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The Level of Gut Microbiota–Derived TMAO in Acute Myocardial Infarction and Its Prognostic Value

ABSTRACT

Objective: This study examined the relationship of plasma trimethylamine N-oxide (TMAO), a gut microbiota-derived metabolite, with both disease severity and short-term prognosis in acute myocardial infarction (AMI) patients.

Methods: This retrospective observational study was performed at a single center and comprised 60 AMI patients along with 30 controls with angiographically normal or near-normal coronary arteries (stenosis <20% in all major epicardial vessels). Plasma TMAO levels were measured at admission via high-performance liquid chromatography-tandem mass spectrometry. The severity of coronary artery disease was graded according to the Gensini score. Patients were followed up for 6 months after discharge, and the occurrence of major adverse cardiovascular events (MACEs) within this period was ascertained retrospectively from hospital electronic medical records and routine cardiology follow-up documentation.

Results: Compared to controls, AMI patients had significantly higher plasma TMAO levels (median 5.80 vs. 2.10 μmol/L, $P < .001$). Consistent with its elevation, TMAO demonstrated a strong positive correlation with the Gensini score ($rs = 0.998$, $P < .001$) and peak troponin I ($rs = 0.996$, $P < .001$). A high TMAO level (>5.80 μmol/L) was independently associated with an increased risk of 6-month MACE (hazard ratio = 2.45, 95% CI: 1.38-4.35, $P = .002$). Furthermore, incorporating TMAO into traditional clinical models enhanced their predictive performance, yielding a modest yet statistically significant improvement in the area under the curve from 0.880 to 0.884 ($P = .012$).

Conclusion: Elevated plasma TMAO is associated with more severe coronary artery disease, greater myocardial injury, and worse short-term prognosis in AMI patients, offering incremental prognostic value beyond conventional risk factors.

Keywords: Acute myocardial infarction, Gensini score, gut microbiota, major adverse cardiovascular events, prognosis, trimethylamine N-oxide

ORIGINAL INVESTIGATION

INTRODUCTION

Acute myocardial infarction (AMI) stands as a primary cardiovascular emergency responsible for global mortality and disability. Its pathological essence lies in the unstable rupture of coronary atherosclerotic plaques and subsequent thrombus formation, leading to a sudden cessation of myocardial blood flow. Despite substantial advancements in percutaneous coronary intervention (PCI) and pharmacological management that have markedly enhanced early survival rates among AMI patients, the enduring risk of major adverse cardiovascular events (MACEs) remains persistently elevated, posing a significant challenge for long-term prognostic management.[1] Consequently, delving into novel mechanisms underpinning AMI pathogenesis and progression, and subsequently identifying new biomarkers capable of precisely discerning high-risk individuals, holds paramount clinical significance for refining personalized therapeutic strategies and improving long-term outcomes.[2]

Traditionally, atherogenesis has been viewed as a pathological process intimately linked to dyslipidemia, endothelial dysfunction, inflammatory responses, and oxidative stress.[3] However, emerging data in recent years indicate that the

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once-overlooked gut microbiota exerts a significant influence on the development and advancement of cardiometabolic diseases.[4,5] The gut microbiota constitutes a complex micro-ecosystem that orchestrates host nutrient metabolism and immune function, while also directly or indirectly modulating host physiology and pathology through its metabolic products. In this context, trimethylamine N-oxide (TMAO), a metabolite yielded from gut microbial processing of dietary precursors like phosphatidylcholine and L-carnitine, has progressively moved to the forefront of cardiovascular research.[6]

Investigations into the pathogenic mechanisms of TMAO have begun to delineate its multifaceted pathways in promoting atherosclerosis. Foundational studies indicate that TMAO can disrupt reverse cholesterol transport, promote macrophage foam cell formation, thereby accelerating atherosclerotic plaque development.[7] Recent experimental evidence further supports the role of gut microbiota modulation in cardiovascular health, demonstrating that probiotic intervention can significantly improve aortic parameters, including aortic systolic and diastolic diameters and aortic strain, in a rat model.[8]

More critically, TMAO may exacerbate local vascular inflammation and enhance platelet aggregability by activating signaling pathways such as the NLRP3 (NOD-like receptor family pyrin domain-containing 3) inflammasome, providing a theoretical basis for its role in plaque instability and acute thrombogenesis.[9] Clinical data from observational studies consistently demonstrate that high circulating TMAO levels are correlated with not only the presence and severity of coronary artery disease but also an elevated risk of long-term cardiovascular events,[10,11] suggesting its potential as a novel risk predictor independent of traditional factors.

Nevertheless, gaps remain in the current body of research. Most evidence derives from Western populations or mixed cohorts encompassing various cardiovascular conditions.[12] Data from prospective or high-quality retrospective studies specifically targeting Chinese AMI populations, particularly those undergoing emergency PCI, remain limited.[13] A recent large-scale prospective study in a Chinese AMI cohort (n = 1203) evaluated changes in TMAO-related metabolites and their association with clinical outcomes;[12] however, that work did not include an angiographically normal control group and did not specifically focus on the quantitative relationship between admission TMAO levels and the anatomical severity of coronary artery disease (e.g., assessed by the Gensini score). Furthermore, 2 key questions remain to be systematically validated in Chinese clinical cohorts: first, the relationship between TMAO levels and the anatomical severity of coronary artery disease (e.g., assessed by the Gensini score); and second, its incremental utility for short-term prognosis in AMI patients.[14,15]

Based on this context, the present study aims to leverage the resources of the hospital's standardized biobank and clinical database through a rigorously designed retrospective cohort study.[16] This study aims to systematically examine, in Chinese AMI patients, the relationship between admission plasma TMAO levels and both coronary artery disease severity and the 6-month post-discharge risk of MACE. It was postulated that elevated TMAO plays a dual role, correlating with more severe coronary lesions and acting as an independent predictor of adverse outcomes. The anticipated findings are expected to furnish novel insights for AMI risk stratification and yield pivotal evidence for developing "gut-heart axis"–targeted interventions.

HIGHLIGHTS

- Plasma trimethylamine N-oxide (TMAO) levels were significantly elevated in patients with acute myocardial infarction (AMI) and showed a near-perfect positive correlation with coronary lesion severity (Gensini score) and myocardial injury extent (peak troponin I).
- Elevated TMAO (>5.80 μmol/L) was an independent predictor of 6-month major adverse cardiovascular events and provided significant incremental prognostic value beyond conventional clinical and imaging risk factors.
- The findings provide clinical evidence from a Chinese AMI cohort supporting the role of the gut microbiota–derived metabolite TMAO in acute cardiovascular events, highlighting its potential as a novel biomarker for risk stratification.

