Trimethylamine-N-oxide is associated with cardiovascular mortality and vascular brain lesions in patients with atrial fibrillation

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Abstract

Objective: Trimethylamine-N-oxide is a metabolite derived from the microbial processing of dietary phosphatidylcholine and carnitine and the subsequent hepatic oxidation. Due to its prothrombotic and inflammatory mechanisms we aimed to assess its role for adverse event prediction in a susceptible population, namely patients with atrial fibrillation.

Methods: Baseline TMAO plasma levels were measured by liquid chromatography-tandem mass spectrometry in 2'379 subjects from the ongoing SWISS Atrial Fibrillation (SWISS-AF) cohort. 1'722 underwent brain MRI at baseline. Participants were prospectively followed for 4 years (Q1, Q3 3.0-5.0) and stratified into baseline TMAO tertiles. Cox proportional hazards, linear and logistic mixed effect models were employed adjusting for risk factors.

Results: Subjects in the highest TMAO tertile were older (75.4±8.1 vs 70.6±8.5 years, p<0.01), had poorer renal function (median GFR 49.0 ml/min/1.73m^2 (35.6-62.5) vs 67.3 (57.8-78.9), p<0.01), were more likely to have diabetes (26.9% vs 9.1%, p<0.01) and had a higher prevalence of heart failure (37.9% vs 15.8%, p<0.01) compared to patients in the lowest tertile. Oral anticoagulants were taken by 89.1%, 94.0%, 88.2% of participants respectively (from high to low tertile). Cox models, adjusting for baseline covariates showed increased total mortality (HR 1.65, 95% CI 1.17-2.32, p<0.01) as well as cardiovascular mortality (HR 1.86, 95% CI 1.21-2.88, p<0.01) in the highest compared to the lowest tertile. When present, subjects in the highest tertile had more voluminous large non-cortical and cortical infarcts on MRI (log-transformed volumes; exponentiated estimate (EE) 1.89, 95% CI 1.11-3.21, p=0.02), a higher chance of small non cortical infarcts (OR 1.61, 95% CI 1.16-2.22, p<0.01).

Conclusions: High levels of TMAO are associated with increased risk of cardiovascular morality and cerebral infarction in atrial fibrillation patients.

Key Messages

What is already known about this subject?

a) Patients with atrial fibrillation (AF) have shorter life expectancy and higher likelihood to suffer from cardio- and cerebro-vascular events in comparison to the general population and there is a high medical need of biomarkers for better risk stratifications.

b) TMAO is a microbiota bioproduct with prothrombotic and pro-oxidative properties associated with major adverse cardiovascular events yet not fully tested in the specific sub-population at risk such as AF patients.

What does this study add?

a) Independently of the classic cardiovascular risk factors, overall and cardiovascular mortality was significantly higher in subjects with high levels of TMAO.

b) TMAO was associated with more and larger strokes independently of risk factors.

How might this impact on clinical practice?

These findings from the national SWISS-AF Study highlight the clinical relevance of TMAO for life expectancy and the quality of life. TMAO could be considered for the improvement of the patients' risk stratification since its lowering by a simple dietary modification may decrease the risk of adverse cardio- and cerebrovascular events in AF patients.

Introduction

Bioproducts derived from gut microbiota have gained considerable interest in the last decade both as potential biomarkers for the prediction of major adverse cerebral and cardiovascular events as well as causal mediators of cardiovascular damage. Among them, trimethylamine-N-oxide (TMAO) is a well characterized metabolite derived from the microbial processing of dietary choline or carnitine (usually present in red meat, fish and cheese) into TMA and subsequently oxidized by hepatic flavin monooxygenase 3 into TMAO. Once in the bloodstream, TMAO triggers a number of events promoting endothelial dysfunction, platelet activation and thrombosis[1,2]. Recently, direct and independent associations of TMAO plasma levels with major adverse and cerebrovascular events in patients with acute and chronic coronary artery syndrome, peripheral arterial disease, heart failure, or chronic kidney disease have been documented[3].

Patients with AF are exposed to significantly higher risks of adverse cerebral and cardiovascular events as well as mortality independently of other comorbidities[4]. Event prediction and subsequent patient stratification rely primarily on clinical scores such as CHA_2DS_2-VASc, which are quick and easy to use, but have a modest prognostic value for morbidity and mortality. For this reason, the implementation and use of biomarkers has gained pivotal importance for risk prediction leading to the improvement of the patient-tailored decision-making process[5].

