Clinical Investigations

Intestinal Microbiota-Dependent Phosphatidylcholine Metabolites, Diastolic Dysfunction, and Adverse Clinical Outcomes in Chronic Systolic Heart Failure

W.H. WILSON TANG, MD,[1,2] ZENENG WANG, PhD,[1] KEVIN SHRESTHA, MD,[2] ALLEN G. BOROWSKI, RDCS,[2] YUPING WU, PhD,[3] RICHARD W. TROUGHTON, MBBS,[4] ALLAN L. KLEIN, MD,[2] AND STANLEY L. HAZEN, MD, PhD[1,2]

Cleveland, Ohio; and Christchurch, New Zealand

ABSTRACT

Background: Trimethylamine-*N*-oxide (TMAO) has been linked to increased cardiovascular risk. We aimed to determine the prognostic value of TMAO and its dietary precursors, choline and betaine, in heart failure (HF).

Methods and Results: In 112 patients with chronic systolic HF with comprehensive echocardiographic evaluation, we measured plasma TMAO, choline, and betaine by mass spectrometry. Median (interquartile range) TMAO levels, choline, and betaine levels were 5.8 (3.6−12.1) μmol/L, 10.9 (8.4−14.0) μmol/L, and 43.8 (37.1−53.0) μmol/L, respectively, and were correlated with each other (all $P < .0001$ for both). TMAO levels were significantly higher in patients with diabetes mellitus (9.4 [4.9−13.2] vs 4.8 [3.4−9.8] μmol/L; $P = .005$) and in subjects with New York Heart Association functional class III or greater (7.0 [4.7−14.8] vs 4.7 [3.4−11.3] μmol/L; $P = .02$). Elevated TMAO, choline, and betaine levels were each associated with higher plasma N-terminal pro−B-type natriuretic peptide (NT-proBNP) levels and more advanced left ventricular diastolic dysfunction, but not systolic dysfunction or inflammatory and endothelial biomarkers. Higher choline (hazard ratio [HR] 1.64, 95% CI 1.22−2.20; $P = .001$), betaine (HR 1.51, 95% CI 1.10−2.08; $P = .01$), and TMAO (HR 1.48, 95% CI 1.10−1.96; $P = .01$) predicted increased risk for 5-year adverse clinical events (death/transplantation). Only higher TMAO levels predicted incident adverse clinical events independently from age, estimated glomerular filtration rate, mitral E/septal Ea, and NT-proBNP levels (HR 1.46, 95% CI 1.03−2.14; $P = .03$).

Conclusion: Elevated plasma TMAO, choline, and betaine levels are each associated with more advanced left ventricular diastolic dysfunction and portend poorer long-term adverse clinical outcomes in chronic systolic HF. However, only higher plasma TMAO was associated with poor prognosis after adjustment for cardiorenal indices. (*J Cardiac Fail 2015;21:91−96*)

Key Words: Intestinal microbiota, trimethylamine-*N*-oxide, diastolic dysfunction, heart failure.

From the [1]Center for Cardiovascular Diagnostics and Prevention, Department of Cellular and Molecular Medicine, Lerner Research Institute, Cleveland, Ohio; [2]Department of Cardiovascular Medicine, Heart and Vascular Institute, Cleveland Clinic, Cleveland, Ohio; [3]Department of Mathematics, Cleveland State University, Cleveland, Ohio and [4]Department of Medicine, Christchurch School of Medicine and Health Sciences, Christchurch, New Zealand.

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Reprint requests: W.H. Wilson Tang, MD, 9500 Euclid Avenue, Desk J3-4, Cleveland, OH 44195. Tel: (216) 444-2121; Fax: (216) 445-6165. E-mail: tangw@ccf.org

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Intestinal microbiota are implicated in the development of metabolic phenotypes such as obesity and insulin resistance.[1] With the use of an unbiased metabolomics approach, our group recently identified 3 metabolites of the dietary lipid phosphatidylcholine—choline, betaine and the gut microbiota–generated metabolite trimethylamine-*N*-oxide (TMAO)—that are associated with atherosclerotic cardiovascular disease.[2] We recently validated those findings in a larger-scale clinical cohort whereby elevated plasma TMAO levels portend greater risk of major adverse cardiac events,[3] and we showed the mechanistic link between TMAO and macrophage activation[2] as well as alterations in cholesterol metabolism and transport.[4] Because choline and betaine are substrates in the formation of TMAO by intestinal microbiota, we have further demonstrated their prognostic values in predicting future major adverse cardiac events to be largely driven by the presentation of elevated TMAO levels.[5]