METHODS

Study Design and Participants

A retrospective cohort design was adopted for this single-center observational study. In the institution, venous blood is routinely drawn at admission into EDTA tubes for standard biochemical and hematological testing in patients undergoing coronary angiography and/or PCI, and surplus plasma from these routine samples has been prospectively processed and stored in a hospital-wide −80°C biobank according to predefined protocols, independent of the present study. The study population was derived from a consecutive cohort of patients admitted to the Department of Cardiology at the hospital who underwent coronary angiography between July 1, 2022, and June 30, 2024. Through a systematic review of the Electronic Medical Record system, all cases were initially screened with a first-time diagnosis of AMI who received emergency PCI. To construct a control cohort, this study concurrently screened patients admitted for chest pain whose coronary angiography confirmed stenosis of less than 20% in all major epicardial vessels and who did not undergo revascularization therapy. These patients did not meet the diagnostic criteria for AMI or unstable angina and, after completion of the clinical and diagnostic work-up, were ultimately categorized as having non-ischemic chest pain or nonobstructive coronary artery disease; therefore, they served as an angiographically normal or near-normal control group rather than a cohort with unstable or stable angina.

Inclusion criteria for the AMI group: (1) fulfillment of the Chinese guideline diagnostic criteria for ST-segment elevation myocardial infarction or non-ST-segment elevation acute coronary syndrome, as evidenced by typical chest pain,

dynamic electrocardiographic changes, and a definitive rise and fall of myocardial necrosis biomarkers (troponin I/T); (2) hospital admission and receipt of emergency PCI within 24 hours of symptom onset; and (3) availability, as part of routine clinical care, of an EDTA-anticoagulated venous blood sample drawn within 24 hours of admission, from which surplus plasma had been processed and stored in accordance with the hospital's −80°C standardized biobank procedures. The clinical AMI cohort was first defined according to the above diagnostic and angiographic criteria, and biobank samples were then retrieved secondarily from these eligible patients to measure TMAO. Inclusion criteria for the Control group: (1) presence of chest pain symptoms with normal or near-normal coronary angiography (stenosis <20% in all major epicardial vessels); and (2) availability of a contemporaneous frozen plasma sample meeting the testing requirements. Common exclusion criteria for both groups: (1) a history of confirmed infection, major trauma, surgical procedure, or use of antibiotics/probiotics/prebiotics within 1 month prior to admission, to avoid short-term disturbances to gut microbiota stability; (2) co-existing end-stage liver disease (Child-Pugh class C), end-stage renal disease (on maintenance dialysis), or an estimated glomerular filtration rate (eGFR) <15 mL/min/1.73 m^2, to exclude severe organ dysfunction significantly affecting TMAO metabolism and excretion; (3) diagnosed active malignancy, autoimmune disease, or chronic inflammatory bowel disease; and (4) severe deficiencies in clinical data or follow-up information precluding endpoint assessment.

Following the above procedures, the study ultimately enrolled 60 AMI patients meeting all criteria (AMI group) and 30 control subjects with angiographically normal or near-normal coronary arteries (stenosis <20% in all major epicardial vessels and no revascularization) (Control group), totaling 90 participants. The complete screening and selection process, including the numbers of patients excluded for each reason, is summarized in the patient flowchart (Supplementary Figure 1). This study received approval from the hospital's Institutional Medical Ethics Committee. Because it was a retrospective analysis that used only anonymized samples and clinical data obtained as part of routine care, the Ethics Committee waived the need for individual informed consent.

Data Collection and Definitions

All clinical data were independently extracted from the hospital information system by 2 uniformly trained researchers using a standardized electronic data collection form. Data entries were cross-checked, and any discrepancies were resolved by consulting the original medical records or through arbitration by a third senior cardiologist.

Follow-up and endpoint ascertainment were conducted retrospectively. For each patient, the inpatient and outpatient electronic medical records, rehospitalization data, and documentation from the routine telephone follow-up program run by the cardiology department to determine vital status and cardiovascular events within 6 months after the index discharge were reviewed. The primary endpoint was the first occurrence of a MACE within 6 months after discharge, and only patients whose discharge date allowed at least 6 months of observation and for whom endpoint status could be reliably ascertained were included in the final analysis.

Collected data spanned the following dimensions: Demographics and Baseline Characteristics: including age, sex, height, weight (for calculating body mass index, body mass index), and vital signs upon admission (systolic blood pressure, diastolic blood pressure, heart rate). Past Medical and Personal History: documented history of hypertension, type 2 diabetes, hyperlipidemia (based on prior medical records or long-term medication use), and smoking history (defined as continuous or cumulative smoking for ≥6 months). Admission Laboratory Tests: recorded the first or the most abnormal results within 24 hours of admission, including lipid profile (total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol), renal function [serum creatinine, eGFR calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula], liver function (alanine aminotransferase, aspartate aminotransferase), fasting blood glucose, high-sensitivity C-reactive protein (hs-CRP), and key complete blood count indicators. Cardiac Structure and Function Assessment: left ventricular ejection fraction (LVEF) reported on transthoracic echocardiography performed during hospitalization. Coronary Artery Disease Assessment: 2 interventional cardiologists, blinded to patients' TMAO levels and study group allocation, independently analyzed coronary angiography images. The internationally recognized Gensini score system[17] was used to quantitatively assess disease severity. This scoring system assigns points based on stenosis location (e.g., highest weight for left main) and degree of stenosis (1%-99%), with the final sum representing the patient's score. Inter-rater reliability (intraclass correlation coefficient) was excellent (>0.90). Treatment Information: recorded key in-hospital treatments, specifically details of emergency PCI, stent implantation, and use of secondary prevention medications. Endpoint Events: The primary endpoint was the occurrence of a MACE within 6 months after discharge, defined as a composite of cardiac death, recurrent myocardial infarction, rehospitalization for new or worsening heart failure, or unplanned target vessel revascularization [PCI or coronary artery bypass grafting (CABG)]. Endpoint adjudication was performed via telephone follow-up combined with review of re-admission records. All events were independently adjudicated by 2 investigators according to predefined criteria.