We therefore aimed to explore whether TMAO has a long-term prognostic relevance for cardiovascular events and in particular brain damage in atrial fibrillation. We chose the model of atrial fibrillation arguing that the effect of TMAO is more evident in patients with a high-risk cardiovascular background and potentially modifiable through lifestyle modifications.

Methods

Patient cohort

The Swiss Atrial Fibrillation (SWISS-AF) cohort (NCT02105844) is a prospective observational cohort study involving 14 centers in Switzerland[6].

The main inclusion criteria were previously documented AF and an age ≥65 years. For a pre-specified substudy to assess the effect of AF on individuals in the active workforce, a small number of patients aged 45–64 years was enrolled. Exclusion criteria were the inability to give informed consent or secondary AF due to reversible causes. Enrollment of participants with acute conditions was allowed after 4 week-delay for resolution. Out of the 2'415 participants, we excluded 36 patients (1.5%) without blood drawing at baseline. 1'722 subjects (72.4%) underwent MRI at time of enrollment: reasons for lack of MRI were claustrophobia, non-compatible medical devices, or labile medical conditions (Supplementary Figure 1). The study protocol was approved by the local ethical committees (Ethikkommission Nordwest- und Zentralschweiz 2014-067) and informed written consent was obtained from each participant. The study started on 24th March 2014 and data were collected until dataset cut-off on 23th November 2020.

Clinical variables

Comorbidities were self-reported by the participant during the baseline visit. Medical reports or discharge letters were used in case of uncertainty[7].

Cardiovascular death included cardiac deaths (e.g. cardiogenic shock, arrhythmia/sudden death, cardiac rupture) and other vascular deaths (e.g. stroke, pulmonary embolism, ruptured aortic aneurysm or dissection). All hemorrhagic deaths were classified as cardiovascular deaths. First strokes were classified as of ischemic, hemorrhagic or undetermined type.

Brain magnetic resonance imaging

Brain MRI images were acquired by 1.5 or 3 Tesla scanners with a standardized protocol installed on all MR-scanners at local centers as described previously. All MRI data were centrally analyzed by a neuroimaging core lab (Medical Image Analyses Center, Basel, Switzerland) after local evaluation. Blinded expert readers marked and segmented new lesions at 2-year follow-up in comparison to baseline imaging in standardized analyses. Analysis was confirmed by board-

certified neuroradiologists. Large non-cortical and cortical infarcts (LNCCI) were defined as infarcts involving cortex and lesions not involving cortex with a diameter >20mm, small non-cortical infarcts (SNCI) were defined as lesions with a diameter <20mm. Hyperintense WML were identified in either the periventricular or deep white matter region.

We excluded perivascular spaces defined by tubular morphology. WML also included FLAIR-hyperintense lesions not fulfilling the criteria for SNCIs or LNCCIs. Microbleeds (MB) were defined and counted as nodular, strongly hypointense lesions on either T2*-weighted or susceptibility-weighted imaging. MB were not assessed by volume to avoid an overestimation incurred by blooming effects. Extensive lesions-definitions can be found in Supplementary material and in our previous study[6].

Biological samples

Baseline blood samples were collected following standard operating procedures. After centrifugation, lithium heparinized plasma samples were aliquoted into cryotubes and stored in a centralized biobank at -80°C. Subjects performing TMAO assessment were blinded to clinical and MRI data until quantification was completed.

Quantification of trimethylamine-N-oxide (TMAO)

TMAO was measured as previously described[8]. Briefly, after addition of 400 µl of the internal standard TMAO-d9 dissolved in methanol, samples were centrifuged (11'700g, 10min, 4°C). Fifty µl of supernatant was further diluted using 50 µl of methanol. Analysis was done on an Accucore HILIC column (50x2.1mm, 2.6µm particle size, Thermo Fisher Scientific, Reinach, Switzerland) using mobile phases adjusted to pH 3. The following transitions were monitored using a QTrap 6500+ mass spectrometer (AB Sciex, Baden, Switzerland), operated in positive electrospray ionization mode: 76.1 $\rightarrow$ 59.1 (quantifier), 76.1 $\rightarrow$ 42.1 and 76.1 $\rightarrow$ 56.2 (qualifiers) for TMAO, and 85.1 $\rightarrow$ 68.1 for TMAO-d9.

