

Ethnic differences in association of outcomes with trimethylamine N-oxide in acute heart failure patients

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Abstract

Aims The aim of this study was to investigate whether ethnicity influences the associations between trimethylamine N-oxide (TMAO) levels and heart failure (HF) outcomes.

Methods and results Trimethylamine N-oxide levels were measured in two cohorts with acute HF at two sites. The UK Leicester cohort consisted mainly of Caucasian ($n = 842$, 77%) and South Asian ($n = 129$, 12%) patients, whereas patients in the Japanese cohort ($n = 116$, 11%) were all Japanese. The primary endpoint was the measurement of all-cause mortality and/or HF rehospitalization within 1 year post-admission. Association of TMAO levels with outcome was compared in the entire population and between ethnic groups after adjustment for clinical parameters. TMAO levels were significantly higher in Japanese patients [median (interquartile range): 9.9 μM (5.2–22.8)] than in Caucasian [5.9 μM (3.6–10.8)] and South Asian [4.5 μM (3.1–8.4)] ($P < 0.001$) patients. There were no differences in the rate of mortality and/or HF rehospitalization between the ethnic groups ($P = 0.096$). Overall, higher TMAO levels showed associations with mortality and/or rehospitalization after adjustment for confounders ($P = 0.002$). Despite no differences between ethnicity and association with mortality/HF after adjustment ($P = 0.311$), only in Caucasian patients were TMAO levels able to stratify for a mortality/HF event ($P < 0.001$).

Conclusions Differences were observed in the association of mortality and/or rehospitalization based on circulating TMAO levels. Elevated TMAO levels in Caucasian patients showed increased association with adverse outcomes, but not in non-Caucasian patients.

Keywords Heart failure; Ethnicity; TMAO; Gut metabolite; Outcomes

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Introduction

The oxidized gut microbiome-derived metabolite, trimethylamine N-oxide (TMAO), has been linked with outcomes in patients with heart failure (HF), with elevated circulating TMAO levels showing association with poor outcomes (mortality/rehospitalization).^{1–8} However, these previous studies enrolled predominantly Caucasian patients, and ethnic influences on the association of TMAO with outcomes have not yet been investigated.^{1–8} The aim of the current study was to investigate whether ethnic

differences influence associations between TMAO levels and HF outcomes.

Methods

Study population

Trimethylamine N-oxide levels were measured in patients with acute HF at two sites (University Hospitals of Leicester,

Glenfield Hospital, UK, and Jichi Medical University Hospital, Shimotsuke, Tochigi, Japan) with identical protocols for sample collection, TMAO measurements, and data management.¹ The UK Leicester cohort has been previously published¹ and consists of two main ethnic groups of Caucasian ($n = 842$, 87%) and South Asian ($n = 129$, 13%) patients, whilst the patients in the acute HF cohort at Jichi Medical University Hospital were all Japanese. The study was approved by the local ethics committee at each participating centre and complied with the Declaration of Helsinki.

Statistical analyses

The primary endpoint was a composite of all-cause mortality and/or rehospitalization (mortality/HF) due to HF within 1 year post-admission. Demographic, laboratory, and clinical data were compared with ethnicity, using the Mann–Whitney U test for continuous variables and the χ^2 test for categorical variables. The Kruskal–Wallis test was used to compare group

differences between the ethnic groups and TMAO levels. B-type natriuretic peptide (BNP) and N-terminal (NT)-prohormone BNP (NT-proBNP) levels were measured using different natriuretic peptide assays in the two cohorts and were therefore log normalized and then z-transformed (normalized to 1 standard deviation) along with TMAO levels for each cohort before analysis. A Cox proportional hazards regression model was used to analyze the associations between ZlogTMAO levels and study outcomes in each ethnic group after adjustment for age, sex, previous history of HF, ischaemic heart disease, hypertension, diabetes mellitus, New York Heart Association class, systolic blood pressure, blood sodium levels, renal function by estimated glomerular filtration rate, haemoglobin, and standardized natriuretic peptide (NT-proBNP or BNP). Interaction analysis was performed to assess whether the association between TMAO levels and mortality/HF differed amongst subgroups stratified according to baseline characteristics. Kaplan–Meier survival curves were generated and the Mantel–Cox log-rank test was used to compare the