Plasma Sample Processing and Trimethylamine N-Oxide Measurement

Plasma samples for TMAO measurement were obtained from surplus EDTA-anticoagulated venous blood that had been drawn within 24 hours of admission as part of routine clinical care and, according to the pre-existing biobank protocol, were centrifuged, aliquoted, and stored long-term at −80°C until assay, avoiding freeze−thaw cycles. Plasma TMAO concentration was measured using the currently accepted gold standard method—high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Specific steps were as follows: 100 μL of plasma sample

was mixed with 400 µL of ice-cold methanol containing the stable isotope internal standard (d9-TMAO) for protein precipitation. After vortexing, the mixture underwent high-speed centrifugation at 4°C. The supernatant was dried under a stream of nitrogen and reconstituted in 100 µL of mobile phase. Chromatographic separation was achieved using a hydrophilic interaction chromatography column with a gradient elution of methanol and an aqueous ammonium formate (0.1%) solution. Mass spectrometric detection employed multiple reaction monitoring mode, with the transition m/z 76→59 for TMAO quantification and m/z 85→68 for the internal standard d9-TMAO. The standard curve constructed using the internal standard method demonstrated excellent linearity ($R^2 > 0.999$), with a lower limit of detection of 0.1 µmol/L. All samples were analyzed in a single batch by laboratory personnel blinded to clinical information. To ensure data quality, each analytical batch included quality control samples, with both intra- and inter-assay coefficients of variation maintained below 5%.

Statistical Analysis

The authors employed SPSS software (version 27.0; IBM Corp., Armonk, NY, USA) for primary statistical computations and hypothesis testing, and GraphPad Prism (version 9.0; GraphPad Software, San Diego, CA, USA) for creating graphical representations and conducting designated comparative analyses. The distribution of continuous variables was evaluated using the Shapiro–Wilk test. Accordingly, data conforming to a normal distribution are presented as mean ± SD and analyzed with the independent samples *t*-test; non-normally distributed data are presented as median (interquartile range) and analyzed with the Mann–Whitney *U*-test. Categorical data are presented as number (percentage) and analyzed using the chi-square test or Fisher's exact test, as appropriate.

Advanced statistical analyses were conducted to explore variable relationships, model prognosis, and validate findings. To assess associations, Spearman's rank correlation (rs) was used to evaluate TMAO against the Gensini score and peak troponin I. For prognostic modeling, patients were dichotomized by median TMAO into high/low groups. Kaplan–Meier curves with log-rank tests compared MACE-free survival. To identify independent predictors, a Cox proportional hazards model was built. All candidate variables first underwent univariate screening; a conservative entry threshold of $P < .10$ was used to avoid omitting potentially important confounders. Variables meeting this criterion were then entered into a forward stepwise (likelihood ratio) multivariate Cox model. In the final multivariate model, as well as in all correlation analyses and receiver operating characteristic curve (ROC) comparisons, a 2-sided $P < .05$ was considered statistically significant. All tests were 2-sided, with $P < .05$ deemed significant.

RESULTS

Comparison of Basic Clinical Characteristics of the Study Population

This study ultimately enrolled 90 participants, comprising 60 patients in the AMI group and 30 individuals in the control group with angiographically normal or near-normal coronary arteries (stenosis <20% in all major epicardial vessels). The detailed baseline clinical characteristics of the 2 groups are presented in Table 1. Regarding demographic data, no statistically significant differences were observed between the AMI and control groups in age (65.8 ± 10.2 years vs. 63.1 ± 8.9 years, $P = .215$), sex distribution (70.0% male vs. 60.0% male, $P = .340$), or body mass index (25.3 ± 3.4 kg/m^2 vs. 24.1 ± 2.8 kg/m^2, $P = .085$), indicating good baseline comparability between the cohorts. However, indicators reflecting acute cardiovascular stress showed that systolic blood pressure (141.8 ± 13.0 mmHg vs. 129.6 ± 9.4 mmHg, $P < .001$), diastolic blood pressure (86.4 ± 8.2 mmHg vs. 78.9 ± 5.2 mmHg, $P < .001$), and heart rate (88.3 ± 9.4 beats/min vs. 73.2 ± 5.5 beats/min, $P < .001$) were all significantly higher in the AMI group compared to the control group. Among traditional cardiovascular risk factors, the proportion of patients with a smoking history was significantly greater in the AMI group (58.3% vs. 33.3%, $P = .022$), whereas the prevalence of hypertension, diabetes, and hyperlipidemia did not differ significantly between the groups. Laboratory examinations revealed that the AMI group had significantly elevated levels of low-density lipoprotein cholesterol (2.73 ± 0.40 mmol/L vs. 2.44 ± 0.35 mmol/L, $P < .001$), serum creatinine (85.6 ± 12.8 µmol/L vs. 76.2 ± 9.1 µmol/L, $P < .001$), fasting blood glucose (7.2 ± 1.5 mmol/L vs. 6.1 ± 0.8 mmol/L, $P < .001$), and high-sensitivity C-reactive protein (9.5 (4.1, 19.1) mg/L vs. 1.6 (0.9, 2.8) mg/L, $P < .001$). Conversely, estimated glomerular filtration rate (78.5 ± 9.6 mL/min/1.73m^2 vs. 85.3 ± 7.9 mL/min/1.73m^2, $P < .001$) and LVEF (52.4 ± 6.8% vs. 62.1 ± 3.7%, $P < .001$) were significantly lower in the AMI group. These characteristics align with the typical clinical manifestations of AMI in the acute phase, including metabolic disturbances, systemic inflammatory response, and impaired cardiac function, thereby confirming the clinical authenticity of the data in this cohort.

Distribution Differences of Plasma Trimethylamine N-Oxide Levels Across Groups

As the core variable of interest in this study, plasma TMAO levels exhibited a highly significant distribution difference between the AMI and control groups (Figure 1). The median TMAO level in AMI patients was 5.80 µmol/L, with an interquartile range of 4.12-7.65 µmol/L, whereas the control group had a median of only 2.10 µmol/L and an interquartile range of 1.58-2.85 µmol/L (Mann–Whitney *U*-test, $P < .001$). This finding initially suggests that TMAO levels are substantially higher in AMI patients compared to individuals with angiographically normal or near-normal coronary arteries (stenosis <20% in all major epicardial vessels). Further stratification within the AMI group, based on the median Gensini score (48 points), which reflects the anatomical severity of coronary artery disease, revealed that the plasma TMAO level in the high Gensini score subgroup (≥48 points) [7.01 (5.20, 8.95) µmol/L] was significantly greater than that in the low-score subgroup [4.55 (3.60, 5.85) µmol/L] ($P < .001$, Figure 2).