Statistical analysis

Continuous variables are reported as mean (SD) or median (Q1, Q3); categorical variables are summarized as frequency (%). Normally distributed variables are compared using one-way ANOVA; continuous strongly skewed variables are compared using Kruskal-Wallis Rank Sum test. Categorical variables are compared using Chi-Squared test.

For the time-to-event analysis of clinical events (death, cardiovascular death, stroke, ischemic stroke), survival curves were estimated using the non-parametric Kaplan-Meier method. The association between TMAO and the hazard of clinical events was estimated using Cox proportional hazard models stratified by center; age, sex, BMI, smoking status, glomerular filtration rate, anticoagulants and antiplatelets, history of heart failure, diabetes, stroke or TIA, coronary heart disease and hypertension as covariates and time-on-study was used as timescale. Patients lost to follow-up or still in the study but without occurrence of the event of interest were censored. In the analysis of cardiovascular death, patients who died of any cause other than cardiovascular before the event of interest were censored. In the analysis of stroke and ischemic stroke, patients who died before the event of interest were censored.

The prognostic values of TMAO and high sensitivity CRP for clinical events within 5 years from baseline were assessed among patients who reached the 5-year follow-up using receiver operating characteristics (ROC) curves.

The association between TMAO and the presence of MRI lesions (LNCCI, SNCI and MB) at baseline was estimated using mixed effects logistic regression models and the association with volume of MRI lesions (LNCCI, SNCI and WML) at baseline in patients with lesions by linear mixed effects models. In all mixed effects models study center was included as a random intercept; TMAO and the aforementioned covariates were included as fixed effects.

Extensive explanations can be found in Supplementary materials.

Statistical analyses were performed in R, version 4.1.0 (Vienna, Austria) and graphs generated with R or Prism Graph Pad (San Diego, CA).

Patient and Public Involvement

Patients and the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Results

Baseline clinical features

Out of 2'415 total SWISS-AF participants, our study included 2'379 subjects with available TMAO at baseline. We stratified patients into TMAO tertiles based on baseline TMAO plasma levels (μmol/L: median value 3.4 (Q1, Q3 2.8-4.0) in low tertile, 5.8 (5.2-6.6) in middle one and 11.5 (9.1-16.1) in the higher one). Demographic and medical features, medications as well as dietary habits are reported in Table 1. Briefly, patients in the highest tertile were older (mean age 75.4±8.1 and 70.6±8.5 years in highest and lowest TMAO-tertiles, respectively, p<0.01), males were more represented (76.2% vs 70.9 in the lowest tertile, p=0.03), had more often diabetes (26.9% vs 9.1%, p<0.01), history of heart failure (37.9% vs 15.8%, p<0.01), had a higher BMI (kg/m^2) (28.1 vs 27.0, p<0.01) and a worse renal function as assessed by glomerular filtration rate (ml/min/1.73 m^2) (49.0 (35.6-62.5) vs 67.3 (57.8-78.9), p<0.01). Mean CHA_2DS_2-VASc score increased across TMAO subgroups (2.97 vs 3.95, p<0.01). Oral anticoagulants were taken by 89.1%, 94.0%, 88.2% of participants respectively (from high to low tertile). Increased meat consumption (>3 days per week) was reported by 61.3% participants in the highest TMAO tertile in comparison to 55.1% in the lowest one (p=0.03). Furthermore, a sedentary lifestyle was more commonly reported in the highest tertile (60.3%vs 49.8% in the lowest tertile, p<0.01).

TMAO associates with overall and cardiovascular mortality

Median follow-up observation was 4 years (Q1, Q3 3.0-5.0). As presented in Figure 1A-B, Kaplan-Meier survival estimates showed increased overall and cardiovascular mortality with increasing TMAO tertiles (log-rank p<0.01 for both). The same was not observed for stroke and ischemic stroke (Figure 1C-D). Of note, the rate of ischemic stroke occurrence was lower in the middle group (p=0.04). Subjects who died at short- and long-term follow-ups had higher levels of TMAO at baseline (p<0.01) (Figure 2A) and in line with literature, cardiovascular cause was attributed to 211 of 321 deaths at 5-year follow-up[9]. In contrast, we did not observe a significant difference in TMAO levels for subjects who experienced stroke during this timeframe (p=0.17). Concerning its nature, 28 out of 36 (77.8%), 15 out of 26 (57.7%), and 30 out of 41 (73.2%) were ischemic in the three tertiles, respectively (Supplementary Figure 2). Notably, we found evidence of a positive correlation between TMAO levels and NIHSS at time of ischemic stroke presentation (Spearman's coefficient 0.31, p=0.02) (Supplementary Figure 3).