Table 1 Baseline patient characteristics according to ethnicity

	Total ($n = 1087$)	Caucasian ($n = 842$)	South Asian ($n = 129$)	Japanese ($n = 116$)	P value
TMAO ($\mu\text{mol/L}$)	5.9 (3.5–11.4)	5.9 (3.6–10.8)	4.5 (3.1–8.4) ^b	9.9 (5.2–22.8) ^a	<0.001
Demographics					
Age	77 (69–83)	79 (71–85)	71 (62–78) ^b	74 (67–81) ^b	<0.001
Male	594 (61%)	508 (60%)	86 (67%)	87 (67%)	0.207
Prior HF	364 (35%)	282 (34%)	44 (34%)	38 (35%) ^a	0.962
Ischaemic heart disease	322 (30%)	225 (27%)	58 (45%) ^a	39 (34%)	<0.001
Hypertension	641 (59%)	480 (57%)	86 (67%) ^a	75 (65%)	0.039
Diabetes mellitus	328 (34%)	261 (31%)	67 (52%) ^a	42 (36%)	<0.001
Dyslipidaemia	274 (25%)	202 (24%)	35 (27%)	37 (32%)	0.156
Atrial fibrillation	491 (45%)	418 (50%)	23 (18%) ^b	50 (43%)	<0.001
NYHA class IV	534 (54%)	451 (54%)	73 (59%)	35 (30%) ^b	<0.001
LV ejection fraction (%)	35 (25–48)	35 (26–48)	34 (23–48)	34 (26–49)	0.782
Clinical signs					
Systolic blood pressure (mmHg)	132 (115–150)	133 (115–150)	135 (116–155)	126 (105–150)	0.240
Diastolic blood pressure (mmHg)	75 (65–85)	74 (65–85)	74 (65–85)	81 (66–94) ^a	0.023
Heart rate (beat/min)	90 (74–106)	88 (74–106)	90 (73–102)	92 (76–112)	0.310
Medication					
Aspirin	458 (42%)	363 (43%)	64 (50%)	31 (28%) ^b	0.001
Beta-blocker	445 (41%)	345 (41%)	57 (45%)	43 (38%)	0.617
ACE inhibitor or ARB	587 (54%)	457 (54%)	70 (54%)	60 (54%)	0.990
Diuretics	647 (60%)	505 (60%)	81 (63%)	61 (55%)	0.371
Laboratory					
Urea (mmol/L)	8.9 (6.5–12.6)	9.0 (6.6–12.7)	8.1 (6.2–12.5)	8.9 (6.4–11.6)	0.383
eGFR (mL/min/1.73 m^2) ^c	49 (36–66)	48 (35–64)	51 (37–68)	56 (36–82) ^a	0.026
Sodium (mmol/L)	138 (135–141)	138 (135–141)	137 (135–140)	140 (137–142) ^a	0.001
Haemoglobin (g/dL)	12.3 (10.8–13.7)	12.4 (10.9–13.8)	11.9 (10.5–13.0) ^b	11.9 (10.7–13.4)	0.012
NT proBNP (pg/mL)	–	2123 (996–3946)	2103 (833–3454)	–	–
BNP (pg/mL)	–	–	–	654 (355–1095)	–
z-transformed log natriuretic peptide	0.17 (–0.43–0.62)	0.18 (–0.40–0.66)	0.18 (–0.53–0.56)	0.05 (–0.59–0.59)	0.460
Outcomes					
Mortality at 1 year	281 (26%)	243 (29%)	25 (19%) ^b	13 (11%) ^b	<0.001
Mortality/HF at 1 year	418 (39%)	332 (39%)	52 (41%)	34 (29%)	0.096

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; HF, heart failure; LV, left ventricular; NT-proBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association; TMAO, trimethylamine-N-oxide. Data are expressed as median (interquartile range) for continuous variables or n (%) for categorical values. Categorical variables were analyzed with χ^2 tests. Continuous variables were analysed with Mann–Whitney U tests.

^aSignificantly higher compared with Caucasian.

^bSignificantly lower compared with Caucasian pairwise analyses.

^cEstimated by Chronic Kidney Disease Epidemiology Collaboration formula.

differences with survival of TMAO levels when stratified by the population median and ethnicity median.

Results

Patient demographics and clinical parameters associated with trimethylamine N-oxide

In total, 1087 patients with acute HF were measured for TMAO levels; of these, 842 (77%) were classified as Caucasian, 129 (12%) as South Asian, and 116 (11%) as Japanese (Table 1). TMAO levels were significantly higher in Japanese patients compared with Caucasian or South Asian patients [median (interquartile range): 9.9 (5.2–22.8), 5.9 (3.6–10.8), and 4.5 μM (3.1–8.4) for the Japanese, Caucasian, and South Asian patients, respectively, $P < 0.001$] (Figure 1). The Caucasian patients were significantly older than the Japanese and South Asian patients. The South Asian patients had higher prevalence of ischaemic heart disease, hypertension, and diabetes mellitus, but lower prevalence of atrial fibrillation. The estimated glomerular filtration rate in Japanese patients was significantly higher than in Caucasian and South Asian patients [56 (mL/min/1.73 m²) (36–82), 48 (mL/min/1.73 m²) (35–64), and 51 (mL/min/1.73 m²) (37–68), respectively, $P = 0.026$]; however, there were no differences in the normalized natriuretic peptide distributions amongst the ethnic groups (Table 1).