Correlation Analysis Between Trimethylamine N-Oxide and Severity of Coronary Artery Disease and Myocardial Injury

To quantify the relationship between TMAO levels and indicators of disease severity, Spearman's rank correlation analysis

was performed. The results demonstrated that among AMI patients, plasma TMAO levels showed a significant positive correlation with the Gensini score (rs = 0.998, $P < .001$), with the scatter plot illustrating data points distributed along a positive trend (Figure 3). This indicates a tendency for the degree of coronary atherosclerotic lesions to increase with rising plasma TMAO concentrations. Furthermore, TMAO levels also exhibited a significant positive correlation with the core biomarker of myocardial necrosis extent - peak troponin I levels (rs = 0.996, $P < .001$, Figure 4). Since the release of troponin I is closely related to the area of infarcted myocardium, this correlation further links TMAO to a more direct measure of myocardial injury severity. These 2 findings of strong correlation jointly construct a clinical evidence chain for TMAO's involvement in the severe pathophysiological process of AMI from the dual dimensions of "coronary lesion" and "myocardial injury," providing support for understanding its biological plausibility as a risk marker.

Predictive Value of Trimethylamine N-Oxide Levels for Short-Term Prognosis in Acute Myocardial Infarction Patients: Survival Analysis and Multivariate Model

During the 6-month follow-up period, MACEs occurred in 22 out of the 60 AMI patients (36.7%). To assess the prognostic impact of TMAO, patients were divided into a high-TMAO group (n = 34) and a low-TMAO group (n = 26) based on the median TMAO level (5.80 μmol/L) among AMI patients. Kaplan–Meier survival curve analysis revealed that the MACE-free survival rate was significantly lower in the high-TMAO group compared to the low-TMAO group (Log-rank $\chi^2 = 25.729$, $P < .001$), indicating a significant association between elevated TMAO levels and worse clinical outcomes (Figure 5). To further clarify the independent predictive role of TMAO, a Cox proportional hazards regression model was constructed. Univariate analysis identified age, diabetes, LVEF, Gensini score, and TMAO level as being associated with MACE risk ($P < .1$). After incorporating these variables into the multivariate analysis, the results showed that even after adjusting for the influences of age, diabetes, LVEF, and Gensini score, a high TMAO level (>5.80 μmol/L) remained an independent risk factor for MACE occurrence in AMI patients, with a hazard ratio (HR) as high as 2.45 (95% CI: 1.38-4.35, $P = .002$). Detailed results are presented in a forest plot (Figure 6 and Table 2). This finding strongly suggests that TMAO provides additional prognostic information beyond traditional clinical and imaging risk factors.

Performance Evaluation and Incremental Value of Trimethylamine N-Oxide in Predicting Major Adverse Cardiovascular Events

To objectively evaluate the discriminatory ability of TMAO as a predictive tool, ROC curves were plotted, and the area under the curve (AUC) was calculated. The AUC for TMAO alone in predicting 6-month MACE was 0.852 (95% CI: 0.737-0.966). To assess its incremental value, it was compared with a traditional clinical prediction model containing age,

Table 1. Baseline Characteristics of the Study Population

Variables	AMI Group (n = 60)	Control Group (n = 30)	$t/\chi^2/z$	*P*
Demographics				
Age, years	65.8 ± 10.2	63.1 ± 8.9	$t = 1.25$	.215
Male, n (%)	42 (70.0)	18 (60.0)	$\chi^2 = 0.91$	.340
BMI, kg/m^2	25.3 ± 3.4	24.1 ± 2.8	$t = 1.74$	.085
Vital signs				
SBP, mm Hg	141.8 ± 13.0	129.6 ± 9.4	$t = 4.55$	**<.001**
DBP, mm Hg	86.4 ± 8.2	78.9 ± 5.2	$t = 4.60$	**<.001**
Heart rate, bpm	88.3 ± 9.4	73.2 ± 5.5	$t = 8.31$	**<.001**
Medical history, n (%)				
Hypertension	38 (63.3)	14 (46.7)	$\chi^2 = 2.29$	.130
Diabetes	22 (36.7)	6 (20.0)	$\chi^2 = 2.58$	.108
Hyperlipidemia	36 (60.0)	18 (60.0)	$\chi^2 = 0.00$	1.000
Smoking	35 (58.3)	10 (33.3)	$\chi^2 = 5.24$	**.022**
Laboratory tests				
LDL-C, mmol/L	2.73 ± 0.40	2.44 ± 0.35	$t = 3.33$	**.001**
Cr, μmol/L	85.6 ± 12.8	76.2 ± 9.1	$t = 3.57$	**<.001**
eGFR, mL/min/1.73 m^2	78.5 ± 9.6	85.3 ± 7.9	$t = -3.36$	**.001**
FBG, mmol/L	7.2 ± 1.5	6.1 ± 0.8	$t = 3.80$	**<.001**
hs-CRP, mg/L^0	9.5 (4.1, 19.1)	1.6 (0.9, 2.8)	$Z = 7.12$	**<.001**
Cardiac structure and function				
LVEF, %	52.4 ± 6.8	62.1 ± 3.7	$t = -7.58$	**<.001**

Data presented as median (interquartile range), analyzed by Mann-Whitney *U*-test (*Z* value).
AMI, acute myocardial infarction; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein; LVEF, left ventricular ejection fraction, DBP, diastolic blood pressure; FBG, fasting blood glucose; Cr, serum creatinine; SBP, systolic blood pressure. Bold values indicate statistically significant between-group differences ($P < .05$).

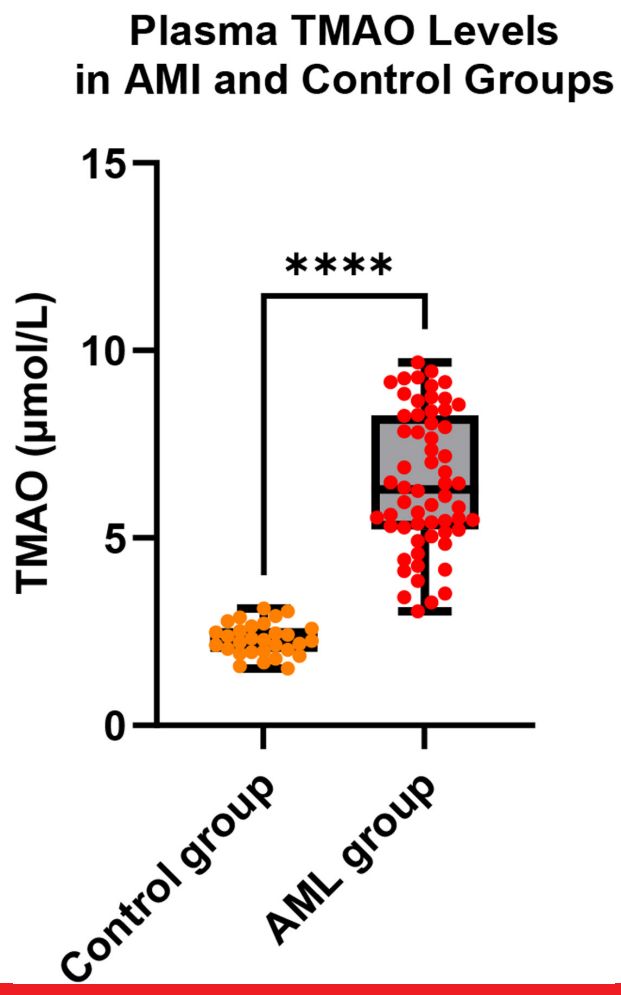

Figure 1. Comparison of plasma trimethylamine N-oxide levels between acute myocardial infarction and control groups (median [interquartile range]: 5.80 [4.12-7.65] vs. 2.10 [1.58-2.85] µmol/L, $P < .001$).

