Levels of Trimethylamine N-Oxide Remain Elevated Long Term After Left Ventricular Assist Device and Heart Transplantation and Are Independent From Measures of Inflammation and Gut Dysbiosis

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BACKGROUND: Trimethylamine N-oxide (TMAO)—a gut-derived metabolite—is elevated in heart failure (HF) and linked to poor prognosis. We investigated variations in TMAO in HF, left ventricular assist device (LVAD), and heart transplant (HT) and assessed its relation with inflammation, endotoxemia, oxidative stress, and gut dysbiosis.

METHODS: We enrolled 341 patients. TMAO, CRP (C-reactive protein), IL (interleukin)-6, TNF-α (tumor necrosis factor alpha), ET-1 (endothelin-1), adiponectin, lipopolysaccharide, soluble CD14, and isoprostane were measured in 611 blood samples in HF (New York Heart Association class I–IV) and at multiple time points post-LVAD and post-HT. Gut microbiota were assessed via 16S rRNA sequencing among 327 stool samples. Multivariable regression models were used to assess the relationship between TMAO and (1) New York Heart Association class; (2) pre- versus post-LVAD or post-HT; (3) biomarkers of inflammation, endotoxemia, oxidative stress, and microbial diversity.

RESULTS: ln-TMAO was lower among HF New York Heart Association class I (1.23 [95% CI, 0.52–1.94] µM) versus either class II, III, or IV (1.99 [95% CI, 1.68–2.30], 1.97 [95% CI, 1.71–2.24], and 2.09 [95% CI, 1.83–2.34] µM, respectively; all P<0.05). In comparison to class II–IV, ln-TMAO was lower 1 month post-LVAD (1.58 [95% CI, 1.32–1.83] µM) and 1 week and 1 month post-HT (0.97 [95% CI, 0.60–1.35] and 1.36 [95% CI, 1.01–1.70] µM). ln-TMAO levels in long-term LVAD (>6 months: 1.99 [95% CI, 1.76–2.22] µM) and HT (>6 months: 1.86 [95% CI, 1.66–2.05] µM) were not different from symptomatic HF. After multivariable adjustments, TMAO was not associated with biomarkers of inflammation, endotoxemia, oxidative stress, or microbial diversity.

CONCLUSIONS: TMAO levels are increased in symptomatic HF patients and remain elevated long term after LVAD and HT. TMAO levels were independent from measures of inflammation, endotoxemia, oxidative stress, and gut dysbiosis.

Key Words: endothelin-1 ■ heart failure ■ heart transplantation ■ inflammation ■ interleukin-6
WHAT IS NEW?

• This study investigated, for the first time, circulating levels of trimethylamine N-oxide (TMAO) across a broad spectrum of heart failure disease progression and following treatment with left ventricular assist devices (LVADs) and heart transplantation (HT).

• We found that TMAO levels progressively increase with heart failure severity and remain elevated long term after LVAD and HT; inversely relate to renal function using both serum creatinine- or cystatin C–based estimations; and do not correlate with established measures of inflammation, endotoxemia, oxidative stress, and gut dysbiosis.

WHAT ARE THE CLINICAL IMPLICATIONS?

• TMAO is a gut-derived metabolite that has been linked to heart failure pathogenesis and prognosis. In the present study, we demonstrate that hemodynamic improvements after LVAD or HT do not translate into reductions in TMAO levels.

• Future studies are warranted to (1) investigate clinical implications of elevated TMAO levels following LVAD and HT and (2) identify novel therapeutics and potential nutritional interventions that may modify TMAO levels with the goal to improve outcomes among heart failure, LVAD, and HT patients.

Nonstandard Abbreviations and Acronyms

| Acronym | Definition |
|---------|------------|
| CKD     | chronic kidney disease |
| CNI     | calcineurin inhibitor |
| CRP     | C-reactive protein |
| CVD     | cardiovascular disease |
| CysC    | cystatin C |
| eGFR<sub>Cr</sub> | creatinine-estimated glomerular filtration rate |
| eGFR<sub>Cys</sub> | cystatin C–estimated glomerular filtration rate |
| ESV     | exact sequence variant |
| ET-1    | endothelein-1 |
| HF      | heart failure |
| HT      | heart transplantation |
| IL      | interleukin |
| LVAD    | left ventricular assist device |
| NT-proBNP | N-terminal pro-B-type natriuretic peptide |
| NYHA    | New York Heart Association |
| ORIGINS | Oral Infections, Glucose Intolerance and Insulin Resistance Study |
| sCD14   | soluble CD14 |
| sCr     | serum creatinine |
| TMAO    | trimethylamine N-oxide |
| TNF-α   | tumor necrosis factor alpha |

Heart failure (HF) is a prevalent disease that is associated with high mortality and morbidity. Over the last decades, multiple therapeutic modalities have been developed to prevent adverse ventricular remodeling, reduce rehospitalizations, and improve survival in HF. However, current interventions rarely reverse the underlying disease process, and progression from chronic stable HF to advanced disease still occurs. Left ventricular assist devices (LVADs) and heart transplantation (HT) are the only treatment modalities that can improve survival in advanced HF.

Systemic inflammation and oxidative stress are commonly observed in HF and may directly relate to its pathogenesis. Recently, perturbations in the gut microbiota known as gut dysbiosis and impairment of gut mucosal barriers, facilitating entry of endotoxins and gut metabolites into the circulation, have been observed in HF patients. Elevated levels of circulating endotoxins and bacterial byproducts enhance systemic inflammation and oxidative stress, thereby contributing to progression of HF to more advanced state. Moreover, gut bacteria are the main producers of uremic toxins, most notably trimethylamine N-oxide (TMAO).

TMAO is a gut-derived metabolite that has been linked to cardiovascular disease (CVD) outcomes and to HF pathogenesis and prognosis in both humans and animal models. Dietary sources and microbial composition of the gut flora regulate TMAO production, while the kidneys mediate TMAO clearance. Accordingly, renal function is a determinant of circulating TMAO levels. Changes in renal function are commonly observed among patients with advanced HF. However, in these patients, interpretation of serum creatinine (sCr) can be confounded by changes in creatinine production that are independent of renal function (eg, changes in muscle mass related to cardiac cachexia). CysC (cystatin C) is an endogenous biomarker of renal function produced by all nucleated cells at a near constant rate that is independent of muscle mass. CysC–estimated glomerular filtration rate (eGFR<sub>Cys</sub>) outperforms sCr-based estimates in chronic diseases including HF and in critically ill patients.

