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Increased plasma trimethylamine-*N*-oxide is associated with incident atrial fibrillation

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ABSTRACT

Background: Plasma trimethylamine-*N*-oxide (TMAO) is associated with cardiovascular disease; however specific relationships with cardiac arrhythmias are unknown. We evaluated the association between plasma TMAO and incident atrial fibrillation (AF).
Methods: Risk associations were explored among 3797 patients with suspected stable angina in the Western Norway Coronary Angiography Cohort (WECAC) and verified in 3143 elderly participants in the community-based Hordaland Health Study (HUSK). Information on endpoints was obtained from nationwide registries.
Results: Median follow-up was 7.3 and 10.8 years in the WECAC and HUSK cohorts, respectively, and 412 (10.9%) and 484 (15.4%) subjects were registered with incident AF. The age and gender adjusted HRs were 1.16, 95% CI 1.05–1.28 and 1.10, 95% CI 1.004–1.19 per 1 SD increase in log-transformed plasma TMAO. Adjusting for hypertension, BMI, smoking, diabetes, or intake of total choline, a TMAO precursor, did not materially influence the risk associations. Among patients in WECAC, further extensive adjustment for other AF risk factors yielded similar results. Adding TMAO to traditional AF risk factors (age, gender, hypertension, BMI, smoking and diabetes) yielded a continuous net reclassification improvement of 0.108, 95% CI 0.015–0.202 and 0.139, 95% CI 0.042–0.235.
Conclusions: Plasma TMAO was associated with and improved reclassification of incident AF in two independent Norwegian cohorts with long-term follow-up. The relationship was independent of traditional AF risk factors, as well as of dietary choline intake. Our findings motivate further studies to explore endogenous metabolic factors influencing the relationship between TMAO and cardiovascular disease.

1. Introduction

Atrial fibrillation (AF) is a highly prevalent cardiac arrhythmia, a major determinant of morbidity and mortality [1], and severely influencing health care expenditure [2]. It is therefore important to identify novel risk relationships and pathophysiological pathways for AF. Important risk markers include higher age, male gender, hypertension, smoking, diabetes, decreased physical activity, and increased alcohol intake, although exact pathophysiological mechanisms connecting these risk factors with AF are largely unknown [3].

Trimethylamine-*N*-oxide (TMAO) is an amine compound produced in the liver from trimethylamine by the flavine monoxidase 3 (FMO3) enzyme [4]. The formation of trimethylamine is believed to be mediated by microorganisms in the small intestine, and dietary choline and carnitine, and to a lesser extent betaine, have been proposed as key substrates for trimethylamine, and ultimately TMAO, production [4–6]. Higher systemic TMAO concentrations predict increased risk of cardiovascular events and all-cause death [6], and have been related to prevalent diabetes [7], as well as to higher age and current smoking [8]. TMAO is also associated with adverse prognosis among patients with heart failure [9]; however the lack of information on patients' history of AF

Abbreviations: ADMA, asymmetric dimethylarginine; AF, atrial fibrillation; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FMO3, flavine monoxidase 3; HR, hazard ratio; HUSK, Hordaland Health Study; ICC, Intraclass correlation coefficient; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; NRI, net reclassification improvement; SD, standard deviation; TMAO, trimethylamine-*N*-oxide; WENBIT, Western Norway B-Vitamin Intervention Trial.

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has been considered a limitation in such studies [10], as heart failure is strongly associated with AF [1].

While elevated TMAO concentrations are associated with adverse cardiovascular prognosis and with several risk factors for AF, no previous studies have reported on relationships between plasma TMAO and prevalent or incident AF. We investigated such associations in a large prospective Norwegian cohort of patients evaluated for stable angina pectoris with long-term follow-up, and validated the prospective findings in a community-based cohort of elderly subjects.