Trimethylamine N-oxide levels as a predictor of mortality/heart failure at 1 year

The mortality rate at 1 year in Caucasian patients was the highest amongst the ethnic groups ($P < 0.001$) whilst for mortality/HF rate at 1 year, no significant differences were observed between the ethnic groups ($P = 0.096$; Table 1).

Higher TMAO levels were significantly associated with mortality/HF for univariate (hazard ratio: 1.28, 95% confidence interval: 1.17–1.40, $P < 0.001$) and multivariable analyses in the entire population (unadjusted $P < 0.001$, adjusted $P = 0.002$) (Table 2). When ethnicity was considered as univariate model, there were no observed differences for associations with mortality/HF between Caucasian and South Asian patients ($P = 0.823$); however, there were differences with Japanese patients ($P = 0.042$), with Japanese patients associated with a lower risk of mortality/HF. However, as multivariate models, there were no observed differences across the ethnic groups and associations with mortality/HF (unadjusted $P = 0.057$, adjusted $P = 0.311$) (Table 2). Kaplan–Meier survival analysis also showed no significant differences for outcome between South Asian and Japanese patients ($P = 0.068$) and between Caucasian and South Asian patients ($P = 0.822$). However, a significant difference was observed between Caucasian and Japanese patients ($P = 0.041$) (Figure 2).

Interactions between ethnicity group and TMAO levels were investigated and showed no significant interaction between the full population (ethnicity groups) and TMAO levels

Figure 1 TMAO levels in patients with acute heart failure according to ethnicity. Box and whisker plot to show the distribution of TMAO levels adjusted for age, sex, and estimated glomerular filtration rate across different ethnicity groups. Boxes indicate median and interquartile range, and whiskers indicate 10th and 90th percentiles. All P values were adjusted for multiple comparisons with Bonferroni correction. TMAO, trimethylamine-N-oxide.

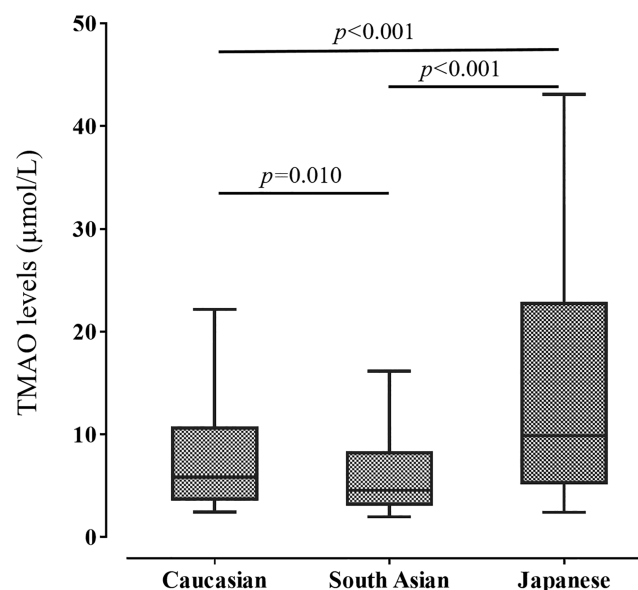


Table 2 Cox regression model for all-cause death and/or hospitalization due to heart failure

	Unadjusted		Adjusted ^a	
	HR (95% CI)	P value	HR (95% CI)	P value
Univariate model				
logTMAO	1.28 (1.17–1.40)	<0.001		
Ethnic group				
Caucasian	Reference			
South Asian	1.03 (0.77–1.39)	0.823		
Japanese	0.69 (0.49–0.99)	0.042		
Multivariate model				
logTMAO		<0.001		0.002
Ethnicity group		0.057		0.311
Ethnic group*logTMAO		0.408		0.125
Caucasian*logTMAO	1.38 (1.24–1.53)		1.23 (1.08–1.40)	
South Asian*logTMAO	1.15 (0.86–1.55)		0.88 (0.61–1.25)	
Japanese*logTMAO	1.21 (0.93–1.57)		1.02 (0.77–1.35)	