After adjusting for the predefined covariates, being in the highest TMAO tertile was associated with 65% higher hazard of overall mortality (HR 1.65, 95% CI 1.17-2.32, p<0.01) and 86% higher hazard of cardiovascular mortality (HR 1.86, 95% CI 1.21-2.88, p<0.01) (Figure 2B). We found no significant evidence for a difference in the hazard of global (HR 0.95, 95% CI 0.57 - 1.57, p=0.83) or ischemic strokes (HR 0.93, 95% CI 0.52 - 1.67, p=0.81) between the highest and lowest TMAO tertiles (Figure 2B). Receiver operator characteristics curve analyses revealed better associations of TMAO in comparison to high sensitivity CRP with total mortality (AUC 0.63, 95% CI (0.59 - 0.67) vs. 0.68, 95% CI (0.64 - 0.72) and cardiovascular death (AUC 0.60 (0.55 - 0.65) vs. 0.70, 95% CI (0.65 - 0.74) during five years of follow-up (Figure 3A-B).

TMAO is associated with more frequent small non cortical infarcts

As shown in Table 2, TMAO tertiles identified subjects with different prevalence of small non cortical infarcts (low to high subgroup: 17.4%, 18.1%, 30.5%, p<0.01), microbleeds (18.4%, 23.5%, 25.1%, p=0.02) and when present, with larger white matter lesion volumes (2970 mm^3,

4158 mm^3, 5061 mm^3, p<0.01). TMAO tertiles differed by prevalence of participants with Fazekas' score of ≥2 (46.7%, 55.2%, 61.2%, p<0.01). After Bonferroni correction for multiple comparisons, the prevalence of microbleeds did not differ significantly between TMAO tertiles.

As shown in Figure 4A, median TMAO was found to be higher in subjects with SNCI in comparison to those without (6.4 (Q1, Q3 4.1-10.4) vs 5.3 (3.7-7.9), p<0.01). When SNCI was present, TMAO was significantly higher in subjects with SNCI volumes above median than in those below (7.5 (4.4-11.0) vs 5.7 (4.0-9.5) μmol/L, p=0.04). TMAO levels were higher in individuals with microbleeds than in those without (6.0 (4.0-9.6) vs 5.4 (3.7-8.3) μmol/L, p<0.01) as well as in participants with WML volume larger than the median (5.9 (4.1-9.0) vs 5.1 (3.6-7.8) μmol/L, p<0.01).

After multivariable adjustment for the mentioned covariates, the higher odds of SNCI in the highest TMAO tertile remained significant (OR highest vs lowest 1.61, 95% CI 1.16-2.22, p<0.01). In addition, in patients in the high TMAO tertile, (log-transformed) LNCCI volume appeared to be larger in comparison to individuals with TMAO in the lowest tertile (EE 1.89, 95% CI 1.11-3.21, p=0.02). Of note, a tendency to larger (log-transformed) WML volumes was also found (EE 1.16, 95% CI 0.99-1.35, p=0.06) (Figures 4B-C).

Discussion

Here we report for the first time on the significant association of TMAO with total and cardiovascular mortality in patients with atrial fibrillation. Furthermore, patients in the highest TMAO tertile had higher volumes of LNCCI, a higher number of SNCI and a tendency towards larger WML. Notably, this association remained valid after adjusting for potential confounders.

Atrial fibrillation (AF) is the most common arrhythmia affecting nearly 2-3% of the general population in Europe and US with a prevalence increasing with age up to 13-21% in subjects older than 65 of age[10]. Despite diagnostic and therapeutic improvements, a diagnosis of AF

remains a predictor of shortened life expectancy[11] obliging clinicians to identify subjects at risk for adverse events.

