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ARTICLE

Elevated trimethylamine *N*-oxide related to ischemic brain lesions after carotid artery stenting

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Abstract

Objectives

To investigate whether the plasma level of trimethylamine *N*-oxide (TMAO), a proatherosclerotic intestinal microbiota metabolite, can be a predictor of ischemic brain injury secondary to carotid artery stenting (CAS).

Methods

In this multicenter, prospective cohort study, we enrolled patients with severe carotid artery stenosis (>70%) who were prepared for CAS. Plasma TMAO level was measured within 3 days before CAS, and MRI was performed 1 to 3 days after CAS.

Results

The mean age of the 268 eligible patients was 64.4 years. New lesions on diffusion-weighted imaging (DWI) were detected in 117 patients (43.7%). TMAO level was higher in patients with new (DWI) lesions than in patients without new lesions (median 5.2 vs 3.2 μmol/L; $p < 0.001$). Increased plasma TMAO levels were associated with an increased risk of having new lesions on DWI after CAS (adjusted odds ratio for the highest vs lowest quartile, 3.85; 95% confidence interval, 1.37–7.56, $p < 0.001$; adjusted odds ratio for the third vs lowest quartile, 1.86; 95% confidence interval, 1.09–4.66, $p = 0.02$). The area under the receiver operating characteristic curve of TMAO was 0.706 for new lesions on DWI, and the optimal cutoff value was 4.29 μmol/L. The sensitivity, specificity, positive predictive value, and negative predictive value of TMAO levels ≥4.29 μmol/L for predicting new lesions on DWI were 61.5%, 74.8%, 65.5%, and 65.5%, respectively.

Conclusions

Increased TMAO levels are associated with an increased risk of new ischemic brain lesions on post-CAS MRI scans.

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Glossary

CAS = carotid artery stenting; **CEA** = carotid endarterectomy; **CI** = confidence interval; **DWI** = diffusion-weighted imaging; **EPD** = embolic protection device; **IQR** = interquartile range; **OR** = odds ratio; **TMAO** = trimethylamine *N*-oxide.

Carotid artery stenting (CAS) is a therapeutic alternative to carotid endarterectomy (CEA) for the treatment of severe cervical carotid artery stenosis,[1,2] which is responsible for up to 20% of cases of stroke.[3] New ischemic lesions occur in approximately half of the patients treated with CAS,[4,5] which may weaken the therapeutic benefits, and cause cognitive impairment even without a corresponding focal deficit.[6,7] CAS causes more ischemic brain lesions than CEA,[8] and an increasing number of patients have been receiving CAS in recent years because of more evidence showing its efficacy.[9–11] Thus, identifying patients who are at risk of new ischemic lesions before CAS is urgently needed.

Trimethylamine *N*-oxide (TMAO), a metabolite generated in the body from a waste product of gut microbes, is associated with cerebrovascular and cardiovascular events in patients with stable cardiovascular disease.[12] It directly contributes to platelet hyperreactivity and enhances thrombosis potential in animal models and healthy volunteers.[13] In patients with coronary artery disease, plasma TMAO level may also reflect coronary plaque vulnerability and progression.[14] We speculate that plasma TMAO levels may be associated with post-procedure new ischemic lesions, which are significantly associated with carotid plaque vulnerability.[15] Thus, in this study, we aimed to examine whether circulating TMAO level predicts ischemic brain injury secondary to CAS in patients with severe carotid stenosis.

Methods

Patients and study design

This multicenter, prospective cohort study was conducted from June 2015 to December 2016. Patients were enrolled at 6 centers in China (The First Affiliated Hospital of Zhengzhou University, Zigong First People's Hospital, Guizhou Provincial People's Hospital, Xiangtan Central Hospital, Xuanwu Hospital Capital Medical University, and Luzhou People's Hospital). All centers had at least one physician or surgeon who had performed a minimum of 100 stenting procedures, with at least 20 cases in the carotid artery.

The inclusion criteria were as follows: age ≥18 years; symptomatic or asymptomatic atheromatous carotid artery stenosis ≥70% by ultrasound, CT angiography, magnetic resonance angiography, or digital subtraction angiography according to the North American Symptomatic Carotid Endarterectomy Trial criteria[16]; prepared for CAS according to the current standard of care; and ability to complete brain MRI examinations and without contraindications to MRI. All women of childbearing age had to have a negative pregnancy test result prior to enrollment. We considered patients to be symptomatic if they had a TIA, amaurosis fugax, or ischemic stroke involving the carotid artery within 6 months before enrollment.

