was an independent predictor of atherosclerotic burden and long-term mortality in patients with coronary artery disease.<sup>7,8</sup> Li *et al.* revealed that TMAO was a prognostic marker for predicting incident cardiovascular events beyond traditional risk factors in acute coronary syndrome.<sup>9</sup> In this multicentre prospective cohort study, we aimed to assess the prognostic value of plasma TMAO in Chinese

patients with chronic heart failure (CHF) after myocardial infarction (MI).

## Methods

### Study population

A total of 1208 consecutive CHF patients after MI admitted to the affiliated hospitals of Soochow University, Jiangsu University, Nanjing Medical University, and Xuzhou Medical University were recruited between January 2014 and March 2018. This study was carried out in accordance with the Declaration of Helsinki and approved by the Clinical Ethics Committee. Patients were included according to the following criteria: history of MI for at least 6 months and CHF diagnosed based on typical symptoms and signs of pulmonary congestion or peripheral oedema, and echocardiographically confirmed left ventricular systolic dysfunction. The exclusion criteria included CHF secondary to other heart diseases, malignant tumour, and end-stage renal disease. Demographical, clinical, and biochemical data were obtained from the medical records. The written informed consent was obtained from each participant before enrolment.

### Measurement of plasma trimethylamine N-oxide

Fasting blood samples were collected using EDTA tubes from CHF patients on admission. Plasma was separated by centrifugation at 3000 rpm for 10 min and then stored at  $-80^{\circ}\text{C}$ . Plasma TMAO levels were measured using stable isotope dilution high performance liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry on an AB Sciex 4000 QTRAP mass spectrometer (Framingham, MA, USA) with trimethylamine-d9 N-oxide as the internal standard.

### Endpoints

The primary composite endpoint was major adverse cardiac events (MACE), including all-cause mortality, HF rehospitalization, or recurrent MI. The secondary endpoint was all-cause mortality. HF rehospitalization was defined as a hospital readmission due to worsening HF requiring intravenous drug therapy. Recurrent MI was diagnosed according to the criteria of European Society of Cardiology.<sup>10</sup> Endpoints were obtained by reviewing the hospital database and by contacting patients or their families.

### Statistical analysis

Continuous variables were expressed as median (interquartile range) and compared with Mann–Whitney  $U$  test. Categorical variables were presented as number (percentage) and compared with  $\chi^2$  test. The association between baseline variables and MACE was assessed using Cox regression analysis. Hazard ratio (HR) and 95% confidence interval (CI) were calculated. Adjustments were made for traditional risk factors, including age, gender, body mass index, hypertension, diabetes, hyperlipidaemia, New York Heart Association class, and left ventricular ejection fraction, and for log-transformed N-terminal pro-B-type natriuretic peptide (NT-proBNP), estimated glomerular filtration rate (eGFR), and high-sensitivity C-reactive protein (hsCRP), to predict MACE and all-cause mortality. Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were calculated to assess the improvement in MACE risk prediction after addition of TMAO to the basic model. The Kaplan–Meier survival analysis was performed to compare the survival rate among patients with different levels of TMAO. In this study,  $P < 0.05$  was considered statistically significant.

## Results

### Baseline characteristics

The baseline characteristics of patients are shown in *Table 1*. Patients were allocated into two groups according to the median levels of TMAO. Patients with elevated TMAO levels were more likely to be older and have histories of atrial fibrillation and diabetes. TMAO seemed to be positively associated with hsCRP and NT-proBNP levels, while inversely correlated with left ventricular ejection fraction and eGFR. The median length of follow-up was 672 days. During the follow-up period, 156 patients died, 384 readmitted with HF, and 67 suffered recurrent MI.

### Trimethylamine N-oxide and major adverse cardiac events

As shown in *Table 2*, elevated TMAO levels were associated with an increased risk of MACE (quartile 4 vs. 1; unadjusted HR = 3.15, 95% CI 2.09–4.73,  $P < 0.01$ ). Following adjustment for traditional risk factors and log-transformed NT-proBNP, eGFR, and hsCRP, plasma TMAO remained a significant predictor of MACE (Model 1: HR = 2.31, 95% CI 1.42–3.59,  $P < 0.01$ ; Model 2: HR = 1.68, 95% CI 1.13–2.81,  $P < 0.01$ ; Model 3: HR = 1.57, 95% CI 1.08–2.64,  $P < 0.01$ ). Moreover, IDI and NRI for MACE were significantly improved by addition of