Heart failure (HF) is a frequent adverse complication of atherosclerotic cardiovascular disease and may present either as myocardial ischemia, vascular dysfunction, and fibrosis leading to progressive diastolic dysfunction or as progressive myocyte damage and cardiac remodeling leading to systolic dysfunction. We recently reported the association between TMAO and long-term mortality risk in a large cohort of patients with a history of chronic HF, independently from renal insufficiency or natriuretic peptide levels.[6] However, the relationship between TMAO and its dietary precursors choline and betaine and myocardial and inflammatory indices, markers of endothelial dysfunction, as well as their relative prognostic values in patients with chronic systolic HF, has not yet been carefully explored. In the present study, our objective was to investigate the relationship between the 3 phosphatidylcholine metabolites TMAO, choline, and betaine and myocardial indices, inflammatory and endothelial biomarkers, and long-term clinical prognosis in subjects with chronic systolic HF.

Materials and Methods

Study Population

This was a single-center prospective cohort study approved by the Cleveland Clinic Institutional Review Board, and every subject provided written informed consent. We enrolled 112 ambulatory subjects ≥18 years of age with stable but symptomatic chronic systolic HF (left ventricular [LV] ejection fraction ≤35%;), who underwent comprehensive echocardiographic evaluation as part of a research study at the Cleveland Clinic. Subjects were excluded if they had significant primary valvular abnormalities. Comprehensive transthoracic echocardiographic evaluation of systolic and diastolic myocardial performance was assessed as previously described.[7] The composite end point of adverse clinical events (all-cause mortality and cardiac transplantation) was prospectively tracked for 5 years by telephone follow-up and medical chart review.

TMAO, Choline, and Betaine Assay

Quantification of TMAO, choline, and betaine was performed with the use of stable isotope dilution liquid chromatography with tandem mass spectrometry (LC/MS/MS), stable isotope dilution assay, and high-performance LC with online electrospray ionization (ESI)/MS/MS on an AB Sciex Qtrap 5500 mass spectrometer as previously described.[8] Arginine metabolites (asymmetric dimethylarginine [ADMA], symmetric dimethylarginine [SDMA], L-arginine, L-ornithine, and L-citrulline) were quantified with the use of stable isotope dilution LC/ESI/MS/MS assays on an upgraded ABI 365 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, California) with Ionics EP 10+ redesigned source (Concord, Ontario, Canada) and ESI needle connected to an Aria LX4 series multiplexed high-performance LC system with Flux pumps (Cohesive Technologies, Franklin, Massachusetts), as previously described.[7] Global arginine bioavailability ratio (GABR) was calculated as the ratio between the substrates (L-arginine) and the products (L-ornithine plus L-citrulline) of nitric oxide production.[9]

Aminoterminal pro–B-type natriuretic peptide (NT-proBNP) levels were measured with the use of the Elecsys platform (Roche Diagnostics, Indianapolis, Indiana).[10] Plasma myeloperoxidase (MPO) and high-sensitivity C-reactive protein (hsCRP) levels were measured by means of CardioMPO assay (Cleveland Heart Labs, Cleveland, Ohio)[11] as well as N Latex Cystatin C CardioPhase assay (Siemens/Dade Behring, Deerfield, Illinois)[12,13] as previously described.

Statistical Analysis

Continuous variables were summarized as mean ± SD if normally distributed and median (interquartile range [IQR]) if nonnormally distributed. Normality was assessed by means of the Shapiro-Wilk *W* test. Spearman rank correlation was used as a nonparametric measure of association between metabolites and dependent variables. Plasma levels of TMAO, choline, and betaine were compared across categoric variables with the use of the Wilcoxon rank sum or Kruskal-Wallis test. The optimal receiver operating characteristic (ROC) curve cutoff value for plasma levels of TMAO, choline, and betaine in predicting adverse clinical events was chosen as the value maximizing sensitivity plus specificity. Kaplan-Meier survival plots were calculated from baseline to time of adverse event and compared with the use of the log rank test. Cox proportional hazards analysis were used to assess the clinical risks associated with higher TMAO, choline, and/or betaine levels, in which the proportional hazards assumption was verified with log (time) versus log [−log (survival)] plots. A 2-sided P value of $<.05$ was considered to be statistically significant. All analyses were performed with the use of JMP 10 Pro (SAS, Cary, North Carolina).

Results

Table 1 illustrates the baseline characteristics of the study population. Overall, the cohort was representative of a stable outpatient cohort of patients with systolic heart failure, with 40 (36%) experiencing at least New York Heart Association (NYHA) functional class III symptoms. Overall, TMAO correlated with choline ($r = 0.40$; $P < .0001$) but not betaine ($r = 0.08$; $P = .43$), and choline correlated with betaine ($r = 0.46$; $P < .0001$).