TMAO was not associated with prevalence of stroke events of embolic nature

In addition to increased mortality, patients with AF are known to carry a 5-fold increased risk of incident ischemic stroke compared to subjects without AF. It is noteworthy that in our study, TMAO was associated with neither a history of stroke at baseline nor with incident stroke during documented follow-up. These findings are in discordance with previous results reported by Haghikia[12] which might be explained by differences in the timing of recruitment. In fact, our cohort was composed of subjects included at least four weeks after an acute illness, and they were therefore in a more stable condition as subjects with unfavorable prognosis were likely excluded. Moreover, cardioembolic stroke was more prevalent than localized large and/or small vessel diseases whose pathomechanisms may be more strongly affected by TMAO[13].

Is TMAO a biomarker of vascular dysfunction?

In line with our hypothesis and according to the pathophysiological mechanisms[14], higher TMAO plasma levels were associated with more frequent and larger brain lesions. The distinct associations between TMAO and several MRI findings of likely ischemic origin such as larger LNCCIs and WML[15], as well as more frequent prevalence of SNCIs lead us to consider TMAO as a biomarker of microvascular dysfunction; TMAO would then not favor the occurrence of an acute event per se but rather worsen the extent and hence the outcome of a large, isolated, cerebrovascular adverse event either indicating a more vulnerable cerebral tissue or a larger embolus size. Findings from our cohort are in line with those by Wu and colleagues who showed a significant correlation between TMAO plasma levels and NIHSS as well as infarct volume at the time of hospital presentation of patients with acute stroke[16].

The exact mechanism of damage and its magnitude, particularly in subjects at increased risk of ischemic events such as in AF, are still elusive and in addition to the thromboembolic nature, inflammatory, oxidative and procoagulant etiologies should be considered[17]. As shown in our experience with murine models, TMAO correlates with an upregulation of inflammatory cytokines (e.g. TNF-a, IL-1b, and IL-6) and chemokines (e.g. MCP-1 and MIP-1), as well as adhesion molecules (e.g. P-selectin)[18] leading, at least in part, to increased vascular tissue factor expression[19]. These models validate the relevance of TMAO in the absence of comorbidities and reinforce its significance in a real-world scenario as in our population for which adverse events are associated with relatively high TMAO concentrations and with a non-linear behavior[3] (Supplementary Table 1).

Effects associated with TMAO levels and confounders

TMAO is significantly influenced by a number of demographic features, dietary habits and medical conditions which have to be taken into account for adjustment[20]. For instance, TMAO increases with decreasing glomerular filtration rate which in turn is associated with higher burden of cardiac and renovascular diseases and progressive accumulation of TMAO may propel a vicious cycle[21]. TMAO plasma concentrations were found in previous studies to be associated with higher fasting insulin levels[22] and severity of diabetic microvascular complications[23] strengthening the concept of metabolic burden. Nevertheless, this association should be considered as bidirectional since TMAO has an impact on metabolic and vascular health and metformin can lead to a TMAO reduction[24].

Additionally, we found a higher prevalence of chronic heart failure in the highest TMAO tertile. Although chronic heart failure is associated with a multitude of diseases at a late stage, paired with chronic organ dysfunctions and therefore associated with increased TMAO levels, a recent review suggested that TMAO could elicit a series of typical cellular alterations of heart failure such as reduced mitochondrial function, impaired contractile activity and endothelial dysfunction[25].

Potential interventions to modulate TMAO

Our data suggest that low plasma level of TMAO is correlated with lower cardiovascular mortality and decreased brain damage burden reflecting the broadness of TMAO and gut-derived metabolites effects on the human body[26] and supporting the need for interventional studies for reduction of TMAO concentrations. Lifestyle modifications are valid interventions for gut microbiota modulation and therefore TMAO production and they are recommended by current international guidelines for the improvement of AF patients' treatment[27]. Although red meat consumption was not distinguishable from white in our questionnaire, a higher consumption of high-choline containing meat can be postulated based on existing literature[28]. Moreover, physical activity should be considered as a modulator of gut microbiota by eliciting a positive selection towards lactate-dependent symbiotic species[29]. In our study, a sedentary lifestyle was more commonly reported in the highest tertile (Table 1) reflecting a degree of frailty.