**Table 1** Baseline characteristics of patients in this study

	Overall (n = 1208)	TMAO <4.5 µmol/L	TMAO ≥4.5 µmol/L	P value
Age (years)	73 (64–80)	71 (61–78)	74 (66–81)	<0.001
Male (%)	828 (68.5)	425 (70.4)	403 (66.7)	NS
BMI (kg/m <sup>2</sup> )	28.1 (25.4–30.2)	27.9 (25.1–29.8)	28.5 (25.6–30.7)	NS
Systolic BP (mmHg)	126 (110–139)	128 (113–142)	123 (108–136)	NS
Diastolic BP (mmHg)	72 (63–82)	74 (65–85)	68 (60–78)	0.003
Heart rate (bpm)	83 (69–98)	85 (72–101)	81 (67–95)	NS
Previous history				
Atrial fibrillation (%)	225 (18.6)	90 (14.9)	135 (22.4)	0.015
Hypertension (%)	540 (44.7)	256 (42.4)	284 (47.0)	NS
Diabetes (%)	345 (28.6)	132 (21.9)	213 (35.3)	<0.001
Hyperlipidaemia (%)	479 (39.7)	248 (41.1)	231 (38.2)	NS
NYHA class III–IV (%)	484 (40.1)	287 (47.5)	197 (32.6)	<0.001
LVEF (%)	39 (35–42)	43 (38–46)	34 (30–38)	<0.001
Revascularization (%)	996 (82.5)	514 (85.1)	482 (79.8)	NS
Medical treatment				
Aspirin (%)	1042 (86.3)	532 (88.1)	510 (84.4)	NS
Clopidogrel (%)	123 (10.2)	57 (9.4)	66 (10.9)	NS
Ticagrelor (%)	170 (14.1)	89 (14.7)	81 (13.4)	NS
Statin (%)	985 (81.5)	521 (86.3)	464 (76.8)	0.021
Beta-blocker (%)	924 (76.5)	488 (80.8)	436 (72.2)	0.032
ACE-I/ARB (%)	879 (72.8)	453 (75.0)	426 (70.5)	NS
Spironolactone (%)	701 (58.0)	340 (56.3)	361 (59.8)	NS
NT-proBNP (pg/mL)	2465 (1589–3627)	1398 (892–2304)	3826 (2680–5245)	<0.001
eGFR (mL/min/1.73 m <sup>2</sup> )	74 (58–86)	80 (65–94)	63 (50–75)	<0.001
hsCRP (mg/L)	3.8 (2.1–6.3)	3.0 (1.5–5.2)	4.7 (3.2–7.1)	0.028

Values are median (interquartile range) or n (%). ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; LVEF, left ventricular ejection fraction; NS, not significant; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; TMAO, trimethylamine N-oxide.

TMAO to the model of traditional risk factors (IDI = 12.6% and NRI = 9.5%,  $P < 0.01$ ).

The Kaplan–Meier survival analysis showed that MACE risk increased with the elevation in TMAO levels and this positive correlation became more significant when TMAO levels were higher than the median (*Figure 1*). Moreover, the risks of MACE were similar between male and female patients, as well as other clinical subgroups (*Figure 2*).

unadjusted HR = 3.02, 95% CI 1.98–4.56,  $P < 0.01$ ). Following adjustment for conventional risk factors and log-transformed NT-proBNP, eGFR, and hsCRP, plasma TMAO remained a significant predictor of all-cause mortality (Model 1: HR = 2.15, 95% CI 1.37–3.24,  $P < 0.01$ ; Model 2: HR = 1.60, 95% CI 1.09–2.67,  $P < 0.01$ ; Model 3: HR = 1.53, 95% CI 1.06–2.51,  $P < 0.01$ ).

## Trimethylamine N-oxide and all-cause mortality Discussion

As shown in *Table 3*, elevated TMAO levels were related to an increased risk for all-cause mortality (quartile 4 vs. 1;

Several recent studies have focused on the role of intestinal microbiota in cardiovascular and metabolic diseases.<sup>2–4</sup>