We excluded participants who met any of the following exclusion criteria: high-risk surgical candidate as defined by the Carotid Revascularization Endarterectomy vs Stenting Trial[17]; previous stenting at the same site; myocardial infarction within the last 30 days; intracranial hemorrhage within the previous 12 months; intolerance or allergic reaction to aspirin or clopidogrel; chronic atrial fibrillation; paroxysmal atrial fibrillation that had occurred within the preceding 6 months or that necessitated anticoagulation therapy; any condition that hampered proper angiographic assessment or made percutaneous arterial access unsafe; and participation in another device or drug trial simultaneously.

The neurointerventionalists themselves chose the stents, embolic protection devices (EPDs), and other devices used for carotid stenting, which had to be approved for use in China. We recommended the use of a compulsory EPD whenever the investigator thought it could be used safely. We recommended use of heparin during the intervention, and local anesthesia and conscious sedation as the preferred method of anesthesia. A combination of clopidogrel and aspirin to cover the stenting procedure was recommended. The patients received medical therapy that was consistent with the current standard of care, including treatment of hypertension and hyperlipidemia.

Standard protocol approvals, registrations, and patient consents

The medical ethics committee and the research board of each participating center approved the study protocol. All patients or their legally acceptable representative provided written informed consent before enrollment.

TMAO measurement

We collected overnight fasting blood samples on the second day of admission at 6:00 to 7:00 AM for routine biochemical examination (complete blood cell count, comprehensive metabolic panel, lipid panel, etc.). For each patient, we collected additional blood samples in EDTA tubes, separated the plasma by centrifugation, and stored it at −80°C until analysis. TMAO was quantified using stable isotope dilution liquid chromatography–tandem mass spectrometry, as described previously.[18] Briefly, we added 80 μL of 10 μmol/L d9-TMAO in methanol to plasma (20 μL) as internal standard to precipitate protein. The samples were vortexed for 1 minute, and the supernatant of each sample was then recovered following

centrifugation at 15,000*g* for 25 minutes at 4°C. Supernatant (10 μL) was analyzed by injection into a silica column (4.6 × 250 mm, 5 μm Luna silica, catalog no. 00G-4274-E0; Phenomenex, Torrance, CA). The column was eluted isostatically at a flow rate of 0.8 mL/min with 80% solvent A (0.1% formic acid in water) and 20% solvent B (methanol). TMAO and d9-TMAO were monitored in the positive multiple reaction monitoring mass spectrometry mode by using characteristic precursor–production transitions including m/z 76/58 and m/z 85/66. For determination of TMAO concentration, we created a standard curve using various known concentrations of TMAO. Researchers blinded to the patient clinical data measured all tests. The median (interquartile range) of reference values in healthy participants was 2.8 (1.9–4.8) μmol/L. The intra-assay and interassay coefficient of variation were 1.9%–5.6% and 2.9%–8.4%, respectively.

Clinical assessment

All patients were scanned at baseline (within 7 days before CAS) and 1 to 3 days after CAS using a 3.0-tesla MRI scanner. Symptoms suggestive of cerebrovascular events triggered a subsequent imaging examination. All patients underwent clinical assessment at baseline, at 24 hours, at 5 to 7 days or at discharge if earlier, and at 30 days. Cerebrovascular events included ischemic stroke, hemorrhagic stroke, and TIA. Ischemic or hemorrhagic stroke was defined as acute focal neurologic symptoms with a cerebral infarct or hemorrhage, as identified on brain imaging, regardless of symptoms duration. TIA was defined as a transient neurologic deficit without evidence of cerebral infarct or hemorrhage on brain imaging. Nondisabling events were defined as NIH Stroke Scale score ≤3 or TIA. Investigators blinded to the baseline characteristics conducted all the assessments.

