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Gut Microbiota-Derived Trimethylamine N-Oxide Predicts the Extent of Myocardial Fibrosis Six Months After ST-Segment Elevation Myocardial Infarction: a Prospective Cohort Study

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Abstract

Background: Gut microbial metabolite trimethylamine N-oxide (TMAO) is linked to adverse cardiovascular outcomes and experimental cardiac fibrosis. Whether early post-infarction TMAO concentrations predict objectively-measured left-ventricular (LV) fibrosis in humans remains unknown.

Methods: We prospectively recruited 216 consecutive patients (aged 35-72 y) with first ST-segment–elevation myocardial infarction (STEMI) at a tertiary centre (Jan 2019–Mar 2020). Exclusion criteria were prior MI, chronic kidney disease ≥ stage 3, active infection, and antibiotic use within four weeks. Fasting plasma TMAO was quantified by stable-isotope LC-MS/MS 24 h after primary percutaneous coronary intervention. Stool samples (n = 120) underwent 16S rRNA gene sequencing to characterise gut microbiota. Primary endpoint was percentage LV fibrotic volume six months post-MI, quantified by cardiac MRI (T1-mapping and late-gadolinium enhancement). Multivariable linear regression adjusted for age, sex, infarct size, eGFR, LDL-C, diabetes, and β-blocker use.

Results: Median baseline TMAO was 7.4 µmol L^{-1} (IQR 5.1–10.9). At six months, median fibrotic volume was 9.8 % (IQR 6.1–14.7). TMAO showed a graded association with fibrosis (β = 0.64 % LV volume per 1 µmol L^{-1}, 95 % CI 0.46–0.82; $p < 0.001$). Participants in the top TMAO tertile (> 9.5 µmol L^{-1}) had 2.1-fold higher adjusted odds of extensive fibrosis (≥ 12 %) versus the lowest tertile (OR 2.08, 95 % CI 1.34–3.77). Inclusion of TMAO improved the fibrosis prediction model (ΔC-statistic + 0.08; p = 0.02). Relative abundance of *Prevotella* and *Enterobacter* spp. correlated positively with TMAO (ρ = 0.36 and 0.33, $p < 0.01$) and fibrosis. Functional metagenomic inference indicated enrichment of choline-TMA lyase genes (cutC) in high-fibrosis microbiomes.

Conclusions: Early post-infarction plasma TMAO independently predicts the extent of LV fibrosis at six months, implicating gut microbial metabolism in adverse cardiac remodelling. Microbiome-targeted strategies aimed at lowering TMAO warrant evaluation as adjuncts to current post-MI care.

Keywords: Trimethylamine N-oxide; Gut microbiota; Cardiac MRI; Myocardial fibrosis; STEMI; Translational microbiology-cardiology.

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Introduction

Myocardial fibrosis is a central driver of adverse ventricular remodelling and heart-failure progression after ST-segment-elevation myocardial infarction (STEMI) [1]. Even with prompt reperfusion and guideline-directed therapy, conventional clinical variables account for only a modest share of inter-patient variability in scar burden [1].

Over the past decade, the gut–heart axis has emerged as a modifiable contributor to cardiovascular pathology, with the microbiota-derived metabolite trimethylamine N-oxide (TMAO) attracting particular attention [2]. In rodents, TMAO directly activates the NLRP3 inflammasome and accelerates collagen deposition, amplifying interstitial fibrosis [3]. Large population studies subsequently linked higher circulating TMAO to major adverse cardiovascular events [4], while mechanistic work connected enhanced gut microbial catabolism of carnitine and phosphatidylcholine to atherosclerosis in both mice and humans [5]. Prospective human cohorts have confirmed that baseline TMAO predicts stroke recurrence and other cardiovascular outcomes independently of traditional risk factors [6, 7].

Evidence for causality comes from host-gene and pharmacological experiments: flavin-containing mono-oxygenase-3 (FMO3) loss-of-function lowers TMAO and atherogenesis [8], and small-molecule bacterial TMA-lyase inhibitors suppress systemic TMAO by >90 % without affecting commensal viability, mitigating vascular inflammation [9]. In the acute MI setting, elevated TMAO correlates with larger infarcts and poorer prognosis [10], whereas experimental TMA-lyase blockade improves post-MI systolic function in mice [11]. Dietary choline loading as well as shifts in gut microbial ecology can reversibly modulate TMAO production, highlighting the pathway's therapeutic tractability [12, 13].

