Combined use of trimethylamine N-oxide with BNP for risk stratification in heart failure with preserved ejection fraction: findings from the DIAMONDHFpEF study

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Circulating levels of Trimethylamine N-oxide (TMAO), a gut microbiome-mediated metabolite related to Western diet[1, 2], have been shown to be associated with risk stratification and outcome in patients with heart failure (HF) with reduced ejection fraction (HFrEF)[3-6]. The aim of the present study was to assess the associations between TMAO with outcomes in patients with HF with preserved ejection fraction (HFpEF).

To investigate the recently identified link between the gut and HF (namely: "gut hypothesis"), TMAO levels were measured in 118 patients with HFpEF, 38 patients with HFrEF, and 40 healthy volunteer participants (sex/age matched) with available baseline plasma samples from the Developing Imaging And plasMa biOmarkers iN Describing Heart Failure With Preserved Ejection Fraction (DIAMONDHFpEF) cohort, a prospective, observational, single-centre study aimed at developing imaging and plasma biomarkers of novel pathophysiological patterns in HFpEF [NCT03050593][7]. Plasma levels of TMAO were measured using liquid chromatography-tandem mass spectrometry, a high throughput, reproducible, and accurate method[3, 5, 8]. BNP was measured using a commercial immunoassay (Siemens, Erlangen, Germany). All patients had blood tests, transthoracic echocardiography (TTE, Philips iE33, Amsterdam, Netherlands), and cardiac magnetic resonance (CMR) imaging (Siemens Skyra Erlangen, Germany) during the same visit. Primary outcomes were defined as the composite endpoint of all-cause mortality or hospitalisation for HF at 18 months (short-term) and at 60 months (long-term).

Baseline patient demographics, blood chemistry, echocardiographic, and CMR measurements are shown in Table 1. Patients with HFpEF had higher body mass index (BMI), higher prevalence of systemic hypertension and atrial fibrillation, and less coronary artery disease. HFpEF patients had lower BNP levels as compared to HFrEF patients [140 (55-252) pg/mL vs. 345 (167-605) pg/mL, $p<0.001$]. HFrEF patients showed higher end-diastolic [140.8 (107.8-164.9) ml/m^2 vs 77.2 (65.9-91.9) ml/m^2] and end-systolic [97.8 (68.7-128.5) ml/m^2 vs 33.1 (27.4-41.9) ml/m^2] volumes on CMR ($p<0.01$), and higher LV filling pressures (E/e' ratio) [13.1 (11.5-19.9) vs. 11.5 (9.1-16.6), $p=0.023$] on TTE (Table 1). HF patients showed elevated circulating TMAO levels when compared to sex/age matched healthy controls [HFpEF 6.6 (4.3-12.2) µmol/L, adj. $p=0.003$; HFrEF 8.4 (3.7-13.8) µmol/L, adj. $p=0.006$; vs. control 4.0 (3.2-5.3) µmol/L], but no differences were observed between HF phenotypes. TMAO was positively