Neuroimaging

The MRI sequences included axial spin-echo T1-weighted, fast-spin T2-weighted, fluid-attenuated inversion recovery, diffusion-weighted imaging (DWI), and apparent diffusion coefficient. DWI was acquired with an echo-planar sequence. The slice thickness was 5 mm, and the gap was 1.5 mm. New ischemic brain lesion was defined as a restricted diffusion signal on DWI sequences with corresponding decreased signal on apparent diffusion coefficient sequences, and this lesion was undetected on the pretreatment scan. Lesions were considered separate when continuity could not be observed between them on adjacent slices or on the same slice. They were manually traced with the internal measuring function of the MRI scanner, and the area was multiplied by slice thickness to calculate volumes of distinct lesions. A neuroradiologist and a neurologist, both blinded to the baseline characteristics, analyzed the image data separately. Two examiners resolved disagreements by consensus; if consensus could not be reached, a third reader resolved the differences.

Statistical analysis

Summaries of categorical variables are reported as proportions, and continuous variables as means ± SD or medians (interquartile ranges [IQRs]). The χ^2 test or Fisher exact test was performed for categorical variables, and the t test or Mann-Whitney U test was used for continuous variables. To investigate whether TMAO can predict new ischemic brain lesions after CAS, we performed different statistical methods. First, logistic regression models were used to investigate the association between TMAO and new ischemic lesions. We used crude and multivariate models adjusted for all other significant predictors and other traditional risk factors and reported odds ratios (ORs). For multivariate analysis, we included any significant imbalances in baseline characteristics and other selected baseline characteristics. Second, a receiver operating characteristic curve was used to test the overall predicted accuracy of TMAO, and results were reported as area under the curve. A 2-tailed p value <0.05 was considered significant. Statistical analyses were performed with SPSS version 19.0 (IBM, Armonk, NY). Sample size calculation was based on the means and SDs observed for plasma TMAO level of patients with and without new lesions on DWI in a pilot study. We calculated that a sample of 250 patients would yield a 90% power at $p = 0.05$ (2-sided).

Results

Patient characteristics and clinical variables

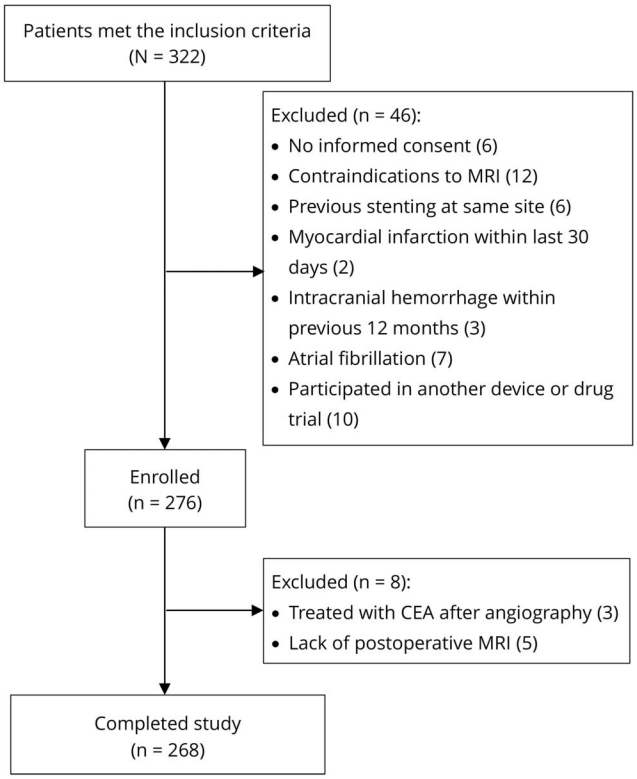
During the study period, 322 patients met the inclusion criteria, and 46 patients were excluded (6 without informed consent, 12 had contraindications to MRI, 6 with previous stenting at the same site, 2 with myocardial infarction within the last 30 days, 3 with intracranial hemorrhage within the previous 12 months, 7 with atrial fibrillation, 10 participated in another device or drug trial). A total of 276 patients were enrolled in the study, and 268 patients completed the study (3 patients were treated with CEA after angiography and 5 patients lacked postoperative MRI) (figure 1).

The mean age of the patients included in this study was 64.4 years, and 43.3% (116/268) were women. Of the 268 patients, 117 (43.7%) had at least one new lesion detected on post-procedure MRI. The baseline characteristics of the 268 patients are summarized in table 1. Patients who had at least one new lesion on DWI after treatment were older (mean 66.7 vs 62.6 years, $p = 0.004$) and more frequently had type III aortic arch (19.2% vs 9.4%, $p = 0.025$). These patients also more frequently had coronary artery disease (30.8% vs 19.2%, $p = 0.028$), more symptomatic carotid artery stenosis (75.2% vs 60.3%, $p = 0.01$), and higher level of low-density lipoprotein (median 2.9 vs 2.4, $p = 0.033$). Both open-cell and closed-cell stents were used for CAS procedures. Two hundred sixty-five of the 268 patients (98.9%) used EPDs (all were filter-type devices) during the procedure, whereas the other 3 patients did not use EPDs because of the tortuous carotid artery. The type of stents and EPDs showed no significant difference between the 2 groups (table e-1, links.lww.com/WNL/A337).