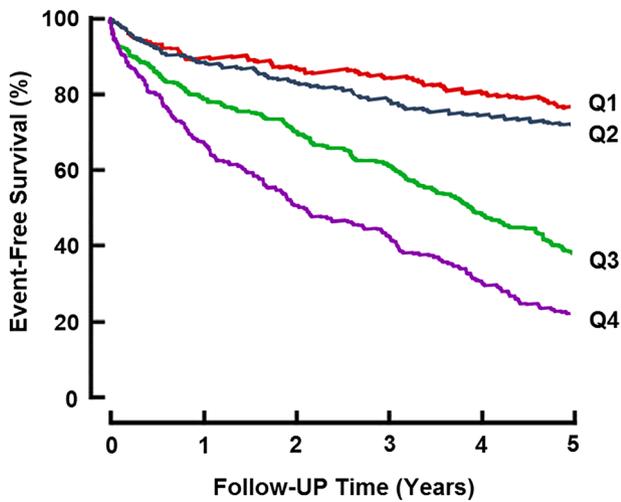
**Table 2** Hazard ratio of plasma trimethylamine N-oxide levels for major adverse cardiac events

	Quartile 1 <2.83 µmol/L	Quartile 2 2.83–4.50 µmol/L	Quartile 3 4.50–7.92 µmol/L	Quartile 4 >7.92 µmol/L
Unadjusted	1	1.26 (0.79–2.01)	1.94 (1.48–2.80)*	3.15 (2.09–4.73)*
Adjusted Model 1	1	1.14 (0.73–1.85)	1.52 (1.07–2.46)*	2.31 (1.42–3.59)*
Adjusted Model 2	1	1.09 (0.62–1.74)	1.30 (0.86–2.09)	1.68 (1.13–2.81)*
Adjusted Model 3	1	1.06 (0.57–1.70)	1.24 (0.76–1.95)	1.57 (1.08–2.64)*

Model 1: adjusted for traditional risk factors, including age, gender, body mass index, hypertension, diabetes, hyperlipidaemia, New York Heart Association class, and left ventricular ejection fraction. Model 2: adjusted for Model 1 plus log-transformed N-terminal pro-B-type natriuretic peptide and estimated glomerular filtration rate. Model 3: adjusted for Model 2 plus log-transformed high-sensitivity C-reactive protein.

\* $P < 0.01$ .

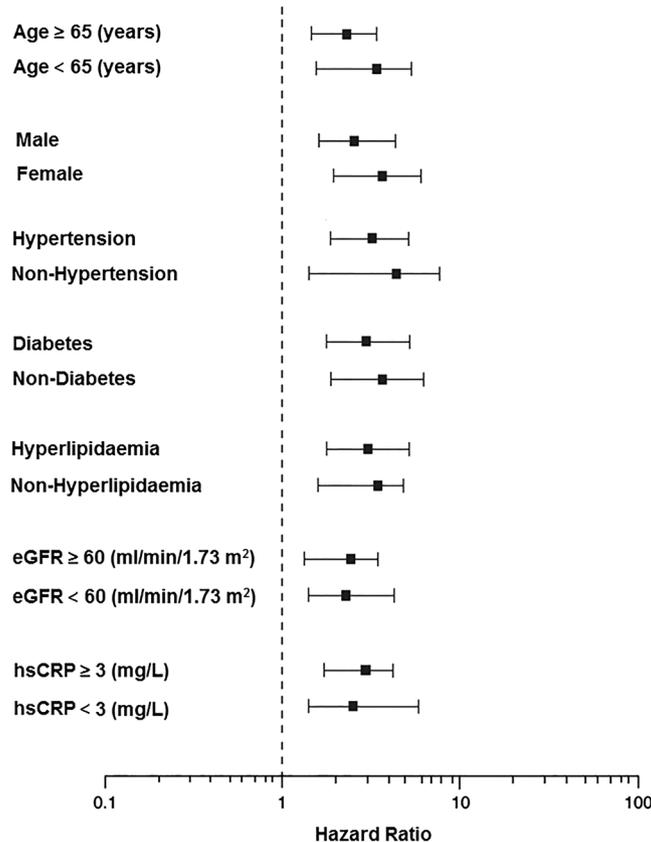
**Figure 1** Kaplan–Meier analysis of event-free survival for major adverse cardiac events in patients with chronic heart failure after myocardial infarction, stratified according to the quartiles of plasma trimethylamine N-oxide levels.



Microbial sequencing analysis has revealed that gut metagenome may result in the development of symptomatic atherosclerosis by regulating host inflammatory pathways.<sup>11</sup> TMAO, which is a metabolite derived from dietary phosphatidylcholine and gut microbes, has been reported to be involved in the pathogenesis of coronary artery disease. In the present study, we included 1208 consecutive patients with CHF after MI in a prospective cohort study and explored the association between plasma TMAO and cardiovascular outcomes using Cox regression analysis. Our results suggested that TMAO might be a valuable predictor of MACE and could be used to improve risk stratification in patients with ischaemic CHF.