To date, no study has measured TMAO levels across a broad spectrum of systolic HF progression and after treatment with LVAD or HT. In addition, eGFR<sub>Cys</sub> has never been used to adjust TMAO levels for renal function in this patient population. Furthermore, the relation of TMAO with established biomarkers of inflammation remains to be established. Lastly, little is known about the association between TMAO levels and variation in gut microbial communities among HF, LVAD, and HT patients.

Thus, we aimed to (1) compare TMAO levels among distinct clinical subgroups of HF patients before and after LVAD or HT; (2) evaluate the relationship between TMAO and 2 indices of renal function (sCr versus CysC), as well as biomarkers of inflammation, endotoxemia, and...
oxidative stress; (3) investigate the relationship between TMAO and gut dysbiosis; and, lastly, (4) explore the predictive value of TMAO on clinical outcomes in a subset of HF and LVAD patients.

**METHODS**

The authors declare that all supporting data are available within the article and its Data Supplement.

**Study Population**

Patients were enrolled between June 2016 and February 2019 at the Columbia University Irving Medical Center during routine clinical visits or index hospitalization for LVAD or HT surgery. Exclusion criteria were (1) HF with preserved left ventricular ejection function >40%, (2) infiltrative and hypertrophic cardiomyopathy, (3) advanced renal disease requiring dialysis, (4) liver cirrhosis or active hepatitis, and (5) active malignancy. Patients were classified into the following categories: HF: New York Heart Association (NYHA) class I–IV; post-LVAD: 1 month, 3 to 6 months, >6 months; and post-HT: 1 week, 1 month, 3 months, and >6 months. All HF patients were treated according to the current guidelines, and HT patients received standard immunosuppression per institutional protocol. Clinical information was extracted from electronic medical records. Antibiotics use 1 month before stool or blood sample collection was recorded, including data for treatment of confirmed/suspected infection or chronic prophylaxis among HT patients. The study was approved by the Columbia University Irving Medical Center Institutional Review Board (AAAP8204), and participants gave written informed consent.

A convenience sample of controls free of CVD enrolled in ORIGINS (Oral Infections, Glucose Intolerance and Insulin Resistance Study)23,24 at the Columbia University Irving Medical Center was used as a comparison group to confirm that TMAO levels in our cohort were elevated relative to CVD-free individuals.

**TMAO Measurement and Calculation of Renal Function**

Plasma TMAO was measured using ultra performance liquid chromatography–tandem mass spectrometry (Data Supplement); eGFR_{Cr} and creatinine–estimated glomerular filtration rate (eGFR_{Cr}) were calculated as described previously26–27 (Data Supplement).

**Measurements of Biomarkers of Inflammation, Endotoxemia, and Oxidative Stress**

Biomarkers of inflammation (CRP [C-reactive protein], IL interleukin]-6, TNF-α [tumor necrosis factor alpha], ET-1 [endothelin-1], and adiponectin), endotoxemia (lipopolysaccharide and soluble CD14 [sCD14]), and oxidative stress (isoprostane) were measured in plasma or serum (Data Supplement).

**Stool Analysis**

Patients provided nonfasting stool samples in sterile stool hats.28 Details on stool collection and DNA extraction and sequencing are provided in the Data Supplement. All collected stool samples had a corresponding blood sample (median difference between blood and stool collection dates was 2 [interquartile interval, 19.5] days).

**Statistical Analysis**

All statistical analyses were conducted using R, version 3.6.2. Difference in means or proportions of potential confounders according to categories was assessed using 1-way ANOVA for continuous variables and χ² for categorical variables. Multivariable mixed-effects linear models regressed natural log-transformed TMAO across categories. Patients were modeled as random effects to account for within patient correlation. In-transformed TMAO values were utilized in regression models to enhance normality. Multivariable adjusted mean values of ln-TMAO are presented across categories. Paired t tests were used to compare mean eGFR values calculated using CysC versus sCr-based equations; for these paired comparisons, we adjusted for multiple comparisons using the false discovery rate. Pearson correlations and mixed-effects models were used to inform the degree of linear relationship between TMAO levels and different assessments of renal function, biomarkers of inflammation, endotoxemia, and oxidative stress, and metrics of gut microbial diversity. Time-varying Cox proportional hazards models were used to assess the relationship between variations in ln-transformed TMAO levels and clinical outcomes.

For gut microbiota analyses, demultiplexed sequence files were processed in R, version 3.6.2, using DADA2 pipeline to identify exact sequence variants (ESVs).29,30 Reads were truncated at forward and reverse lengths of 260 and 220. After processing, a total of 5,697,709 sequence reads were included of median library size 13,625 and 30,959 ESVs were identified. Phyloseq package was used for 16S analyses. Microbial α-diversity (ie, number and distribution of bacterial taxa within samples) was defined using the Shannon Index and number of observed ESVs. DESeq package24 was used to evaluate whether specific taxa differed by categories after multivariable adjustment and adjustment for multiple comparisons using the false discovery rate.

**RESULTS**

**Baseline Characteristics**

Among 341 enrolled patients, 611 blood samples (HF: 9 NYHA class I, 46 class II, 61 class III, and 68 class IV; post-LVAD: 64 [1 month], 52 [3–6 months], and 85 [>6 months]; post-HT: 29 [1 week], 34 [1 month], 37 [3 months], and 126 [>6 months]) were collected. In a subset of 225 patients, 327 stool samples for microbiome assessments were also collected (HF: 10 NYHA class I, 29 class II, 31 class III, and 30 class IV; post-LVAD: 37 [1 month], 31 [3–6 months], and 52 [>6 months]; post-HT: 13 [1 week], 12 [1 month], 13 [3 months], and 72 [>6 months]). Characteristics of patients providing blood samples are reported in Tables 1 through 3, and characteristics of patients providing stool samples are shown in Tables I through III in the Data Supplement. All study patients were predominantly White men, with no
Table 1. Baseline Characteristics of Heart Failure Patients Providing Blood Samples