New ischemic brain lesions and TMAO

Of the 117 patients who had at least one new lesion on posttreatment DWI, 33.3% (39/117) had a single lesion and

Figure 1 Trial profile



CEA = carotid endarterectomy.

66.7% (78/117) had multiple lesions. The median number of lesions was 3 (IQR, 1–8), and the median total lesion volume was 0.19 mL (IQR, 0.05–0.62). In 10 of the 117 patients (8.5%) with positive DWI findings after stenting, lesions were associated with symptoms of ischemic events between start of stenting and predetermined posttreatment scans. In the remaining 107 patients, no cerebrovascular events occurred up to the time of the MRI scan. The location of lesions and clinical events of these patients are summarized in table 2.

Plasma TMAO levels were higher in patients with new lesions than in patients without new lesions (median 5.2 vs 3.2 μmol/L, $p < 0.001$) on DWI. Compared with patients in the lowest quartile of TMAO levels, those in the highest quartile (unadjusted OR for highest quartile vs lowest quartile, 5.49; 95% confidence interval [CI], 2.64–11.43; $p < 0.001$) and third quartile (unadjusted OR for third quartile vs lowest quartile, 2.94 [95% CI, 1.44–6.03]; $p = 0.003$) had a higher risk of developing new ischemic brain lesions (table 3). In multivariate logistic analysis, after adjusting for age, sex, proportion of symptomatic carotid artery stenosis, coronary artery disease, systolic blood pressure, serum glucose, low-density lipoprotein, high-density lipoprotein, homocysteine, and proportion of type III aortic arch, elevated plasma levels of TMAO remained a significant predictor of the risk of new ischemic brain lesions (table 3).

Based on the receiver operating characteristic curve, the optimal cutoff value of plasma TMAO level as an indicator for predicting new lesions on DWI after treatment was projected to be 4.29 μmol/L, which yielded a sensitivity of 61.5% and a specificity of 74.8%, with the area under the curve at 0.706 (95% CI, 0.642–0.769; $p < 0.001$; figure 2). The positive and negative predictive values of TMAO levels ≥4.29 μmol/L for predicting new lesions on DWI were 65.5% and 71.5%, respectively. We also found that TMAO levels ≥4.29 μmol/L were associated with an increased risk of new lesion on DWI (table 3).

In a predefined subgroup analysis, the median plasma TMAO levels were 4.21 (2.35–5.86) in 179 patients with symptomatic carotid artery stenosis and 3.24 (2.14–5.24) in 89 patients with asymptomatic artery stenosis ($p = 0.012$). TMAO remained associated with the risk of new lesions on DWI in 179 patients with symptomatic carotid artery stenosis (unadjusted OR for highest quartile vs lowest quartile, 6.18 [95% CI, 2.32–16.43]; $p = 0.002$) and 89 patients with asymptomatic artery stenosis (unadjusted OR for highest quartile vs lowest quartile, 4.93 [95% CI, 2.02–12.05]; $p < 0.001$).

Clinical events of 30-day follow-up and new ischemic brain lesions

A total of 107 of 117 patients (91.5%) in the new DWI lesion group and 125 of 151 patients (82.8%) in the without new DWI lesion group completed the 30-day follow-up. Thirty-six patients were lost to follow-up. Of the 232 patients, 9 (3.8%) experienced stroke (8 ischemic stroke and 1 hemorrhagic stroke), and 3 (1.3%) experienced TIA. One patient experienced hemorrhagic stroke without new lesion on the posttreatment DWI, and hemorrhage occurred 5 days after the CAS (3 days after the MRI scan). One patient had TIA with multiple lesions on post-CAS scan, and the TIA occurred 17 days after the CAS due to subacute stent thrombus. No fatal cerebrovascular events occurred; most events were nondisabling (9 of 12, 75%). When stroke was combined with TIA, 7.4% of patients (8/107) in the new DWI lesion group and 3.2% (4/125) in the without new DWI lesion group had a stroke or TIA in the 30-day follow-up ($p = 0.143$).