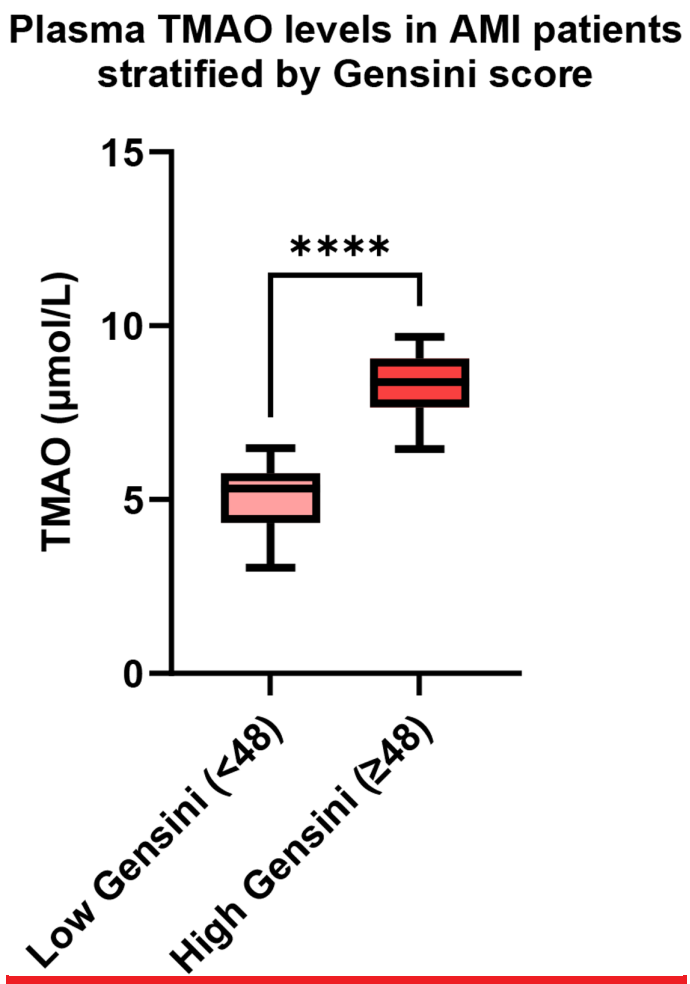

Figure 2. Plasma trimethylamine N-oxide levels in acute myocardial infarction patients stratified by Gensini score (high-score vs. low-score subgroup: 7.01 [5.20-8.95] vs. 4.55 [3.60-5.85] µmol/L, $P < .001$).

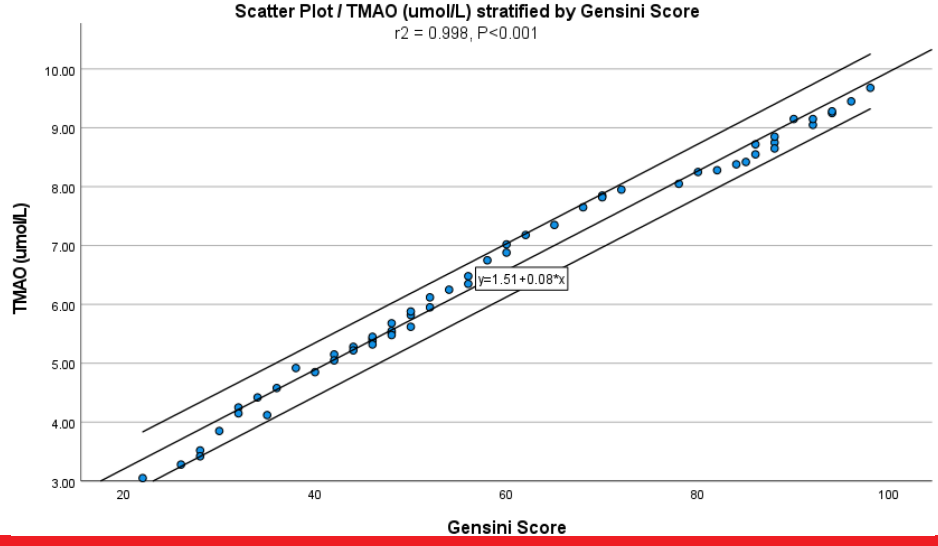

Figure 3. Correlation between plasma trimethylamine N-oxide levels and Gensini score in acute myocardial infarction patients ($rs = 0.998$, $P < .001$).

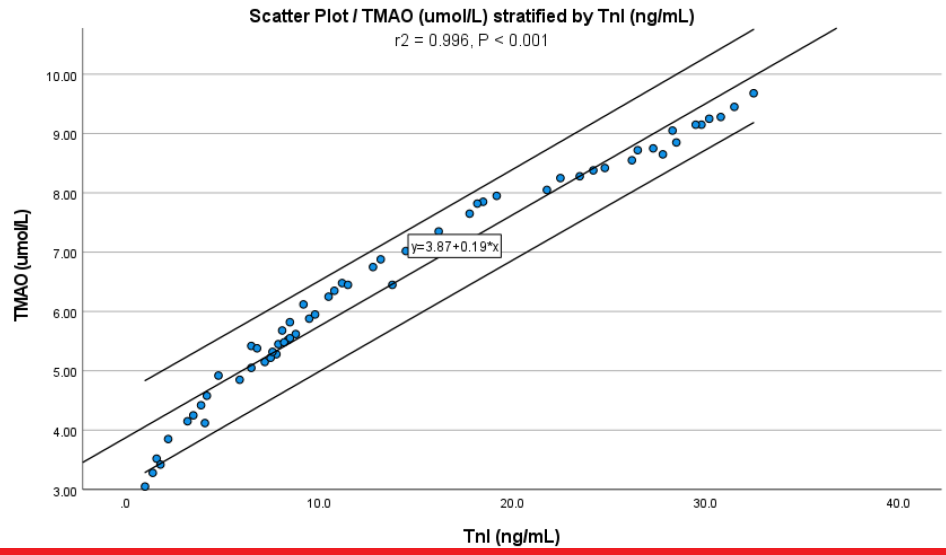

Figure 4. Correlation between plasma trimethylamine N-oxide levels and peak troponin 1 in acute myocardial infarction patients (rs = 0.996, *P* < .001).