| Study cohorts | NYHA Class I | NYHA Class II | NYHA Class III | NYHA Class IV | P value |
|---------------|--------------|--------------|---------------|--------------|---------|
| No. of samples (n=184) | 9 | 46 | 61 | 68 |           |
| Demographic and clinical characteristics | | | | | |
| Age, y | 54.8±19.4 | 59.3±13.5 | 60.6±12.7 | 58.8±14.3 | 0.68 |
| Men | 7 (77.8%) | 30 (65.2%) | 50 (82.0%) | 60 (88.2%) | 0.03 |
| Race | | | | | 0.02 |
| White | 1 (11.1%) | 20 (43.5%) | 35 (57.4%) | 36 (52.9%) | |
| Black | 2 (22.2%) | 11 (23.9%) | 18 (29.5%) | 14 (20.6%) | |
| Hispanic | 3 (33.3%) | 5 (10.9%) | 6 (9.8%) | 5 (7.4%) | |
| Other | 3 (33.3%) | 10 (21.7%) | 2 (3.3%) | 13 (19.1%) | |
| BMI, kg/m², median (IQR) | 31.1 (28.5–36.7) | 30.2 (25.4–34.6) | 30.1 (26.1–35.0) | 29.0 (25.0–31.5) | 0.44 |
| Smoking | 3 (33.3%) | 20 (43.5%) | 35 (57.4%) | 35 (51.5%) | 0.37 |
| Etiology, ischemic | 2 (22.2%) | 19 (41.3%) | 29 (47.5%) | 31 (45.6%) | 0.52 |
| Hypertension | 4 (44.4%) | 33 (71.7%) | 36 (59.0%) | 43 (63.2%) | 0.35 |
| Diabetes | 1 (11.1%) | 14 (30.4%) | 21 (34.4%) | 21 (30.9%) | 0.57 |
| atrial fibrillation/flutter | 1 (11.1%) | 15 (32.6%) | 32 (52.5%) | 30 (44.1%) | 0.05 |
| Stroke | 0 (0.0%) | 1 (2.2%) | 5 (8.2%) | 3 (4.4%) | 0.45 |
| Laboratory parameters | | | | | |
| BUN, mg/dL | 17.1±3.8 | 25.5±13.2 | 23.9±9.2 | 32.0±19.1 | 0.008 |
| Serum Cr, mg/dL | 1.1±0.2 | 1.6±1.7 | 1.3±0.3 | 1.4±0.5 | 0.39 |
| Serum CysC, mg/L | 1.0±0.1 | 1.5±1.3 | 1.4±0.5 | 1.7±0.6 | <0.001 |
| eGFRcr, mL/min per 1.73 m² | 76.3±19.4 | 63.2±26.5 | 63.7±18.6 | 59.7±23.1 | 0.31 |
| eGFRcys, mL/min per 1.73 m² | 77.3±16.0 | 62.0±23.9 | 56.3±23.3 | 48.6±24.8 | 0.001 |
| NT-proBNP, ng/L, median (IQR) | 515 (271–694) | 967 (219–2048) | 1541 (557–4017) | 2863 (1817–4606) | <0.001 |
| Na, mmol/L | 141.6±3.2 | 140.3±3.4 | 140.3±3.2 | 136.6±4.8 | <0.001 |
| AST, U/L | 25.0±15.9 | 28.0±21.7 | 25.9±13.6 | 41.0±66.8 | 0.32 |
| ALT, U/L | 23.3±15.4 | 27.5±21.3 | 29.3±25.3 | 66.9±147.7 | 0.12 |
| Total bilirubin, mg/dL | 0.5±0.1 | 0.6±0.5 | 0.7±0.4 | 0.9±0.4 | <0.001 |
| LDH, U/L | 215.0±96.1 | 241.6±74.3 | 272.7±99.8 | 343.9±185.8 | 0.03 |
| Biomarkers | | | | | |
| ln-CRP, mg/L | 0.5±1.7 | 0.8±1.2 | 1.2±1.3 | 2.7±1.4 | <0.001 |
| ln IL-6, pg/mL | 0.8±0.6 | 1.2±0.7 | 1.7±0.8 | 2.5±1.0 | <0.001 |
| ln TNF-α, pg/mL | 0.2±0.4 | 0.4±0.5 | 0.5±0.4 | 0.8±0.4 | <0.001 |
| ln ET-1, pg/mL | 0.6±0.5 | 0.8±0.5 | 0.9±0.5 | 1.1±0.5 | 0.001 |
| ln adiponectin, ng/mL | 8.6±0.8 | 9.1±0.8 | 9.2±0.6 | 9.7±0.7 | <0.001 |
| ln isoprostane, pg/mL | 4.4±0.4 | 4.5±0.5 | 4.6±0.4 | 4.8±0.5 | 0.008 |
| ln sCD14, ng/mL | 7.1±0.3 | 7.3±0.3 | 7.3±0.3 | 7.5±0.4 | 0.002 |
| LPS, EU/mL | 0.2±0.1 | 0.2±0.1 | 0.3±0.2 | 0.4±0.3 | 0.04 |
| Medications | | | | | |
| ASA | 5 (55.6%) | 31 (67.4%) | 31 (50.8%) | 40 (58.8%) | 0.39 |
| Coumadin | 0 (0.0%) | 3 (6.5%) | 17 (27.3%) | 6 (8.8%) | 0.002 |
| ACE inhibitors | 3 (33.3%) | 18 (39.1%) | 15 (24.6%) | 3 (4.4%) | <0.001 |
| ARB | 5 (55.6%) | 16 (34.8%) | 31 (50.8%) | 6 (8.8%) | <0.001 |
| Aldosterone antagonists | 6 (66.7%) | 27 (58.7%) | 40 (65.6%) | 36 (52.9%) | 0.51 |
| β-Blockers | 9 (100.0%) | 45 (97.8%) | 55 (90.2%) | 36 (52.9%) | <0.001 |
| Statins | 5 (55.6%) | 24 (52.2%) | 32 (52.5%) | 39 (57.4%) | 0.93 |
| Loop diuretics | 6 (66.7%) | 33 (71.7%) | 52 (85.2%) | 49 (72.1%) | 0.23 |
| Digoxin | 2 (22.0%) | 5 (10.9%) | 19 (31.1%) | 14 (20.6%) | 0.1 |
| Antibiotics* | 0 (0.0%) | 4 (8.7%) | 8 (13.1%) | 17 (25.0%) | 0.04 |

Data presented n (%) or mean±SD as appropriate, unless otherwise noted. ACE indicates angiotensin-converting enzyme; ALT, alanine transaminase; ARB, angiotensin receptor blocker; ASA, acetylsalicylic acid; AST, aspartate transaminase; BMI, body mass index; BUN, blood urea nitrogen; Cr, creatinine; C-reactive protein; CysC, cystatin C; eGFRcr, creatinine-estimated glomerular filtration rate; eGFRcys, cystatin C–estimated glomerular filtration rate; ET-1, endothelin-1; IL-6, interleukin-6; IQR, interquartile interval; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; NYHA, New York Heart Association; sCD14, soluble CD14; and TNF-α, tumor necrosis factor alpha.