Discussion

In this multicenter clinical study, we found that plasma TMAO levels before CAS were significantly higher in patients with new lesions on post-CAS DWI scans than in patients without new lesions. After adjusting for possible confounders, elevated plasma levels of TMAO remained an independent predictor for new lesions on DWI secondary to CAS. The association was consistent across the predefined subgroup that was defined according to clinical symptom.

The rate of new lesions detected on DWI at a median of 1 day after CAS in our trial was similar to that in previous trials,[4,8] i.e., as in about half of CAS patients. New lesions on DWI may

Table 1 Baseline characteristics of the patients

Characteristic	Total (n = 268)	At least one new lesion (n = 117)	No new lesion (n = 151)	*p* Value
Age, y	64.4 ± 11.6	66.7 ± 10.3	62.6 ± 12.1	0.004
Women	116 (43.3)	47 (40.1)	69 (45.7)	0.365
Symptomatic carotid artery stenosis	179 (66.8)	88 (75.2)	91 (60.3)	0.01
Right carotid artery stenosis	140 (52.2)	65 (55.6)	75 (49.7)	0.339
Vascular risk factors				
Hypertension	195 (72.8)	80 (68.4)	115 (76.2)	0.151
Diabetes	90 (33.6)	36 (30.8)	54 (35.8)	0.391
Dyslipidemia	96 (35.8)	40 (34.2)	56 (37.1)	0.588
Coronary artery disease	65 (24.3)	36 (30.8)	29 (19.2)	0.028
Peripheral artery disease	38 (14.2)	18 (15.4)	20 (13.2)	0.618
Metabolic syndrome[a]	103 (38.4)	47 (40.2)	57 (37.8)	0.686
Smoking	145 (54.1)	65 (55.6)	80 (52.9)	0.675
Family history of stroke	58 (21.6)	24 (20.5)	34 (22.5)	0.693
Clinical findings				
Systolic blood pressure, mm Hg	141.1 ± 16.5	139.2 ± 17.7	142.5 ± 15.6	0.107
Heart rate, beats/min	87.2 ± 10.5	87.6 ± 11.2	86.8 ± 9.7	0.532
BMI, kg/m^2	25.1 ± 3.1	25.2 ± 3.3	25.0 ± 2.9	0.599
Laboratory findings				
Serum glucose, mmol/L	5.6 (5.1–6.4)	5.6 (4.9–6.2)	5.7 (5.1–6.4)	0.152
TG, mmol/L	1.4 (1.1–1.7)	1.4 (1.1–1.8)	1.5 (1.0–1.7)	0.157
LDL, mmol/L	2.6 (2.0–3.4)	2.9 (2.2–3.6)	2.4 (2.0–3.2)	0.023
HDL, mmol/L	0.9 (0.7–1.2)	0.9 (0.8–1.3)	0.8 (0.7–1.2)	0.432
TC/HDL	4.6 (3.0–5.7)	4.5 (2.9–5.5)	4.7 (3.2–5.8)	0.587
LDL/HDL	3.0 (2.0–3.7)	3.2 (1.9–3.9)	2.9 (2.1–3.5)	0.223
Homocysteine, μmol/L	11.3 (9.6–14.8)	11.8 (9.5–13.6)	10.9 (9.6–14.2)	0.441
TMAO, μmol/L	3.6 (2.3–5.6)	5.2 (3.2–7.2)	3.2 (2.1–4.3)	<0.001
Medical treatment				
Antiplatelet therapy 48 h before stenting	268 (100)	117 (100)	151 (100)	NA
Heparin during procedure	263 (98.1)	114 (97.4)	149 (98.7)	0.773
Aspirin plus clopidogrel after procedure	256 (95.5)	110 (94.0)	146 (96.7)	0.294
Type III aortic arch	30 (14.9)	29 (19.2)	11 (9.4)	0.025
General anesthesia	15 (5.6)	6 (5.1)	9 (6.0)	0.769
Interval between pre-CAS scan and CAS, d	1 (1–3)	1 (1–3)	1 (1–3)	0.594
Interval between CAS and post-CAS scan, d	1 (1–2)	1 (1–2)	1 (1–2)	0.658
Presence of ischemic lesion on DWI before treatment	37 (13.8)	19 (16.2)	18 (11.9)	0.309