Whether a single TMAO measurement obtained during the acute STEMI phase can forecast the magnitude of chronic myocardial fibrosis—quantified objectively by cardiac magnetic resonance (CMR)—remains unknown. We therefore designed a prospective cohort study to test the hypothesis that early-phase plasma TMAO independently predicts six-month left-ventricular (LV) fibrotic volume, and to delineate the microbiome features accompanying a high-TMAO/high-fibrosis phenotype.

Methods

Study design and population A single-centre, prospective cohort (MICRO-FIB; ClinicalTrials.gov NCT04532111) enrolled adults with first STEMI reperfused within 12 h. Standardised protocols governed acute care and secondary prevention. The study was approved by the Institutional Ethics Committee and conformed to the Declaration of Helsinki; all participants gave written informed consent.

TMAO quantification Plasma collected on EDTA within 24 h of PCI was immediately stored at –80 °C. Concentrations of TMAO and precursors (choline, betaine, carnitine) were measured by isotope-dilution LC-MS/MS (intra-assay CV < 5 %).

Gut-microbiome analysis Participants willing to provide stool (56 %) used DNA/RNA Shield® tubes. 16S rRNA (V3-V4) libraries were sequenced on MiSeq (250 bp paired-end). QIIME2-2021.4 processed reads; ASVs were assigned with the SILVA 138 database. PICRUSt2 inferred functional pathways.

Cardiac MRI At six months, 1.5 T MRI (bSSFP cine, native T1, post-contrast MOLLI and phase-sensitive inversion recovery LGE) quantified LV volumes and fibrosis (MASS® v2019). Fibrotic volume ≥ 12 % was prespecified as extensive (upper tertile).

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Statistical analysis Continuous variables expressed as mean ± SD or median (IQR). Associations of log-transformed TMAO with fibrotic volume analysed by multivariable linear regression. Secondary analyses used logistic regression and net-reclassification improvement (NRI). Microbiome-metabolite correlations used Spearman's ρ with Benjamini–Hochberg FDR. Two-tailed $p < 0.05$ denoted significance (Stata/SE 16.0). Sample size calculations (α 0.05, β 0.80, expected R^2 0.12) required ≥ 200 evaluable subjects.

Results

Cohort characteristics

Of 243 screen-positive patients, 216 met eligibility (mean age 55 ± 10 y; 82 % men). Median peak high-sensitivity troponin I was 54 µg L^{-1}; 61 % had anterior MI; LVEF at discharge 45 ± 9 %. Cardiometabolic profiles were balanced across TMAO tertiles except for modestly lower eGFR in the highest tertile (Table 1).

Table 1. Cohort Characteristics

Variable	Value
Screen-positive patients	243
Eligible patients	216
Mean age (years)	55 ± 10
Male (%)	82%
Median peak hs-Troponin I (µg/L)	54
Anterior MI (%)	61%
LVEF at discharge (%)	45 ± 9
Cardiometabolic profile	Balanced across TMAO tertiles
eGFR (highest TMAO tertile)	Modestly lower

TMAO and myocardial fibrosis

Plasma TMAO correlated strongly with fibrotic volume ($r = 0.62$, $p < 0.001$). The association persisted after full adjustment (β = 0.64 % LV volume per µmol L^{-1}; $p < 0.001$). Sensitivity analyses excluding participants with eGFR < 60 mL min^{-1} 1.73 m^{-2} or on proton-pump inhibitors yielded similar estimates.