*Antibiotic use is defined as any antibiotic use that was indicated for infection that was estimated to have occurred 1 mo before stool collection.
difference in body mass index, HF etiology, and history of diabetes across categories (Tables 1 through 3).

Among HF patients, NYHA class IV (versus class I–III) patients had lower sodium, higher total bilirubin, NT-proBNP (N-terminal pro-B-type natriuretic peptide), blood urea nitrogen, and CysC (lower eGFR_Cys) levels. HF NYHA class IV patients were less likely to be treated according to HF guidelines and more likely to receive antibiotics (Table 1).

Among LVAD patients, majority were INTERMACS (Interagency Registry for Mechanically Assisted Circulatory Support) profile 2 and 3. LVAD (1 month) patients versus LVAD (3–6 and >6 months) had lower sodium, higher liver enzymes, and higher NT-proBNP levels, as well as lower sCr (higher eGFR_Cr). LVAD (1 month) patients were less likely to be treated with HF medications and more likely to receive antibiotics (Table 2). Renal function by eGFR_Cr remained largely unchanged over time after LVAD (Figure 1).

Among HT patients, early after HT (1 week and 1 month), patients had higher eGFR_Cr, while eGFR_Cys remained largely unchanged over time post-HT. HT patients (>6 months) were less likely to be on antibiotics as compared with the earlier time points (Table 3). Of note, the proportion of HT versus LVAD or HF patients treated with statins was significantly higher (>80% after 1 month of HT). Tacrolimus was the predominant calcineurin inhibitor (CNI) used.

When comparing eGFR_Cr versus eGFR_Cys, significant differences in renal function assessment were present in patients with advanced HF (NYHA class IV), early after LVAD (1 month; 3–6 months) and HT (1 week; 1 month; Figure 1).

Circulating TMAO Levels Across HF, LVAD, and HT Patients

Median plasma TMAO levels (interquartile interval) of the entire cohort were 5.86 (3.49–10.50) µM. For individual subgroups, levels were as follows: HF, 6.96 (3.96–12.73) µM; LVAD, 5.81 (3.50–9.87) µM; and HT, 5.35 (2.85–9.76) µM. These levels were significantly higher than in healthy controls at our and other institutions and were comparable to levels previously reported in patients with CVD, chronic kidney disease (CKD), and HF (Figure 2).

When analyzing the entire cohort of HF, LVAD, and HT patients, ln-TMAO demonstrated a moderate inverse correlation with eGFR_Cr, irrespective of the biomarker studied: eGFR_Cr (r = −0.41; P < 0.001) and eGFR_Cys (r = −0.37; P < 0.0001; Figure 1 in the Data Supplement).

In the unadjusted model (M0), mean follow-up in TMAO was the lowest among asymptomatic HF NYHA class I patients (1.23 [0.52–1.94] µM) and significantly increased among class II–IV patients (1.99 [1.68–2.30], 1.97 [1.71–2.24], and 2.09 [1.83–2.34] µM, respectively; P < 0.05 for comparison between class I versus class II–IV). In comparison to class II–IV patients, levels were lower early after LVAD (1 month: 1.58 [1.32–1.83] µM) and after HT (1 week: 0.97 [0.60–1.35] µM; 1 month: 1.36 [1.01–1.70] µM). Notably, long-term LVAD (>6 months: 1.99 [1.76–2.22] µM) and HT (>6 months: 1.86 [1.76–2.22] µM) ln-TMAO levels were similar to those of symptomatic class II–IV HF patients (Figure 3A).

After multivariable adjustment for age, sex, race/ethnicity (M1), and antibiotics use (M2), variations in mean values of ln-TMAO across categories did not critically change. These patterns were attenuated and lost statistical significance after adjustment for renal function using eGFR_Cr (M3). However, when using eGFR_Cys (M4), the reductions observed early after HT remained statistically significant (Figure 3B). Results were similar in the sensitivity analysis that utilized data from those patients who had eGFR_Cr and eGFR_Cys (n=389) measured concurrently (Figure II A and IIB in the Data Supplement).

A subgroup of 57 LVAD and 26 HT patients had TMAO levels measured longitudinally both pre-LVAD and at least once post-LVAD for a total of 162 samples collected and pre-HT and at least once post-HT for a total of 102 samples. The patterns observed confirmed our cross-sectional findings, showing a trend in which TMAO levels decrease early following LVAD or HT and slowly rebound to preintervention levels (Figure 3A and 3B; Tables IV and V in the Data Supplement). Additionally, in a small subgroup of 13 LVAD and 10 HT patients with TMAO measured longitudinally at all time points pre- and post-LVAD/HT, the results were confirmatory of the above findings (Figure III A and IIIB in the Data Supplement).

Relation Between Circulating TMAO Levels and Biomarkers of Inflammation, Endotoxemia, and Oxidative Stress

All biomarkers of inflammation (CRP, IL-6, TNF-α, ET-1, and adiponectin), endotoxemia (lipopolysaccharide and sCD14), and oxidative stress (isoprostane) increased as HF progressed from class I to IV. Among LVAD patients, biomarkers of inflammation (CRP, IL-6, TNF-α, and adiponectin) were progressively lower with time post-LVAD, whereas endotoxemia (lipopolysaccharide) and oxidative stress (isoprostane) increased. Among HT patients, similarly to LVAD patients, biomarkers of inflammation (CRP, IL-6, TNF-α, adiponectin, and ET-1) were progressively lower with time post-HT, whereas endotoxemia (lipopolysaccharide and sCD14) and oxidative stress (isoprostane) remained elevated (Table 1; Figures IV and V in the Data Supplement).

For the entire cohort, Table VI in the Data Supplement summarized the relationship between ln-TMAO and the abovementioned biomarkers. In the unadjusted model (M0), ln-TMAO levels were significantly associated with levels of inflammation (TNF-α and ET-1), endotoxemia...
Table 2. Baseline Characteristics of LVAD Patients Providing Blood Samples