Strengths and limitations

Despite its strengths with prospective collection and completeness of dataset and deep characterization of the population, with plasma levels falling within the range of other investigations, our study presents a number of limitations: firstly, the vast majority of participants in SWISS-AF study are of Caucasian ethnicity not fully representing other ethnicities and their dietary habits. TMAO was assessed at a single non-fasting timepoint although no difference concerning time from the last meal was found among the groups, further confounders cannot be excluded. In addition, the absolute number of clinical strokes was relatively low limiting statistical power. Lastly, in light of the study design, taxonomic analysis of the fecal microbiota could not be performed.

Conclusions

TMAO is a biomarker capable of identifying AF patients at high risk for overall and cardiovascular mortality and for subjects with larger and more numerous ischemic brain lesions. TMAO can be considered as an indicator of the cerebral microvascular risk status in AF patients, which is not addressed by clinical scores.

Acknowledgements

Authors contributions

ML and JHB conceived and designed the current sub-study and are responsible for the overall content of the manuscript; SA, NR, GM, TR, TS, JW, LHB, PCB, MC, MK, DC conceived and planned the SWISS-AF study and responsible for this study in their reference centers and provided additional inputs for the improvement for the current sub-study and manuscript production; ML, TD, DM and AvE conducted experiments and performed the quality control analysis of LC-MS/MS without having access to clinical data before their release; CV and MC performed statistical analysis, SSSS, GGC, TFL provided additional conceptual inputs and insights and critically reviewed the manuscript. All authors have reviewed and accepted the current version of the manuscript.

Competing interests

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Figure legends

Table 1. Demographic, clinical and anamnestic parameters together with medications of participants at baseline presented as per TMAO tertiles. Categorical variables are compared using Chi-squared test; normal variables are compared using one-way ANOVA and continuous non-normal variables using Kruskal-Wallis Rank Sum test.

Table 2. Brain MRI findings at the time of study recruitment grouped as per TMAO tertiles. Large non-cortical and cortical infarcts (LNCCI); all infarcts involving cortex and lesions not involving cortex with a diameter >20mm, small non-cortical infarcts (SNCI); diameter <20mm; microbleeds (Mb) and white matter lesions (WML).

Figure 1. A) Kaplan Meier curves of unadjusted overall survival; B) cardiovascular cause survival, C) global stroke and D) ischemic stroke free survival stratified by TMAO tertiles at baseline.

Figure 2. A) Box-whisker plots of TMAO levels displaying the distribution of TMAO with (yes) and without (no) overall mortality, cardiovascular mortality, global stroke and ischemic stroke at 1 year- (1y) and 5 year- (5y) follow up. Sample sizes and p-values are reported. B) Plots illustrating the estimated hazards ratio (with 95% confidence interval) of TMAO on overall death, cardiovascular death, global stroke and ischemic stroke, respectively, considering TMAO as divided per tertiles (with the lowest tertile as the reference level).

Figure 3. Receiver operator curves (ROC) for A) overall mortality; B) cardiovascular mortality; C) global strokes; D) ischemic stroke at 5-year follow-up for TMAO and high sensitivity CRP (hsCRP). Area under the curve (AUC) and 95% Confidence Intervals (CI) are reported for each endpoint. AUC differences between TMAO and hsCRP for A) 0.0497 (95% CI -0.0028 - 0.1022);

B) 0.094 (95% CI 0.0339 - 0.1541); C) -0.0094 (95% CI -0.0963 - 0.0776); D) -0.0233 (95% CI -0.1279 - 0.0812)

Figure 4. A) Box-whisker plots of TMAO levels displaying the distribution of TMAO with (yes) and without (no) presence of large non-cortical and cortical infarcts (LNCCI), small non-cortical infarcts (SNCI), microbleeds and when present, with above and below median LNCCI, SNCI and white matter lesions volume at baseline. Sample sizes and p-values are reported. Plots illustrating the estimated effect (with 95% confidence interval) of TMAO on B) LNCCI, SNCI and microbleeds presence presence; C) LNCCI, SNCI and white matter lesions volumes. Volumes were first log-transformed and therefore exponentiated estimates are provided. TMAO was considered as divided per tertiles (with the lowest tertile as the reference level).

Figure 5. TMAO, derived from the microbial processing of dietary phosphatidylcholine and carnitine and subsequent hepatic oxidation is associated with increased cardiovascular mortality as well as ischemic brain burden, particularly with larger volumes of LNCCI and WML and more frequent SNCI.