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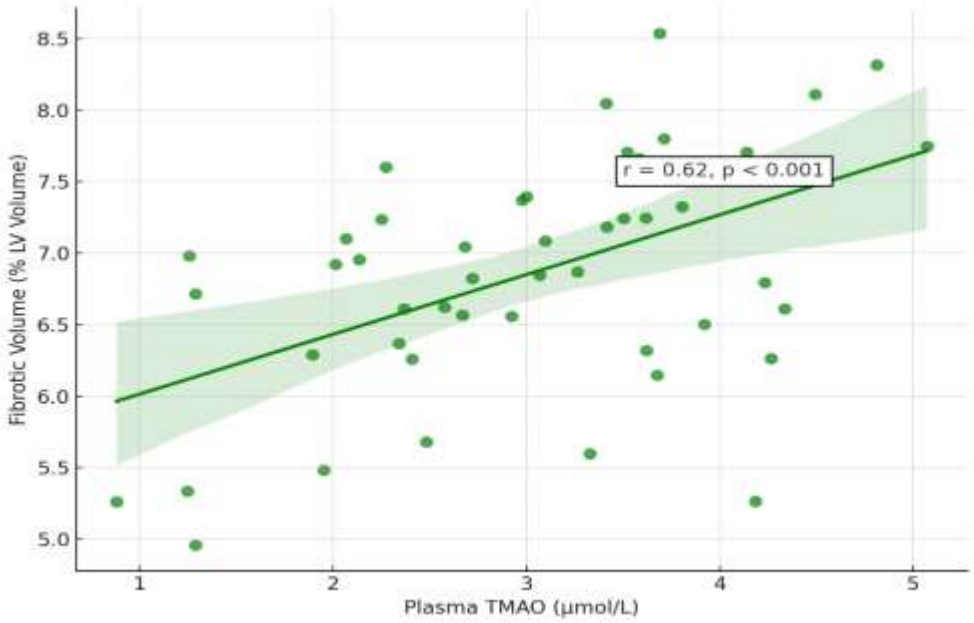
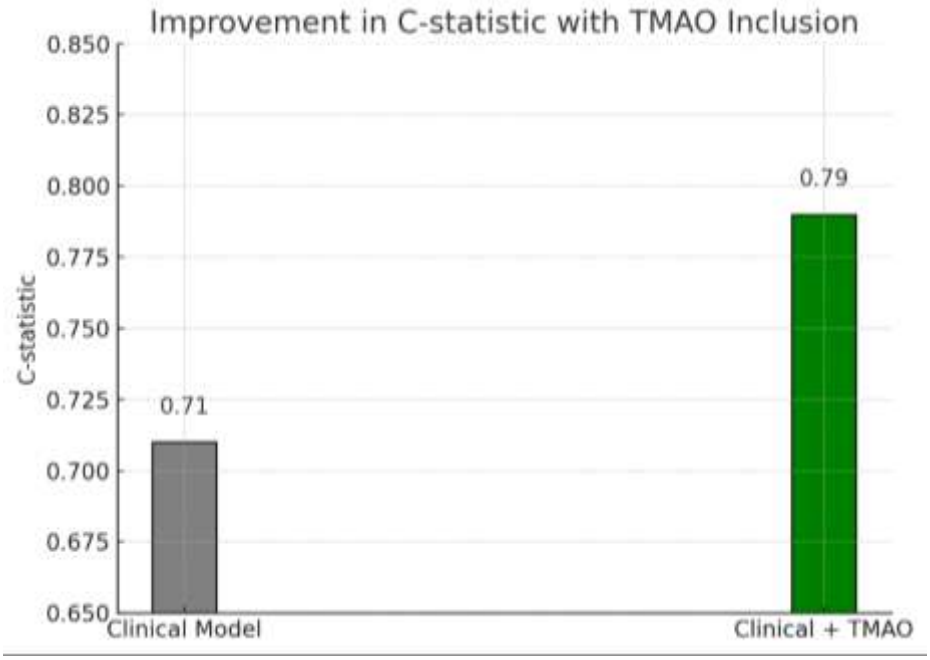