2. Material and methods

2.1. Study design

The primary study population has been described in detail elsewhere [11]. In short, 4164 patients underwent coronary angiography due to suspected stable angina pectoris at two Norwegian university hospitals in the period 2000–2004 and were included in the Western Norway Coronary Angiography Cohort (WECAC). Approximately 2/3 of the patients were enrolled into the Western Norway B-Vitamin Intervention Trial (WENBIT; ClinicalTrials.gov Identifier: NCT00354081), a randomized controlled trial investigating B-vitamin treatment as secondary cardiovascular disease (CVD) prevention [12]. In the present study, we excluded patients without valid measurements on plasma TMAO ($n = 23$), leaving 4141 eligible patients for the final baseline analyses. For the prospective analyses, we excluded 344 patients with previous AF, defined as having a history of paroxysmal or chronic AF based on either patient self-report or an electrocardiogram confirming AF at baseline.

The validation cohort consisted of 3327 participants (born 1925–27) in the community-based Hordaland Health Study (HUSK; http://husk.b.uib.no) whose baseline examinations were conducted during 1997–1999 [13]. Those without baseline data on plasma TMAO ($n = 125$) or with a history of previous AF ($n = 59$) were excluded, leaving a total of 3143 participants.

All patients provided written informed consent. The study was carried out according to the Declaration of Helsinki, and was approved by the Regional Committee for Medical and Health Research Ethics, Norwegian Health Region West (Approval numbers 2010/1880 and 2013/2324 for WECAC, and 2015/876 for HUSK).

2.2. Clinical and biochemical data

The collection of anamnestic, clinical and anthropometric data in WECAC has been reported previously [11]. In brief, patients provided self-reported anamnestic data, which were checked against medical records when available. In HUSK baseline data on demographics, anthropometry and lifestyle were collected by questionnaires and by physical examination.

Subsets of 1968 patients in WECAC and 2773 subjects in the HUSK cohorts also provided detailed information on dietary intakes using a validated Food Frequency Questionnaire, enabling us to estimate daily intake of energy, as well as intake of total choline and betaine, as previously reported [7,14].

2.3. Laboratory analyses

In WECAC, venous blood samples were drawn 1–3 days prior to or immediately after angiography, and the majority of patients ($n = 2832$) were in a non-fasting state. Routine laboratory analyses were performed on fresh blood samples at each recruiting hospital. In HUSK, non-fasting blood samples were collected at the baseline survey. Study specific analyses for the two cohorts were performed at the laboratory of BEVITAL AS, Bergen, Norway (www.bevital.no) on samples stored at -80 °C and later thawed, as described previously [11]. Plasma TMAO and asymmetric dimethylarginine (ADMA) were analyzed by liquid chromatography–tandem mass spectrometry [15]. Plasma TMAO had a within-day Coefficient of Variation 2.1–3.1%.

2.4. Endpoint data

The endpoint was receiving a diagnosis of AF during hospitalization (according to the International Classification of Diseases 10th edition I48) or death due to AF throughout 31 December 2009. Information on endpoints from hospitals was obtained from the Cardiovascular Disease in Norway project (CVDNOR; https://cvdnor.b.uib.no), which provides data on cardiovascular disease (CVD) diagnoses at discharge from Norwegian hospitals in the period 1994–2009 [16]. Information on death due to AF was obtained from the Norwegian Cause of Death Registry. Endpoint data were linked to each patient using the 11-digit personal identification number unique to each Norwegian resident.

2.5. Statistical analyses

Continuous and categorical variables are reported as median (25th–75th percentiles) and counts (%), respectively. Linear trends across quartiles of plasma TMAO were tested by median linear regression for continuous variables, and by logistic regression for dichotomous and ordinal variables, respectively. Between-group differences were tested by the Mann-Whitney *U* test and Chi square test for continuous and categorical variables, respectively.