diabetes, and LVEF. The traditional model had an AUC of 0.880 (95% CI: 0.780-0.981). When TMAO was added to the traditional model, the predictive performance of the combined model improved significantly, with the AUC increasing to 0.884 (95% CI: 0.781-0.987). The DeLong test confirmed that the AUC of the combined model was significantly higher than that of the traditional model alone (*P* = .012). A comparison of the ROC curves is clearly presented in Figure 7. This analysis demonstrates that plasma TMAO level has good predictive capability for MACE and provides significant incremental value to risk assessment based on conventional clinical indicators, suggesting that incorporating it into existing evaluation systems could potentially enhance the accuracy of identifying high-risk patients.

Subgroup Analysis to Verify the Robustness of Results

To ensure the consistency and reliability of the main study findings, exploratory analyses were conducted in different pre-specified subgroups to examine the robustness of the association between high TMAO levels (>median) and MACE risk. The subgroup analysis encompassed key stratifications including sex (male/female), age (≤65 years/>65 years), and the presence or absence of diabetes (Table 3). The results showed that across all subgroups, high TMAO levels consistently demonstrated a trend toward increased MACE risk, with all point estimates of the HR being greater than 1. Interaction tests did not reveal statistically significant interactions (all *P* for interaction >.05), indicating that the adverse prognostic impact of TMAO may be universally

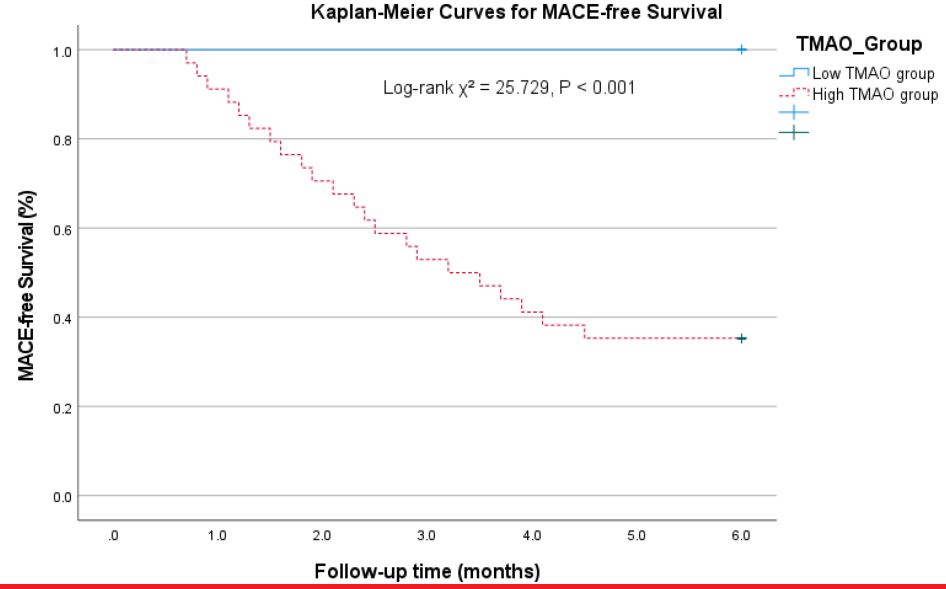

Figure 5. Major adverse cardiovascular event–free survival curves in acute myocardial infarction patients stratified by trimethylamine N-oxide levels (Log-rank χ^2 = 25.729, *P* < .001).

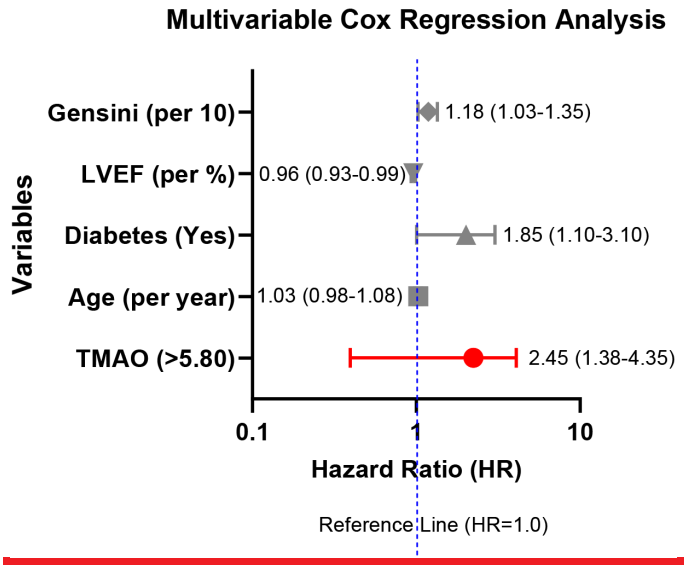

Figure 6. Forest plot of multivariate Cox regression analysis for major adverse cardiovascular events in acute myocardial infarction patients.

present across patient populations with different characteristics. Although the sample size of this study limits the statistical power of the subgroup analyses, this consistent trend enhances the credibility of TMAO as a universal risk marker and provides preliminary support for its potential application across diverse clinical contexts.

DISCUSSION

The core findings can be summarized into 3 points. First, plasma TMAO levels were significantly higher in AMI patients compared to controls with angiographically normal or near-normal coronary arteries (stenosis <20% in all major epicardial vessels), and within the AMI group, TMAO levels showed a strong positive correlation with the anatomical severity of coronary atherosclerosis (Gensini score). Taken together, these findings suggest that TMAO levels are not merely associated with the presence of AMI, but also reflect the severity of coronary artery lesions, such that higher TMAO concentrations may signify a more extensive and severe atherosclerotic burden in the coronary tree. Second, TMAO levels were significantly correlated with the peak concentration of the myocardial necrosis marker (troponin I), suggesting a potential link to more extensive myocardial injury. Finally, and most clinically significant, a high TMAO level was an independent predictor of MACEs within 6 months after discharge in AMI patients and provided significant incremental value to traditional clinical risk prediction models. Collectively, these results indicate that TMAO is not merely a "bystander" marker of AMI disease severity but likely an active mediator involved in disease progression with important prognostic stratification value.