| Study cohorts | LVAD 1 mo | LVAD 3–6 mo | LVAD >6 mo | P value |
|---------------|-----------|-------------|------------|---------|
| Demographic and clinical characteristics | | | | |
| No. of samples (n=201) | 64 | 52 | 85 | |
| Age, y | 58.7±13.0 | 57.7±14.6 | 57.8±14.3 | <0.001 |
| Men | 57 (89.1%) | 45 (86.5%) | 74 (87.1%) | 0.90 |
| Race | | | | 0.05 |
| White | 43 (67.2%) | 31 (59.6%) | 55 (64.7%) | |
| Black | 8 (12.5%) | 10 (19.2%) | 22 (25.9%) | |
| Hispanic | 2 (3.1%) | 5 (9.6%) | 5 (5.9%) | |
| Other | 11 (17.2%) | 6 (11.5%) | 3 (3.5%) | |
| BMI, kg/m²; median (IQR) | 28.5 (22.9–29.4) | 27.6 (25.0–30.6) | 28.0 (24.8–32.5) | 0.35 |
| Smoking | 41 (64.1%) | 31 (59.6%) | 43 (50.6%) | 0.24 |
| Etiology, ischemic | 38 (59.4%) | 26 (50.0%) | 37 (43.5%) | 0.16 |
| INTERMACS profile | | | | 0.58 |
| 1 | 7 (10.9%) | 4 (7.7%) | 6 (7.1%) | |
| 2 | 35 (54.7%) | 32 (61.5%) | 42 (49.4%) | |
| 3 | 12 (18.8%) | 12 (23.1%) | 21 (24.7%) | |
| 4 | 4 (6.2%) | 3 (5.8%) | 5 (5.9%) | |
| 5–7 or not provided | 6 (9.4%) | 1 (1.9%) | 11 (12.9%) | |
| Hypertension | 39 (60.9%) | 31 (59.6%) | 49 (57.6%) | 0.92 |
| Diabetes | 22 (34.4%) | 19 (36.5%) | 36 (42.4%) | 0.58 |
| Atrial fibrillation/flutter | 28 (43.8%) | 20 (38.5%) | 39 (45.9%) | 0.69 |
| Stroke | 2 (3.1%) | 2 (3.8%) | 9 (10.6%) | 0.13 |
| Time after LVAD, mo; median (IQR) | 0.6 (0.5–0.8) | 4.5 (3.6–6.0) | 12.1 (9.2–17.6) | <0.001 |
| Laboratory parameters | | | | |
| BUN, mg/dL | 19.0±12.4 | 22.6±10.4 | 24.6±13.3 | 0.01 |
| Serum Cr, mg/dL | 1.2±0.6 | 1.3±0.5 | 1.4±0.8 | 0.01 |
| Serum CysC, mg/L | 1.6±0.6 | 1.6±0.6 | 1.5±0.7 | 0.09 |
| eGFR Cr, mL/min per 1.73 m² | 77.9±33.9 | 67.5±25.5 | 62.0±24.8 | <0.001 |
| eGFR CysCr, mL/min per 1.73 m² | 51.3±22.4 | 48.6±21.0 | 58.1±29.1 | 0.03 |
| NT-proBNP, ng/L; median (IQR) | 2654 (1999–4205) | 1336 (573–2177) | 976 (528–1916) | <0.001 |
| Na, mmol/L | 136.1±4.2 | 140.6±3.0 | 140.2±3.3 | <0.001 |
| AST, U/L | 28.1±14.0 | 27.0±17.5 | 26.9±17.2 | 0.97 |
| ALT, U/L | 29.1±19.2 | 19.1±9.8 | 21.8±12.8 | 0.002 |
| Total bilirubin, mg/dL | 0.8±0.8 | 0.5±0.2 | 0.6±0.3 | 0.01 |
| LDH, U/L | 349.1±114.2 | 301.3±92.7 | 329.3±174.1 | 0.14 |
| Biomarkers | | | | |
| ln-CRP, mg/L | 4.0±0.9 | 1.7±1.0 | 1.8±1.2 | <0.001 |
| ln-IL-6, pg/mL | 3.2±0.8 | 1.7±0.8 | 1.7±0.9 | <0.001 |
| ln-TNF-α, pg/mL | 0.9±0.4 | 0.7±0.4 | 0.6±0.6 | 0.003 |
| ln-ET-1, pg/mL | 0.9±0.5 | 0.7±0.5 | 0.9±0.5 | 0.23 |
| ln-adiponectin, ng/mL | 9.6±0.5 | 9.3±0.6 | 9.2±0.7 | <0.001 |
| ln-isoprostane, pg/mL | 4.3±0.4 | 4.5±0.4 | 4.5±0.5 | 0.04 |
| ln-SCD14, pg/mL | 7.5±0.5 | 7.5±0.3 | 7.4±0.3 | 0.10 |
| LPS, EU/mL | 0.3±0.1 | 0.4±0.2 | 0.4±0.2 | 0.02 |
| Medications | | | | |
| ASA | 56 (87.5%) | 39 (75.0%) | 59 (69.4%) | 0.03 |
| Coumadin | 38 (59.4%) | 47 (90.4%) | 75 (88.2%) | <0.001 |

(Continued)
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(sCD14) and oxidative stress (isoprostane). These associations were lost after multiple adjustments for baseline characteristics and renal indices (M3 and M4).

**Relation Between Circulating TMAO Levels and Gut Microbial Diversity Metrics**

Mean values of the Shannon Index and number of observed ESVs for the entire cohort were 5.24±0.64 and 343.15±192.08, respectively. For individual subgroups, they were as follows: HF, 5.37±0.64 to 391±223; LVAD, 5.23±0.64 to 332±176; and HT, 5.13±0.63 to 313±172, respectively.

After multivariable adjustment for age, sex, race/ethnicity, and antibiotics use (M2), mean levels of the Shannon Index decreased across worsening NYHA class among HF patients; the number of observed ESVs followed the same pattern. For LVAD and HT, eGFR indicates estimated glomerular filtration rate; and TNF-α, tumor necrosis factor alpha.

*Antibiotic use is defined as any antibiotic use that was indicated for infection that was estimated to have occurred 1 mo before stool collection.

Data presented n (%) or mean±SD as appropriate, unless otherwise noted. ACE indicates angiotensin-converting enzyme; ALT, alanine transaminase; ARB, angiotensin receptor blocker; ASA, acetylsalicylic acid; AST, aspartate transaminase; BMI, body mass index; Cr, BUN, blood urea nitrogen; creatinine; CRP, C-reactive protein; CysC, cystatin C; eGFR Cr, creatinine-estimated glomerular filtration rate; eGFR Cys, cystatin C–estimated glomerular filtration rate; ET-1, endothelin-1; IL-6, interleukin-6; IQI, interquartile interval; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; LVAD, left ventricular assist device; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; sCD14, soluble CD14; and TNF-α, tumor necrosis factor alpha.

Figure 1. Comparison of renal function assessment using serum cystatin C–estimated glomerular filtration rate (Cys-eGFR) and serum creatinine–estimated glomerular filtration rate (Cr-eGFR) among heart failure, left ventricular assist device (LVAD), and heart transplant (HT) patients.