correlated with age, BMI, E/e', and BNP levels, and negatively correlated with eGFR (Table 1). A total of 27 events (13 deaths and 14 HF hospitalisation) were recorded in the HFpEF cohort over a follow-up of 18 months (short-term), and 55 events (32 deaths and 23 hospitalisation) over a follow up of 60 months (long-term). For short-term outcomes, Cox proportional hazards regression showed that HFpEF patients with a circulating TMAO level exceeding a cut-off of 5 µmol/L (derived from the upper quartile of the control population in this study as a whole integer) showed a 4-fold increase in risk of an event [HR (95% CI) 3.82 (1.15-12.69), p=0.029]. TMAO remained significantly associated with outcome when adjusted for age, sex, BMI, and eGFR [3.66 (1.03-13.04) p=0.045]. However, TMAO was not significantly associated with events as a continuous, univariate marker (p=0.109). For long-term outcomes, at the cut-off point of 5 µmol/L, TMAO was significantly associated with outcomes [2.74 (1.34-5.61) p=0.006], and showed associations when adjusted for confounders [2.45 (1.12-5.35) p=0.025]. As a continuous variable, TMAO was associated with outcome as a univariate marker [1.98 (1.11-3.55) p=0.021], but did not retain this when adjusted for confounders. When mortality was investigated, as a continuous variable, TMAO was significantly associated with outcome both as a univariate marker [2.40 (1.11-5.18) p=0.025], and after adjustment [3.53 (1.34-9.33) p=0.11]. As elevation of BNP levels is less pronounced in HFpEF patients, Kaplan-Meier survival analyses were performed to compare the use of TMAO as a predictor of outcome in HFpEF patients with low BNP levels (cut-off value of 140 pg/mL and 250 pg/mL, derived from the ESC guidelines [9]) and compared to those with more pronounced elevations of circulating BNP (≥140 pg/mL and ≥250 pg/mL) (Figure 1). When all HFpEF patients were stratified by TMAO and BNP levels, those with lower levels of both biomarkers reported the greatest survival, with a gradual increase of risk when one or both biomarkers were elevated for short- (log-rank test, p≤0.004, Figure 1A and 1C) and long-term outcomes (log-rank test, p≤0.010, Figure 1B and 1D). When compared to patients with lower levels of both biomarkers, BNP alone, when stratified at 140 pg/mL, showed significant changes in survival for short-term (p=0.04), but not long-term (p=0.11) outcomes, whereas TMAO alone, when stratified at 5 µmol/L, showed significant changes in survival for both short- and long-term outcomes (p≤0.04, Figure 1A and 1B). When stratified at 250 pg/mL, both BNP alone and TMAO alone showed significant changes in survival for both outcomes (p≤0.03, Figure 1C and 1D).

The present study indicates that TMAO levels were elevated to a similar extent in HFpEF and HFrEF patients in comparison to cardiovascular disease-free participants, in line with a previous report[10]. Furthermore, our findings suggest the possible use of TMAO for risk stratification of long-term mortality. In addition, our results support the hypothesis that TMAO levels could aid in stratifying HFpEF patients who would otherwise be classified as low-risk based on BNP levels. Indeed, when a cut-off level was used for TMAO, associations with adverse outcomes in HFpEF patients in which BNP, the 'gold standard' biomarker in HFrEF, was less elevated were observed. TMAO levels also increased with higher LV filling pressure and BNP levels, suggesting association with worsening of HF with progressive diastolic dysfunction, in line with a previous report showing association between TMAO levels, HF severity, and diastolic dysfunction[11]. To date, only one study has investigated the prognostic value of TMAO in HFpEF[10], with inconclusive results. The present report suggests a role for TMAO in the clinical management of HFpEF patients, allowing for better risk stratification, unachievable with BNP alone[12, 13]. Further, therapeutic intervention of the gut microbiome may offer potential additive treatments for HF. Our data supports the notion of combined multimarker strategies, in particular if circulating and imaging biomarkers from different pathophysiological patterns are combined[14]. As study limitations, patients were recruited from a single centre with a relatively small sample size that does not allow extensive prediction modelling to be performed. In addition, data regarding dietary intake and antibiotic treatment that could influence TMAO levels were not available.

In conclusion, as natriuretic peptides are not as highly elevated in HFpEF compared to HFrEF, elevated circulating levels of TMAO may provide utility in risk stratification of HFpEF where this and other biomarkers show equivocal levels. Therefore, the combined use of BNP and TMAO may be useful in patients with HFpEF.

Conflict of interest statement

The authors declare no conflicts of interest.

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Author Contribution

AS and MZI contributed to conception, design, data acquisition, analysis, and interpretation of the study, drafted and critically revised the manuscript. YY contributed to data analysis and interpretation, and critically revised the manuscript. LMH contributed to conception, data acquisition and analysis of the study, and critically revised the manuscript. ASi contributed to design, data acquisition and interpretation of the study, drafted and critically revised the manuscript. PK, AJR, GGS contributed to the design, data acquisition and interpretation of the study and critically revised the manuscript. IBS, MGP, NLL contributed to the conception and design of the study, data acquisition and interpretation, and critically revised the manuscript. TS contributed to the conception, design, data analysis and interpretation of the study and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work ensuring integrity and accuracy.