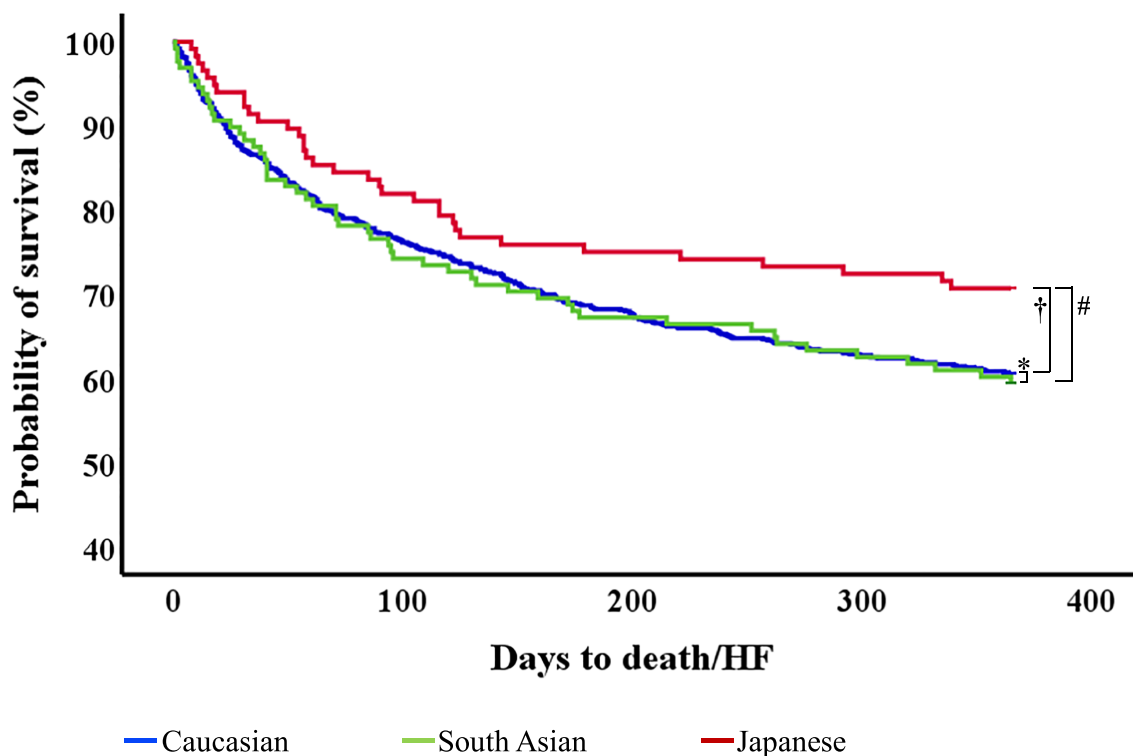
CI, confidence interval; HR, hazard ratio; TMAO, trimethylamine-N-oxide.

^aAdjusted for age, sex, previous history of heart failure, ischaemic heart disease, hypertension, diabetes, systolic blood pressure, New York Heart Association class, sodium, haemoglobin, estimated glomerular filtration rate, and standardized natriuretic peptide.

after adjustment (unadjusted $P = 0.408$, adjusted $P = 0.125$). When each ethnic group was considered, the hazard ratios of TMAO with mortality/HF were similar across the three ethnic groups (hazard ratio unadjusted 1.15–1.38, adjusted 0.88–1.23) (Table 2).