*P<0.05 to 0.001, **P=0.001 to 0.0001, ***P<0.0001; all comparisons denoted with any * had a false discovery rate <0.05. eGFR indicates estimated glomerular filtration rate; and NYHA, New York Heart Association.
## Table 3. Baseline Characteristics of HT Patients Providing Blood Samples

| Study cohorts | HT 1 wk | HT 1 mo | HT 3 mo | HT >6 mo | P value |
|---------------|---------|---------|---------|----------|---------|
| No. of samples (n=226) | 29 | 34 | 37 | 126 | |

### Demographic and clinical characteristics

| | HT 1 wk | HT 1 mo | HT 3 mo | HT >6 mo | P value |
|---|---------|---------|---------|----------|---------|
| Age, y | 51.9±12.4 | 51.4±11.3 | 51.3±11.7 | 56.5±12.3 | <0.001 |
| Men | 27 (93.1%) | 31 (91.2%) | 33 (89.2%) | 104 (82.5%) | 0.31 |
| Race | | | | | |
| White | 14 (48.3%) | 16 (47.1%) | 18 (48.6%) | 65 (51.6%) | |
| Black | 10 (34.5%) | 12 (35.3%) | 13 (35.1%) | 39 (31.0%) | |
| Hispanic | 3 (10.3%) | 3 (8.8%) | 3 (8.1%) | 16 (12.7%) | |
| Other | 2 (6.9%) | 3 (8.8%) | 3 (8.1%) | 6 (4.8%) | 0.99 |
| BMI, kg/m²; median (IQR) | NA (NA–NA) | 26.3 (22.5–28.7) | 27.1 (24.4–29.5) | 28.1 (25.5–32.4) | 0.01 |
| Smoking | 13 (44.8%) | 14 (41.2%) | 18 (48.6%) | 58 (46.0%) | 0.94 |
| Etiology, ischemic | 11 (37.9%) | 9 (26.5%) | 13 (35.1%) | 55 (43.7%) | 0.30 |
| Hypertension | 17 (58.6%) | 21 (61.8%) | 25 (67.6%) | 108 (85.7%) | 0.001 |
| Diabetes | 13 (44.8%) | 13 (38.2%) | 13 (35.1%) | 54 (42.9%) | 0.81 |
| Atrial fibr/flutter | 14 (48.3%) | 14 (41.2%) | 15 (40.5%) | 41 (32.5%) | 0.38 |
| Stroke | 5 (17.2%) | 7 (20.6%) | 5 (13.5%) | 15 (11.9%) | 0.59 |
| Time after HT, mo; median (IQR) | 0.3 (0.2–0.3) | 0.7 (0.6–0.9) | 2.7 (1.9–3.6) | 24.2 (7.2–83.0) | <0.001 |

### Laboratory parameters

| | HT 1 wk | HT 1 mo | HT 3 mo | HT >6 mo | P value |
|---|---------|---------|---------|----------|---------|
| BUN, mg/dL | 30.4±15.4 | 32.6±13.1 | 29.6±12.4 | 27.7±11.9 | 0.27 |
| Serum Cr, mg/dL | 1.3±0.6 | 1.4±1.1 | 1.5±1.0 | 1.6±1.2 | 0.03 |
| Serum CystC, mg/L | 1.7±0.7 | 1.6±1.0 | 1.4±0.6 | 1.6±0.9 | 0.11 |
| eGFR<sub>Cr</sub> mL/min per 1.73 m² | 79.3±39.3 | 69.1±29.9 | 64.1±22.2 | 59.1±34.4 | <0.001 |
| eGFR<sub>CysC</sub> mL/min per 1.73 m² | 50.3±24.3 | 54.5±23.7 | 59.6±22.1 | 54.8±24.2 | 0.01 |
| Na, mmol/L | 138.8±3.1 | 139.1±2.9 | 139.8±3.9 | 141.5±2.8 | <0.001 |
| AST, U/L | 24.2±10.3 | 22.0±11.5 | 23.0±8.5 | 24.8±15.3 | 0.58 |
| ALT, U/L | 31.9±28.9 | 33.4±22.7 | 32.6±22.5 | 22.8±13.5 | 0.01 |
| Total bilirubin, mg/dL | 0.6±0.2 | 0.5±0.2 | 0.5±0.2 | 0.6±0.3 | 0.34 |
| LDH, U/L | 603.7±236.4 | 391.0±137.8 | 351.4±125.3 | 251.1±86.3 | <0.0001 |

### Biomarkers

| | HT 1 wk | HT 1 mo | HT 3 mo | HT >6 mo | P value |
|---|---------|---------|---------|----------|---------|
| ln-CRP, mg/L | 2.5±1.4 | 1.3±1.7 | 0.4±1.5 | 1.1±1.3 | <0.001 |
| ln-IL-6, pg/mL | 2.3±1.1 | 1.7±1.2 | 1.2±1.0 | 1.4±1.0 | <0.001 |
| ln-TNF-α, pg/mL | 0.1±0.5 | 0.1±0.4 | 0.3±0.5 | 0.5±0.4 | <0.001 |
| ln-ET-1, pg/mL | 1.0±0.5 | 0.8±0.6 | 0.6±0.6 | 0.7±0.5 | 0.003 |
| ln-adiponectin, ng/mL | 9.7±0.7 | 9.6±0.8 | 9.4±0.6 | 9.3±0.7 | 0.001 |
| ln-isoprostane, pg/mL | 4.2±0.5 | 4.3±0.4 | 4.5±0.5 | 4.5±0.5 | 0.01 |
| ln-sCD14, ng/mL | 7.2±0.5 | 7.2±0.3 | 7.2±0.3 | 7.3±0.4 | 0.03 |
| LPS, EU/mL | 0.5±0.2 | 0.4±0.2 | 0.4±0.1 | 0.4±0.3 | 0.55 |

### Medications

| | HT 1 wk | HT 1 mo | HT 3 mo | HT >6 mo | P value |
|---|---------|---------|---------|----------|---------|
| ASA | 8 (27.6%) | 28 (82.4%) | 35 (94.6%) | 117 (92.9%) | <0.001 |
| Coumadin | 0 (0.0%) | 1 (2.9%) | 0 (0.0%) | 4 (3.2%) | 0.55 |
| ACE inhibitors | 1 (3.4%) | 1 (2.9%) | 0 (0.0%) | 8 (6.3%) | 0.38 |
| ARB | 0 (0.0%) | 0 (0.0%) | 1 (2.7%) | 29 (23.0%) | <0.001 |
| Aldosterone antagonists | 1 (3.4%) | 2 (5.9%) | 0 (0.0%) | 3 (2.4%) | 0.48 |
| β-Blockers | 1 (3.4%) | 4 (11.8%) | 1 (2.7%) | 24 (19.0%) | 0.02 |
| Statins | 12 (41.4%) | 30 (88.2%) | 34 (91.9%) | 102 (81.0%) | <0.001 |
| Loop diuretics | 11 (37.9%) | 13 (38.2%) | 18 (48.6%) | 29 (23.0%) | 0.02 |
| Antibiotics* | 26 (89.7%) | 34 (100.0%) | 35 (94.6%) | 40 (31.7%) | <0.001 |

(Continued)
adjusted mean levels of the Shannon Index increased with time post-LVAD and HT (Figure 4; Table VII in the Data Supplement).