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Table 1. Patient demographics.

	HF (n=156)	HFpEF (n=118)	HFrEF (n=38)	Control (n=40)	HFpEF vs. HFrEF	Correlation with TMAO full population (rs)	p value
Demographics							
Age (years)	73 (67-78)	73 (65-78)	72 (62-77)	72 (69-77)	ns	0.154	0.031
Male (%)	49	49	50	53%	ns	-	-
Body Mass Index (kg/m^2)	32.2 (26.4-38.5)	33.8 (28.4-39.7)	27.5 (24.7-32.3)	-	< 0.001	0.158	0.027
NYHA (I/II/III/IV)	53/59/40/4	37/46/31/4	16/13/9/0	-	ns	-	-
Hypertension (%)	81	91	54	-	<0.001	-	-
Coronary Artery Disease (%)	29	21	50	-	<0.001	-	-
Diabetes Mellitus (%)	46	49	39	-	ns	-	-
Atrial Fibrillation (%)	38	79	24	-	0.017	-	-
eGFR (mL/min/1.73m^2)	68 (48-85)	69 (50-85)	65 (48-78)	-	ns	-0.527	<0.001
CMR							
LVEDVi (ml/m^2)	85.9 (69.6-106.8)	77.2 (65.9-91.9)	140.8 (107.8-164.9)	80.4 (72.34-89.6)	<0.001	0.028	0.736
LVESVi (ml/m^2)	39.2 (29.7-57.6)	33.1 (27.4-419)	97.8 (68.7-128.5)	32.6 (29.2-37.8)	<0.001	0.005	0.953
LVEF (%)	52.8 (41.8-58.6)	55.4 (51.8-60.1)	28.3 (20.7-36.4)	57.3 (55.4-59.77)	<0.001	-0.001	0.991
Echocardiography							
E/e’	12.3 (9.7-19.9)	11.5 (9.1-16.6)	13.1 (11.5-19.9)	-	0.023	0.287	<0.001
Biomarkers							
BNP (pg/mL)	170 (80-336)	140 (55-252)	345 (167-605)	-	<0.001	0.221	0.006
TMAO (µmol/L)	7.0 (4.2-12.5)	6.6 (4.3-12.2)	8.4 (3.7-13.8)	4.0 (3.2-5.3)	ns	-	-

HF = heart failure; HFpEF = heart failure with preserved ejection fraction; HFrEF = heart failure with reduced ejection fraction; TMAO = trimethylamine N-oxide; BNP = B-type natriuretic peptide; NYHA = New York Heart Association functional class; eGFR = estimated glomerular filtration rate; LVEDVi: Left ventricular end diastolic volume indexed; LVESVi: Left ventricular end systolic volume indexed; LVEF = left ventricular ejection fraction; E/e'= Peak modal transmitral flow velocity in early diastole / Pulse wave tissue Doppler imaging peak modal velocity in early diastole; r_s = Spearman's rho. Data expressed as median (IQR) for continuous variables and % of total for categorical variables.

Figure 1.