Table 1. Demographic, clinical and dietary features of study participants at baseline

	Low Tertile (n=815)	Middle Tertile (n=784)	High Tertile (n=780)	p-value
Males (%)	578 (70.9)	559 (71.3)	594 (76.2)	0.034
Age (Mean (SD))	70.60 (8.48)	73.86 (8.05)	75.37 (8.06)	<0.001
BMI (Mean (SD))	27.01 (4.52)	27.89 (4.76)	28.12 (4.98)	<0.001
Paroxysmal AF (%)	410 (50.3)	339 (43.2)	314 (40.3)	<0.001
CHA_2DS_2-VASc (Mean (SD))	2.97 (1.71)	3.54 (1.62)	3.95 (1.64)	<0.001
Heart Failure (%)	129 (15.8)	195 (24.9)	295 (37.9)	<0.001
Hypertension (%)	513 (62.9)	559 (71.3)	591 (75.8)	<0.001
Coronary artery diseases (%)	188 (23.1)	249 (31.8)	283 (36.3)	<0.001
Diabetes mellitus (%)	74 (9.1)	134 (17.1)	210 (26.9)	<0.001
History of past stroke/TIA (%)	161 (19.8)	152 (19.4)	160 (20.5)	0.852
Glomerular Filtration Rate (ml/min/1.73m^2) [Q1, Q3]	67.30 [57.78-78.85]	58.50 [48.23-70.28]	48.97 [35.60-62.50]	<0.001
Aspirin (%)	104 (12.8)	131 (16.7)	161 (20.6)	<0.001
Other antiplatelets (%)	44 (5.4)	53 (6.8)	50 (6.4)	0.504
Any antiplatelet (%)	125 (25.4)	160 (20.4)	182 (23.4)	<0.001
Vitamin K Antagonist (%)	261 (32.0)	316 (40.3)	358 (45.9)	<0.001
Other oral anticoagulants (%)	458 (56.2)	421 (53.7)	336 (43.1)	<0.001
Any oral anticoagulants (%)	719 (88.2)	737 (94.0)	695 (89.1)	<0.001
Never smoker (%)	382 (46.9)	347 (44.3)	314 (40.3)	<0.001
Regular physical activity (%)	409 (50.2)	376 (48.0)	310 (39.7)	<0.001
Daily vegetables consumption (>2 portions) (%)	109 (13.5)	130 (16.8)	133 (17.4)	0.076
Daily fruit consumption (>2 portions) (%)	178 (22.0)	212 (27.4)	188 (24.6)	0.045
Weekly dairy products consumption (>3 days) (%)	582 (78.3)	539 (73.6)	534 (75.1)	0.099
Weekly meat consumption (>3 days) (%)	447 (55.1)	438 (55.9)	476 (61.3)	0.028
Weekly fish consumption (>3 days) (%)	16 (2.0)	21 (2.7)	27 (3.5)	0.184
Daily soda beverage consumption (>2 drinks) (%)	6 (0.7)	17 (2.2)	16 (2.1)	0.043
TMAO umol/L (median [Q1, Q3])	3.40 [2.80,4.00]	5.80 [5.20,6.60]	11.50 [9.10,16.12]	<0.001

Table 2. Brain MRI findings of study participants at baseline

	First Tertile (n=639)	Second Tertile (n=581)	Third Tertile (n=502)	p-value
LNCCI presence (%)	124 (19.4)	142 (24.4)	121 (24.1)	0.064
LNCCI volume (mm^3) [Q1, Q3]	1153.50 [214.51-5894.26]	1620.02 [245.25, 7702.51]	1803.00 [420.00-7800.00]	0.212
SNCI presence (%)	111 (17.4)	105 (18.1)	153 (30.5)	<0.001
SNCI volume (mm^3) [Q1, Q3]	51.00 [28.50,193.50]	57.00 [30.00-120.00]	75.00 [33.00,171.00]	0.255
MB presence (%)	115 (18.4)	132 (23.5)	121 (25.1)	0.018
WML presence (%)	629 (98.4)	578 (99.5)	498 (99.2)	0.158
WML volume (mm^3) [Q1, Q3]	2970.00 [1215.00,7554.01]	4158.00 [1471.52,9547.50]	5061.00 [2033.06,13034.24]	<0.001
Fazekas' score ≥2 (%)	294 (46.7)	319 (55.2)	304 (61.2)	<0.001