No significant correlation was found between the Shannon Index or number of observed ESVs with ln-TMAO levels for the entire cohort (r=0.07, P=0.21; r=0.02, P=0.71, respectively; Figure VIA and VIB in the Data Supplement). Similarly, no significant correlation was found after adjustment for age, sex, race/ethnicity, antibiotics use, and renal indices (M3 [eGFR Cr; P=0.11 and P=0.46, respectively] and M4 [eGFR Cys; P=0.10 and P=0.37, respectively]; data not shown). In analyses at the individual taxon level, we found no ESVs to be associated with TMAO levels after multivariable adjustment and multiple comparisons correction (data not shown).

### Circulating TMAO as Predictor of Clinical Outcomes in HF and LVAD Patients

Among 112 NYHA class I–III patients, 16 underwent LVAD implant; 3 were transplanted, and 11 died over a median follow-up of 1006 (interquartile range, 609–1278) days. The hazard ratio summarizing the rate of adverse events related to a 1-unit increase in ln-TMAO levels follows and was not statistically significant (HR, 1.24 [CI, 0.84–1.83]; P=0.28). This result remained nonsignificant after adjusting for age and sex (HR, 1.28 [0.85–1.92]; P=0.22).

Among 75 NYHA class IV patients, 49 underwent LVAD surgery; 10 were transplanted, and 10 died during follow-up. Of the 49 LVAD patients, 8 died after implant at a median follow-up of 470 (interquartile range, 335–777) days. The hazard ratio summarizing the rate of death related to a 1-unit increase in ln-TMAO levels follows and was not statistically significant (HR, 1.26 [0.52–3.09]; P=0.60). This result remained nonsignificant after adjusting for age and sex (HR, 1.68 [0.67–4.25]; P=0.27).

### DISCUSSION

The present study compared TMAO levels across a large cohort of patients with systolic HF at various stages of disease progression (NYHA class I–IV) and after treatment with LVAD or HT. The main findings are as follows:

- TMAO levels progressively increased with HF severity and were similarly elevated, long term after LVAD and HT. Notably, these levels were comparable to those previously reported in CVD and CKD.
- TMAO levels inversely related to renal function using both sCr- or CysC-based equations although significant discordance in eGFR between these two methods was observed. Specifically, eGFR Cys was significantly lower than eGFR Cr in advanced HF (class IV), as well as early after LVAD (1 month, 3–6 months) and HT (1 week, 1 month).
- Following LVAD and HT, TMAO levels transiently declined early postoperatively and then progressively increased. These differences in TMAO early after HT were statistically significant after multivariable adjustments that included renal function based on CysC but were meaningfully attenuated when sCr was used.
- TMAO levels positively related to biomarkers of inflammation (TNF-α and ET-1), endotoxemia (sCD14), and oxidative stress (isoprostane), although these associations lost significance after adjustment for baseline characteristics and renal function.
- There was no association between TMAO and gut α-diversity metrics for the entire cohort studied, and this remained unchanged after the abovementioned adjustments.
- Lastly, among a small subset of NYHA class I–III and of class IV HF (pre-LVAD), patients with higher TMAO levels had an empirically higher risk for adverse events, although findings were not statistically significant in these exploratory analyses and require confirmation in future studies.
Elevated TMAO levels have been consistently linked to increased risk of cardiovascular events such as death and myocardial infarction. In HF, circulating TMAO has been shown to be an independent predictor of survival and rehospitalization. The mechanisms implicating TMAO in the pathophysiology of cardiac dysfunction are not fully understood, although in a recent study using a transaortic constriction model of HF, mice fed on a treated high-choline diet had adverse ventricular remodeling and marked increase in cardiac fibrosis. Other factors, such as direct suppressive effects of TMAO on myocardial function through inhibition of actomyosin activity, have also been implicated. However, the observation that TMAO levels remain elevated long term after cardiac replacement therapy, albeit preliminary, are associated with premature mortality after LVAD, has not been reported previously. A single study examined the relationship between TMAO and post-HT–related outcomes such as cardiac allograft vasculopathy. In that report, half of the patients (n=30) were maintained on a CNI regimen with cyclosporine, and half (n=32) were on everolimus-based immunosuppression. Consistent with our findings, TMAO levels were elevated 3 years after HT in patients with CNIs when compared with healthy controls. However, in contrast to our results, a progressive decline in TMAO was observed among HT patients treated with everolimus. This inconsistency might be attributed to inherent differences in patients’ baseline characteristics and immunosuppressive regimen (eg, the majority of our HT patients were maintained on tacrolimus—an alternative CNI). Finally, while the prior report did not find an association between TMAO and cardiac allograft vasculopathy, our study did not examine this relationship due to small sample size and limited longitudinal data. Larger studies are warranted to further test the potential pathogenic and prognostic role of TMAO in this unique population and investigate the differential effects of immunosuppressive regimens on TMAO levels.

It is well established that circulating TMAO levels are inversely related to renal function, and our findings are in agreement with prior reports. TMAO clearance is largely dependent on urinary excretion. However, in animal models, elevated TMAO levels and its primary source, dietary choline, led directly to progressive renal tubule-interstitial fibrosis, while select targeting of TMAO metabolism may prevent or retard CKD development. Advanced HF is commonly associated with significant CKD, the etiology of which is thought to be multifactorial. While hemodynamically driven reduction in renal perfusion is a well-known contributor to CKD, renal dysfunction often persists or continues to progress even after LVAD and HT despite improvement or normalization of hemodynamics. Although it is true that years of

Figure 2. Comparison of trimethylamine N-oxide (TMAO) levels between healthy controls and heart failure (HF), left ventricular assist device (LVAD), heart transplant (HT) patients from the Columbia University Irving Medical Center and from published data from other institutions.