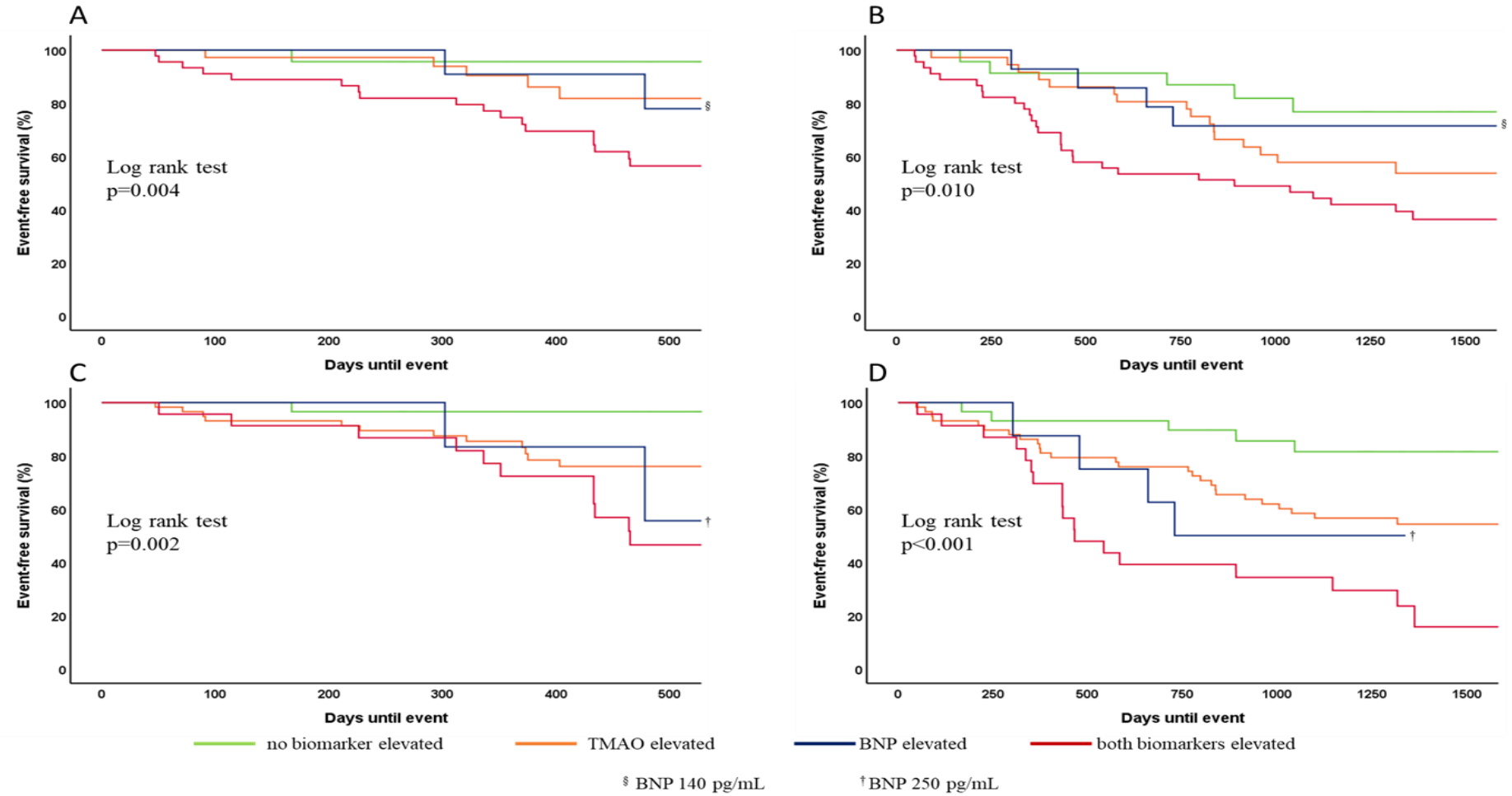
A
Event-free survival (%)
Log rank test
p=0.004
§
Days until event
B
Log rank test
p=0.010
§
Days until event
C
Log rank test
p=0.002
†
Days until event
D
Log rank test
p<0.001
†
Days until event
no biomarker elevated
TMAO elevated
BNP elevated
both biomarkers elevated
§ BNP 140 pg/mL
† BNP 250 pg/mL

Figure 1. Kaplan-Meier analysis of survival at 18 months (short term), **(A)** and **(C),** and 60 months (long-term), **(B)** and **(D),** for all-cause mortality or heart failure hospitalisation in patients with preserved ejection fraction (HFpEF) with neither biomarker elevated, only trimethylamine N-oxide (TMAO) elevated (5 µmol/L), only B-type natriuretic peptide (BNP) elevated (>140 pg/mL in **(A)** and **(B)** or >250 pg/mL in **(C)** and **(D)**) or both biomarkers elevated.