Survival was studied using Kaplan-Meier plots and the difference in survival across quartiles of TMAO was assessed by the log-rank test. Univariate and multivariate Cox regression models were used to obtain hazard ratios (HRs) [95% confidence intervals (CIs)] and reported as per 1 standard deviation (SD) increment in log-transformed plasma TMAO. Risk associations in the primary WECAC cohort were explored unadjusted and adjusted for age and gender (Model 1), and potential effect modifications investigated by adding interaction product terms. Model 1 was further adjusted for fasting status, as well as serum carbamide, as TMAO may counteract destabilizing effects by carbamide on intracellular proteins [17]. A Model 2 was created adjusting for age, gender, hypertension, diabetes, body mass index and smoking. In WECAC patients this model was further extended in a Model 3, additionally adjusted for estimated glomerular filtration rate (eGFR), C-reactive protein (CRP), anamnestic heart failure, left ventricular ejection fraction (LVEF), left ventricular end-diastolic pressure (LVEDP), the extent of coronary artery disease (0–3 significant [>50%] stenotic epicardial coronary arteries at coronary angiography), alcohol intake (0 – >5 units/week), or any history of valvular disease, acute myocardial infarction, or coronary revascularization. No materially different estimates for TMAO in Model 2 were obtained when substituting height and weigth for body mass index, or systolic and diastolic blood pressure for a history of hypertension (data not shown). We fitted a generalized additive model smoothed spline using the crude Cox model to investigate potential non-linear risk relationships between plasma TMAO as a continuous variable and incident AF.

In the validation cohort we investigated the TMAO-AF risk relationships in Models 1 and 2 which were similar to the models used among WECAC patients.

In the subsets of patients with information on dietary intake, we also adjusted the TMAO-AF relationship for energy adjusted dietary intakes of total choline and betaine.

Model fit when adding TMAO to the Cox Model 2 was assessed by comparing the Akaike's information criterion. We tested model discrimination by calculating the difference in C-statistics and the integrated discrimination improvement between logistic regression models with and without plasma TMAO, also containing age, gender, diabetes, hypertension, smoking and body mass index. Reclassification of patients was investigated by adding plasma TMAO to the same multivariate logistic model and calculating the continuous net reclassification improvement (NRI > 0).

In WENBIT participants, the within-person reproducibility of plasma TMAO after one year was studied by 1-way random effects modelling to calculate the intraclass correlation coefficient (ICC). We did not have data on repeated biosampling in HUSK.

All tests were two-sided and the significance level set to 0.05. We used the software IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp., the SAS statistical program (version 9.4; SAS Institute, Inc., Cary, NC), and R for Windows (R Core Team 2016, Vienna, Austria), utilizing the packages #survival, #Hmisc, #PredictABEL, #ROCR, and #ICC).

3. Results

3.1. Baseline characteristics

Characteristics according to plasma TMAO quartiles are shown in Table 1 and Appendix Table A.1 for the WECAC, and in Appendix Table B.1 for HUSK. Higher TMAO was associated with male gender, diabetes, higher plasma ADMA and pyridoxal 5′-phosphate (a B6 vitamer), as well as with most plasma metabolites in the choline oxidation pathway. Negative associations were seen for TMAO with eGFR, current smoking and physical activity. Moreover, in the WECAC patients, TMAO was positively related to previous AF, heart failure, aortic stenosis and coronary heart disease, as well as to LVEDP, the extent of coronary artery disease and serum high sensitive cardiac troponin T, carbamide, and plasma riboflavin. A relationship between higher TMAO and an adverse CVD risk profile was also reflected by more prevalent use of most cardiovascular medications, with the exception of statins, among subjects with higher TMAO. We found a weak inverse relationship between TMAO and the low-density lipoprotein cholesterol/apolipoprotein B ratio in WECAC patients, but no significant associations with other lipid parameters. In HUSK TMAO showed weak inverse relationships with total-, low-density- and high-density lipoprotein cholesterol.

No relationships between plasma TMAO with dietary intakes of choline or betaine were found among patients in WECAC, whereas a moderate positive association with choline intake was observed among subjects in HUSK.

3.2. The prospective association between plasma TMAO and atrial fibrillation

Among the 3797 patients in WECAC with no previous AF, 412 (10.9%) were diagnosed with incident AF during median (25th–75th percentiles) 7.3 (6.3–8.6) years. Baseline characteristics according to