Consistent with a growing body of evidence, elevated plasma TMAO has been identified as a risk marker for cardiovascular events in population-based and stable coronary disease cohorts. This study's data contribute to the existing literature by extending this association to the acute, high-risk setting of AMI.[18,19] However, evidence specifically within the acute, high-risk context of AMI, and particularly from Chinese populations, has been insufficient.[20] The present research addresses this gap and further links TMAO to a more precise quantitative indicator of coronary artery disease (Gensini score).[21] This strong correlation (rs = 0.998) surpasses the simple association between TMAO and the "presence or absence of coronary heart disease" observed in prior studies, providing more robust clinical anatomical evidence for the "gut-heart axis" theory.[22] The underlying pathophysiological mechanisms warrant in-depth exploration. Trimethylamine N-oxide may exacerbate the pathological process of AMI through several pathways.[23] First, TMAO has been shown to promote macrophage polarization toward a pro-inflammatory M1 phenotype and activate the NLRP3 inflammasome, thereby amplifying local plaque inflammation, leading to plaque instability and rupture—the initiating event in AMI.[24] Second, TMAO can enhance platelet calcium release and

Table 2. Cox Proportional Hazards Regression Analysis for Major Adverse Cardiovascular Event Prediction

Variables	Reference Category	Univariate HR (95% CI)	*P*	Multivariate HR (95% CI)	*P*
TMAO (>5.80 μmol/L)	≤5.80 μmol/L	3.82 (2.31-6.32)	<.001	**2.45 (1.38-4.35)**	**.002**
Age (per 1-year increase)	–	1.05 (1.01-1.09)	.018	1.03 (0.98-1.08)	.210
Diabetes (yes)	No	2.35 (1.48-3.73)	<.001	**1.85 (1.10-3.10)**	**.020**
LVEF (per 1% increase)	–	0.94 (0.91-0.97)	<.001	**0.96 (0.93-0.99)**	**.015**
Gensini score (per 10-point increase)	–	1.32 (1.16-1.50)	<.001	**1.18 (1.03-1.35)**	**.018**
Sex (female)	Male	1.18 (0.87-1.60)	.285	–	–
Hypertension (yes)	No	1.28 (0.97-1.69)	.085	–	–
Smoking (yes)	No	1.85 (1.33-2.57)	<.001	–	–
Peak Troponin 1 (per 1 ng/mL)	–	1.09 (1.04-1.14)	<.001	–	–
eGFR (per 1 mL/min/1.73 m^2)	–	0.98 (0.96-1.00)	.120	–	–
hs-CRP (per 1 mg/L)	–	1.03 (1.01-1.05)	.002	–	–

Variables with $P < .10$ in univariate analysis were included in the multivariate Cox proportional hazards model (forward stepwise method). Bold indicates statistical significance ($P < .05$) in the final multivariate model.
eGFR, estimated glomerular filtration rate; HR, hazard ratio; hs-CRP, high-sensitivity C-reactive protein; LVEF, left ventricular ejection fraction; TMAO, trimethylamine N-oxide.

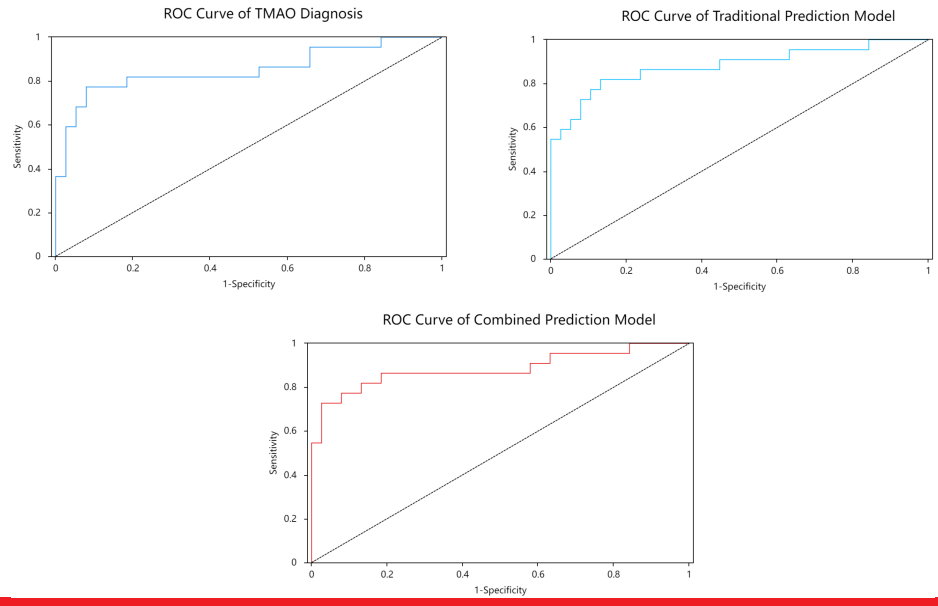

Figure 7. Receiver operating characteristic curves of different prediction models for 6-month major adverse cardiovascular events.

hyper-reactivity, increasing thrombotic risk, which may directly influence the burden and stability of intracoronary thrombi during the acute phase of AMI.[25] Third, the positive correlation between TMAO and the extent of myocardial injury (troponin I) found in this study may suggest that, beyond influencing coronary events, TMAO could directly aggravate myocardial damage through mechanisms such as exacerbating ischemia-reperfusion injury or affecting cardiomyocyte metabolism.[26] These mechanisms collectively outline a potential pathway from "gut microbiota dysbiosis → elevated TMAO → exacerbated inflammation and thrombosis → promotion of plaque rupture and myocardial injury → adverse prognosis."[27]

From a clinical translation perspective, the findings of this study hold dual significance. In terms of risk prediction, TMAO demonstrated predictive performance (AUC = 0.852) superior to traditional inflammatory markers like hs-CRP.[28] This aligns with emerging evidence that novel biomarkers derived from routine laboratory parameters, such as the prognostic nutritional index, can independently predict the functional significance of coronary artery stenosis, highlighting the expanding role of accessible biomarkers in coronary artery disease assessment.[29] Its prognostic value, independent of strong predictors such as age, cardiac function, and severity of coronary lesions, is particularly notable. Incorporating TMAO into existing risk assessment frameworks could significantly enhance the identification of high-risk patients,[30] providing clinicians with a potential new tool for more precisely selecting individuals requiring intensive management and follow-up after the acute phase of AMI. At the level of therapeutic intervention, TMAO, as a modifiable metabolite, opens up a novel "gut-heart axis" target for the secondary prevention of AMI.[31] Future research

Table 3. Subgroup Analysis of the Association Between High Trimethylamine N-Oxide Level and Major Adverse Cardiovascular Events Risk