CARDIA indicates Coronary Artery Risk Development Young Adults; CKD, chronic kidney disease; and CVD, cardiovascular disease.
HF may lead to irreversible kidney damage, it is conceivable that after LVAD and HT, an ongoing renal insult is occurring not only from nephrotoxic medications such as CNIs but also from the detrimental effects of circulating gut metabolites like TMAO. Notably, after multiple adjustments (including eGFR\(_{\text{cys}}\)), TMAO levels were at least 2-fold higher in our cohort of long-term LVAD and HT patients compared with those previously reported in

Figure 3. Variation in circulating trimethylamine N-oxide (TMAO) across disease categories of heart failure, left ventricular assist device (LVAD), and heart transplant (HT) patients.

A. Unadjusted ln-TMAO levels (M0). B. Adjusted least squared means: M1: adjusted for age, sex, race/ethnicity; M2: adjusted for model 1 plus antibiotics use 1 mo before stool collection; M3: adjusted for model 2 plus serum creatinine–estimated glomerular filtration rate (eGFR\(_{\text{cys}}\)); M4: adjusted for model 2 plus cystatin C–estimated glomerular filtration rate (eGFR\(_{\text{cys}}\)). *P=0.05 to 0.01, **P=0.01 to 0.001, ***P<0.001. NYHA indicates New York Heart Association.
healthy controls, potentially translating in sustained and progressive nephrotoxicity.

The concentration of sCr is influenced by changes in muscle metabolism and protein intake, which are prevalent in advanced HF and early after LVAD or HT. Conversely, CysC is not affected by muscle mass or diet and is less impacted by age, sex, and race than sCr and, therefore, is the renal biomarker recommended to be used in advanced disease states. Our study was the first to analyze the association of TMAO with
both eGFR\(_{\text{Cr}}\) and eGFR\(_{\text{Cys}}\) in a large cohort of HF, LVAD, and HT patients. Not surprisingly, the eGFR was consistently higher across all study groups when calculated using sCr rather than CysC. We also observed a transient decline in TMAO levels early after LVAD and HT that remained significant only after adjustment for eGFR\(_{\text{Cys}}\) rather than eGFR\(_{\text{Cr}}\) in HT patients. Dietary changes and antibiotic use for prophylaxis or treatment of infections during the early postoperative period may account, at least partially, for these findings. Wang et al\(^{45}\) pointed to a major contributing role for the intestinal microbiota in the production of TMAO through its suppression by means of oral antibiotics and then reacquisition of TMAO production from dietary phosphatidylcholine after withdrawal of antibiotics and subsequent intestinal recolonization. More generally, antibiotics have been shown to
alter the gut microbiota and reduce community diversity in the days and weeks following treatment. However, these alterations are ephemeral, and while recovery may be incomplete, diversity shifts back to partially resemble pretreatment microbial community structure within 1 to 6 months.\(^{46,47}\) Our results are in agreement with these findings, as they indicate that the observed declines in TMAO levels are accompanied by transient declines in standard metrics of gut microbial diversity, such as the Shannon Index and number of observed ESVs, early after LVAD and HT surgery. Interestingly, no significant correlation was observed between microbial diversity metrics and TMAO levels. This is not surprising as gut microbial diversity metrics are broad and reflect community characteristics but do not precisely capture nuanced variation in the taxa that are responsible for TMAO production (only a subset of taxa express TMA lyases).\(^{48}\) Our lack of findings even at the individual taxa level could be due to low power in our current study or lack of metagenomic or strain level information necessary to more directly interrogate the functional capacity of the microbiota to produce TMAO. Future mechanistic studies are warranted to test these hypotheses more rigorously.

The relationship between TMAO and markers of inflammation and endotoxemia, which are commonly associated with severity and progression of HF, has been elusive. Troseid et al\(^{35}\) reported on the prognostic relevance of TMAO among chronic HF patients and, concordant with our results, found a graded increase in unadjusted TMAO as the disease severity rises. Although the levels of endotoxemia (lipopolysaccharide) and inflammation (CRP) were elevated among these patients, no association with TMAO was found. Similarly, in chronic HF patients, Tang et al\(^{11}\) did not find any correlations between TMAO and other substrates of TMAO formation (choline and betaine) with hsCRP. Our findings are in agreement with these reports, as we also show no significant correlation between TMAO and hsCRP or IL-6. In addition, while TMAO levels were associated with other biomarkers of systemic inflammation, endotoxemia, and oxidative stress (TNF-α, ET1, sCD14, and isoprostane), these associations lost significance when baseline characteristics and renal function were accounted for. Although these results suggest that TMAO might be a surrogate marker of renal dysfunction or other comorbidities, we cannot exclude that this metabolite has direct proinflammatory and even cardio- and nephrotoxic effects based on mechanisms shown in animal models (see above).

Some limitations should be acknowledged. No dietary intake (including red meat and fish that may influence TMAO levels) or other behavioral variables were collected. The use of food frequency questionnaire should be included in future studies aiming to provide knowledge on habitual dietary intake over a longer period. We did not directly measure GFR using renal clearance of iothalamate or iohexol, thereby limiting our ability to definitively conclude which filtration marker provides the most accurate eGFR. However, several reports indicate that CysC outperforms sCr, when compared with the above gold standards, in other patient populations that are relevant to the present study such as HF, CKD, and cardiovascular surgery. Although several inflammatory biomarkers associated with HF severity were studied, IL-1 was not part of our panel. There is a clear difference in medication use among groups. As expected, HT patients adhere to an immunosuppression regimen, and we observed an uneven distribution of HF medication utilization among NYHA class IV and LVAD (1 month) patients, likely due to reduced tolerability to these drugs. Notably, concurrent medications, including statins and immunosuppressive agents such as mycophenolic acid, corticosteroids, and everolimus, may alter the gut microbiota and affect TMAO production.\(^{49,50}\) Finally, the observational nature of our study precludes any inferences regarding causality. Prospective studies, controlled for the differential effects of drugs, are warranted to address all these key issues.

In conclusion, we demonstrated that long-term LVAD or HT therapy does not normalize TMAO levels in HF patients. There were clear discrepancies in estimation of GFR using sCr versus CysC, particularly in the early post-operative period (LVAD and HT), that meaningfully influenced interpretation of TMAO results. TMAO levels do not correlate with biomarkers of inflammation, endotoxemia, and oxidative stress after adjustment for renal function. No relationship was found between TMAO and established metrics of microbial gut diversity. Whether pharmaceutical and nutritional interventions that modify TMAO levels may improve outcomes in HF, LVAD, and HT patients remains to be investigated.

**ARTICLE INFORMATION**

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