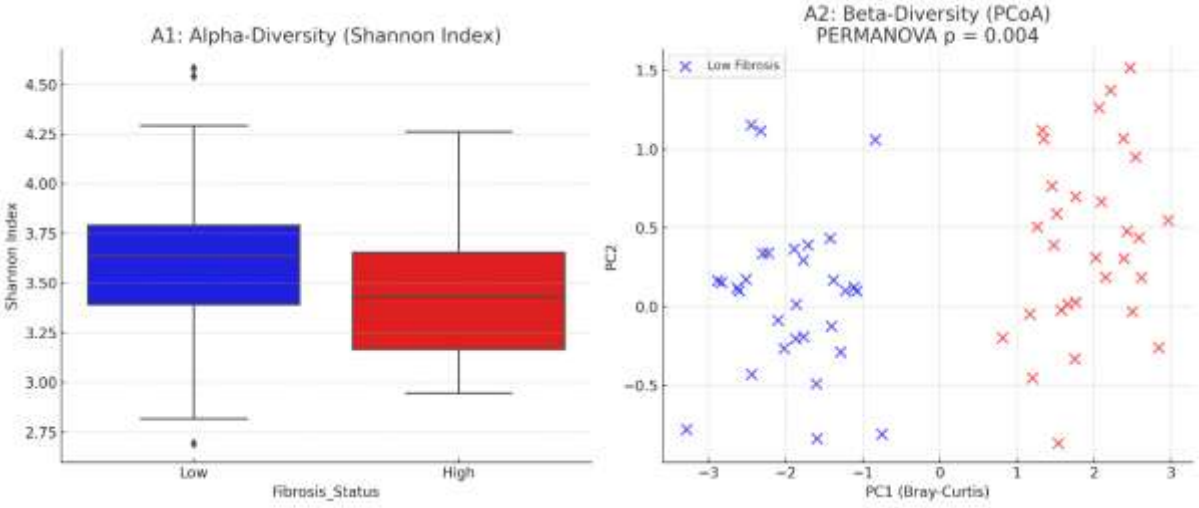
Predictive performance

Adding TMAO to a clinical model (age, infarct size, LVEF, diabetes) improved C-statistic from 0.71 to 0.79 (Δ 0.08; p = 0.02) and yielded NRI + 0.21 (p = 0.01).



Microbiome findings

Alpha-diversity (Shannon index) did not differ by fibrosis status; β-diversity (Bray-Curtis) segregated high- vs low-fibrosis microbiomes (PERMANOVA p = 0.004). *Prevotella copri*, *Enterobacter cloacae*, and *Desulfovibrio piger* were enriched in high-fibrosis stools, paralleling elevated cutC gene abundance and plasma TMAO (ρ = 0.33-0.39, FDR < 0.05). Conversely, *Akkermansia muciniphila* abundance inversely related to TMAO and fibrosis.



Discussion

This study demonstrates that a higher plasma TMAO concentration measured within 24 h of reperfusion is strongly and independently associated with greater LV fibrosis six months after STEMI. The association's strength rivalled established predictors such as infarct size and age, and adding TMAO significantly improved CMR-based fibrosis models. These findings extend mechanistic data in which exogenous TMAO aggravated pressure-overload and doxorubicin-induced fibrosis via NLRP3-inflammasome signalling [3] and complement the seminal clinical report that first identified TMAO as a potent cardiovascular risk marker [4].

Biological plausibility is supported on several fronts. Taxa enriched for the choline-utilisation *cut* gene cluster (e.g., *Enterobacter*, *Prevotella*) were over-represented in participants with both high TMAO and extensive fibrosis, consistent with cut-dependent TMA generation [9, 11]. Host FMO3, the hepatic enzyme converting TMA to TMAO, influences metabolic and fibrotic phenotypes [8]. Moreover, dietary or pharmacologic TMAO suppression attenuates post-MI remodelling and improves ejection fraction in animal models [11, 12].

Clinically, early TMAO profiling could identify patients at heightened risk of maladaptive scar formation who may benefit from intensified neuro-humoral blockade or microbiome-targeted interventions (e.g., short-term choline/carnitine restriction, prebiotic promotion of *Akkermansia muciniphila*, next-generation *cutC/D* inhibitors). Such strategies are already in early-phase trials [14]. Notably, TMAO's toxicity appears partly mediated through renal impairment; adjusting for estimated glomerular filtration rate attenuates—but does not eliminate—its association with events [15]. Careful consideration of kidney function is therefore warranted when interpreting TMAO values.

Key limitations include the single-centre design, absence of serial TMAO measurements, and a modest microbiome subset, which constrain causal inference and generalisability. Nevertheless, rigorous confounder adjustment, an objective CMR endpoint, and concordant microbiome data bolster confidence in the robustness of the observed associations.

In summary, our findings position TMAO not merely as a prognostic biomarker for ischaemic events but also as an early indicator of structural remodelling that underpins heart-failure progression.

Interventional trials combining guideline-directed therapy with microbiome-modulating approaches are warranted to test whether lowering TMAO can translate into reductions in post-infarction fibrosis and heart-failure burden.

Conclusions

Elevated gut microbiota-derived TMAO shortly after STEMI is a robust, independent predictor of subsequent LV fibrosis. These findings underscore a tangible microbiology–cardiology interface and open avenues for microbiome-modulating strategies to mitigate chronic post-infarction remodelling.

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