Characteristic	Subgroup	Reference Category	N	Events	HR (95% CI)	*P*	*P* for Interaction
Overall	–	–	60	18	**3.82 (2.31-6.32)**	<.001	–
Gender	Male	(reference)	40	13	**3.65 (2.05-6.50)**	<.001	.452
	Female		20	5	**4.25 (1.85-9.75)**	.001	
Age (years)	≤65	(reference)	32	10	**3.40 (1.75-6.60)**	<.001	.318
	>65		28	8	**4.10 (2.05-8.20)**	<.001	
Diabetes	No	(reference)	30	8	**3.90 (1.95-7.80)**	<.001	.521
	Yes		30	10	**3.75 (1.88-7.50)**	<.001	

High TMAO was defined as >5.80 μmol/L. Cox proportional hazards models were performed in each subgroup. Hazard ratios are for high vs. low TMAO level. Reference categories are indicated in the third column; *P*-values for reference categories have been omitted as requested. The *P* for interaction values were obtained from separate Cox proportional hazards models that included the stratification factor (gender, age, or diabetes), the TMAO group (high vs. low), and their product term (TMAO group × stratification factor). The *P* for interaction corresponds to the Wald test for the product term, testing whether the effect of TMAO on MACE differs across levels of the stratification factor. Bold values indicate statistically significant associations between high TMAO levels and MACE risk ($P < .05$).
HR, hazards ratio.

directions may include exploring dietary modifications (e.g., reducing intake of foods rich in choline and L-carnitine like red meat and egg yolks), using prebiotics/probiotics to modulate gut microbiota composition,[32] or developing small-molecule drugs specifically inhibiting trimethylamine (TMA)-generating enzymes to lower TMAO levels, followed by prospective validation of whether these interventions ultimately translate into a reduction in cardiovascular events.[33]

It is, of course, essential to view the limitations of this study with caution. First, the retrospective design precludes establishing a causal relationship between TMAO and AMI prognosis; the observed associations may be influenced by unknown confounding factors. Second, the single-center nature and moderate sample size limit the generalizability of the conclusions, necessitating validation in larger-scale, multicenter prospective cohorts. Third, the control group consisted of symptomatic patients with angiographically normal or near-normal coronary arteries, rather than community-based healthy volunteers; consequently, the between-group difference in TMAO may be attenuated, and extrapolation of the present findings to truly healthy populations should be made with caution. Fourth, although admission blood sampling was standardized within 24 hours of hospitalization, TMAO was measured only once; levels may fluctuate during the acute coronary syndrome phase because of hemodynamic, metabolic, and treatment-related changes, and dynamic patterns over time could not be captured in this study. Fifth, because inclusion required the availability of a stored EDTA-anticoagulated plasma sample, a certain degree of selection bias related to biobank coverage and pre-analytic handling cannot be completely ruled out. Sixth, although multiple confounding factors were adjusted for statistically, detailed dietary history or previous antibiotic exposure were not systematically collected; key information potentially influencing TMAO levels, such as habitual intake of TMAO-rich foods, recent dietary changes, antibiotic use, and the resulting gut microbiota composition, was therefore not included in the analysis, and residual confounding from these unmeasured factors cannot be excluded. ded in the analysis, and residual confounding from these unmeasured factors cannot be excluded. Future prospective studies should incorporate comprehensive data collection on potential confounders and consider emerging therapeutic approaches in cardiovascular disease management, as the field continues to evolve with novel interventional strategies.

CONCLUSION

This study confirms within a Chinese AMI cohort that elevated plasma TMAO is not only associated with greater coronary disease severity and myocardial injury but also independently predicts worse short-term prognosis, adding incremental value to conventional risk models. These findings substantiate the relevance of the gut-heart axis in acute cardiovascular events and position TMAO as a promising prognostic biomarker. Future large-scale prospective and interventional studies are warranted to establish causality and explore therapeutic modulation of this pathway.

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Informed Consent: Informed consent was waived by the Institutional Medical Ethics Committee because this was a retrospective study involving only anonymized clinical data and stored biological samples obtained during routine clinical care.

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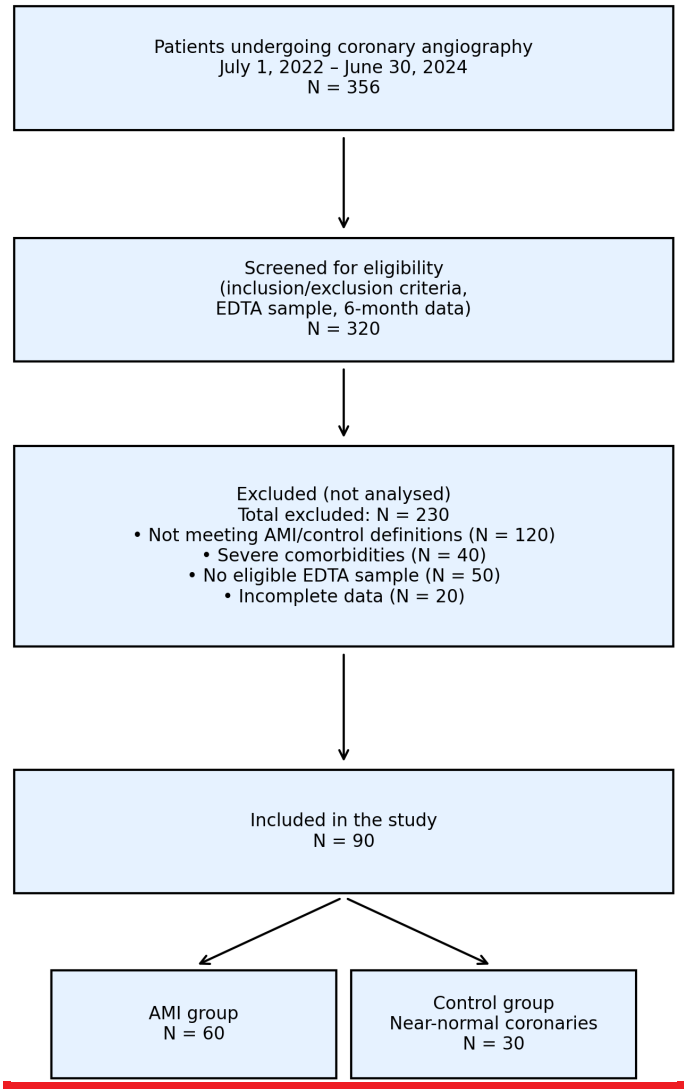

Supplementary Figure 1. Flowchart of participant screening and enrollment.