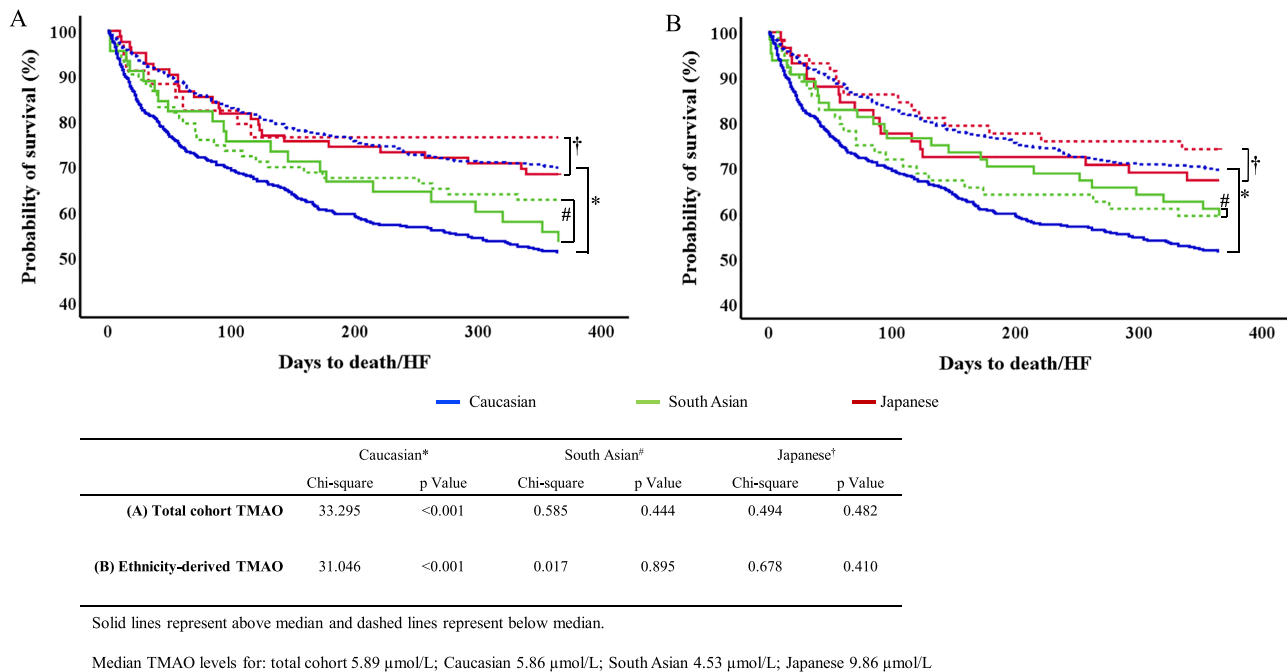
Survival analysis showed that when the median TMAO level for the total population was considered, Caucasian patients showed increasing incidence of event for all-cause death and/or rehospitalization due to HF with elevated circulating levels ($P < 0.001$), whereas Japanese and South Asian

Figure 2 Kaplan–Meier curve showing the relationship of ethnicity and all-cause death and/or rehospitalization due to heart failure at 1 year. *Log rank $P = 0.822$; † $P = 0.041$; # $P = 0.068$. HF, heart failure.



Log rank * $p=0.822$; † $p=0.041$; # $p=0.068$

Figure 3 Kaplan–Meier curve showing the relationship of each ethnic group and all-cause death and/or rehospitalization due to heart failure at 1 year after stratification by TMAO median. HF, heart failure; TMAO, trimethylamine-N-oxide.



patients showed no associations between TMAO and survival ($P \geq 0.444$) (Figure 3A). Furthermore, when TMAO was stratified by the median for each ethnic group, we again found that Caucasian patients showed increasing incidence of event with elevated circulating levels ($P < 0.001$), whereas Japanese and South Asian patients showed no associations between TMAO and mortality/HF event ($P \geq 0.410$) (Figure 3B). Similar results were observed for mortality (Supporting Information, Figures S1A and S2) and rehospitalization due to HF (Supporting Information, Figures S1B and S3).

Discussion

Trimethylamine N-oxide levels in patients with acute HF differed by ethnicity. Caucasian and non-Caucasian patients presented with a similar probability of survival for mortality/HF; however, when dichotomized by TMAO median, only Caucasian patients showed the ability to be stratified based on elevated TMAO levels but not in Japanese and South Asian patients. In conjunction with previous findings, TMAO shows ethnicity-selective contributions with tailored risk selective to Caucasian patients demonstrating that TMAO measurements, at least in our investigated cohorts, are applicable for risk associations with adverse events in Caucasian but not in non-Caucasian patients.

Trimethylamine N-oxide levels are known to be affected by diet/lifestyle, with consumption of choline-rich/carnitine-rich

foods (e.g. fish and red meat) being associated with higher TMAO levels^{9,10} and vegetarians showing lower levels.¹¹ Although diet was not investigated in the present cohort, our findings might reflect cultural and ethnic differences in dietary contributions.¹² Different associations for cardiovascular disease between Caucasians and Asians have been previously reported,¹³ and our findings are consistent with these. A previous study has also shown that the association of TMAO levels with cardiovascular risk differed between Caucasian and Blacks in haemodialysis patients,¹⁴ with a linear increase in adverse events in Caucasian patients, but not in Blacks.

We did not have any information regarding the dietary records and gut microbiota composition to investigate impact on TMAO levels, which is a limitation of the present study. Differences in the standard of care between the UK and Japanese cohorts may have also contributed.

In conclusion, ethnic differences affect TMAO levels and their risk stratification with adverse outcomes in patients with acute HF. Our findings add to our present understanding of outcomes associated with acute HF through the identification of a hitherto unknown ethnicity-selective contribution of the gut microbiome through TMAO.

Conflict of interest

None declared.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Supporting Information

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