Koeth *et al.* indicated that chronic dietary supplementation of L-carnitine in mice could alter caecal microbial composition, increase TMAO synthesis, and promote atherosclerosis.<sup>12</sup> Wang *et al.* performed a prospective cohort study and showed that elevated levels of choline and betaine were associated with incident MACE risk dependent on intestinal microbiota-generated TMAO.<sup>13</sup> Suzuki *et al.* revealed that increased TMAO levels were correlated with a poor prognosis

**Figure 2** Relationship between plasma trimethylamine N-oxide levels and major adverse cardiac events, stratified according to baseline characteristics. Forest plot of the hazard ratio of major adverse cardiac events comparing first and fourth quartiles of plasma trimethylamine N-oxide levels. eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein.



**Table 3** Hazard ratio of plasma trimethylamine N-oxide levels for all-cause mortality

	Quartile 1	Quartile 2	Quartile 3	Quartile 4
	<2.83 $\mu\text{mol/L}$	2.83–4.50 $\mu\text{mol/L}$	4.50–7.92 $\mu\text{mol/L}$	>7.92 $\mu\text{mol/L}$
Unadjusted	1	1.21 (0.75–1.96)	1.87 (1.34–2.73)*	3.02 (1.98–4.56)*
Adjusted Model 1	1	1.12 (0.70–1.82)	1.39 (0.95–2.18)	2.15 (1.37–3.24)*
Adjusted Model 2	1	1.06 (0.59–1.68)	1.23 (0.79–1.91)	1.60 (1.09–2.67)*
Adjusted Model 3	1	1.04 (0.55–1.63)	1.19 (0.72–1.80)	1.53 (1.06–2.51)*

Model 1: adjusted for traditional risk factors, including age, gender, body mass index, hypertension, diabetes, hyperlipidaemia, New York Heart Association class, and left ventricular ejection fraction. Model 2: adjusted for Model 1 plus log-transformed N-terminal pro-B-type natriuretic peptide and estimated glomerular filtration rate. Model 3: adjusted for Model 2 plus log-transformed high-sensitivity C-reactive protein.

\* $P < 0.01$ .

in patients with acute HF, and the combination of TMAO and NT-proBNP could provide additional prognostic information.<sup>14</sup> Moreover, a recent single-centre study by Suzuki *et al.* reported that TMAO has important prognostic value in patients with acute MI and is superior to contemporary biomarkers.<sup>15</sup>

We carried out a multicentre prospective cohort study and found that TMAO was an independent predictor of MACE and all-cause mortality in patients with CHF after MI following adjustment for conventional risk factors. Addition of TMAO to the traditional model contributed to an improvement in IDI and NRI for MACE prediction. The modifiable nature of TMAO highlights the importance of our findings and provides new perspectives for treatment strategies in the secondary prevention.

In recent years, several animal experiments have been conducted to elucidate the pathological mechanisms of TMAO in the development of atherosclerosis and myocardial remodelling. TMAO was found to exert proatherogenic effects in mice and up-regulate CD36 and scavenger receptor A expression in macrophages.<sup>12</sup> In addition, TMAO could alter platelet calcium signalling and promote thrombosis *in vivo*,<sup>16</sup> which suggested that TMAO might be a promising biomarker for coronary plaque vulnerability and progression. Li *et al.* revealed that TMAO promoted cardiac hypertrophy involving Smad3 signalling, indicating that suppression of TMAO production might be a potential target for the treatment of cardiac hypertrophy.<sup>17</sup> Moreover, Organ *et al.* generated a mouse model of pressure overload-induced HF and found

that dietary supplementation of TMAO aggravated myocardial fibrosis and left ventricular remodelling and dysfunction.<sup>18</sup>

There are some limitations in our study. Firstly, TMAO levels are influenced by intestinal microbiota composition, which is affected by diet and other factors, such as age, gender, and ethnicity. Secondly, we did not perform serial measurements of plasma TMAO after patients were included. Thirdly, although this is a prospective cohort study, there is still a lack of causality between TMAO levels and cardiovascular outcomes in patients with ischaemic CHF.

In conclusion, our study suggests that TMAO is a prognostic marker of MACE in CHF patients after MI, independent of traditional risk factors, which may improve the risk stratification in patients with ischaemic CHF.

## Conflict of interest

None declared.

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