Abbreviations: BMI = body mass index; CAS = carotid artery stenting; DWI = diffusion-weighted imaging; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NA = not applicable; TC = total cholesterol; TG = triglyceride; TMAO = trimethylamine *N*-oxide.
Data are mean ± SD, n (%), or median (interquartile range).
[a] Metabolic syndrome was diagnosed according to the guidelines of China (any 3 of the following 4 items: BMI ≥25.0 kg/m^2; fasting plasma glucose ≥6.1 mmol/L and/or 2-hour plasma glucose ≥7.8 mmol/L and/or diabetes; systolic blood pressure/diastolic blood pressure ≥140/90 mm Hg and/or hypertension; fasting TG ≥1.7 mmol/L and/or fasting HDL <0.9 mmol/L [male], <1.0 mmol/L [female]).

Table 2 New DWI lesions on posttreatment scans

Variable	Total (n = 117)	Single lesion (n = 39)	Multiple lesions (n = 78)
Location of lesions			
Ipsilateral carotid circulation only	71 (60.7)	32 (82.1)	39 (50)
Nonipsilateral (ipsilateral vertebrobasilar or contralateral carotid or vertebrobasilar) carotid circulation only	37 (31.6)	7 (17.9)	30 (38.5)
Ipsilateral carotid and nonipsilateral carotid circulation	9 (7.7)	0	9 (11.5)
Ischemic events in patients with new DWI lesions[a]			
Hemispheric stroke	8 (6.8)	2 (5.1)	6 (7.6)
Retinal infarct	1 (0.9)	0	1 (1.3)
TIA	1 (0.9)	0	1 (1.3)
None	107 (91.5)	37 (94.9)	70 (89.7)
Hemorrhagic events in patients with new DWI lesions[a]	0	0	0

Abbreviation: DWI = diffusion-weighted imaging.
Data are n (%).
[a] Events occurring between start of stenting and predetermined posttreatment scans only.

negatively affect neuropsychological function, although most of them were not associated with a cerebrovascular event.[19] In a previous study, new ischemic lesions detected on DWI after CAS seem to be associated with an increased risk of recurrent cerebrovascular events in a median follow-up of 4.1 years.[20] In our trial, no statistical differences were found in cerebrovascular events between patients with and those without new lesions on DWI in a 30-day follow-up. However, the more than 2-fold data difference may suggest that patients with new lesions on DWI tend to have a cerebrovascular event. Different follow-up periods may explain the difference in cerebrovascular events. Whether lesions detected on DWI after CAS are associated with poor prognosis warrants further studies.

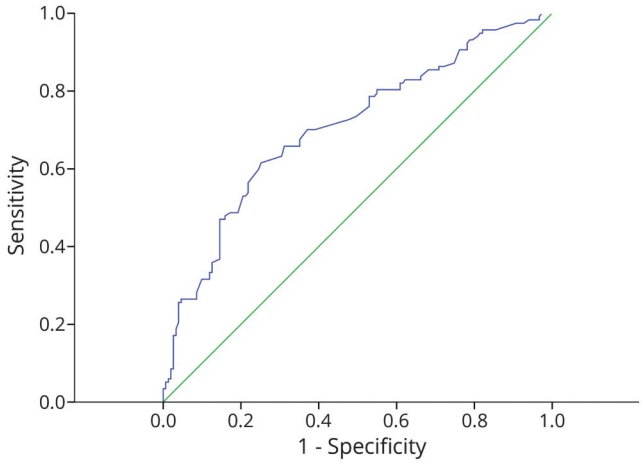
Several studies have suggested a higher rate of posttreatment DWI lesions after CAS compared with CEA.[8,21–23] In a meta-analysis, the risk of new lesions on DWI was almost 7-fold after CAS, compared with CEA.[8] The present results reveal a previously unrecognized association between TMAO and DWI lesions after CAS. In general, 0.7 < area under the curve < 0.9 indicates a moderate diagnostic value. This finding shows that increased TMAO levels (≥4.29 μmol/L) had a moderate diagnostic role for lesions on DWI after CAS. The

Table 3 Risk of new ischemic brain lesion

	Odds ratio (95% CI)			
TMAO level	**Unadjusted**	***p* Value**	**Adjusted[a]**	***p* Value**
Quartile of TMAO level				
Quartile 1	1		1	
Quartile 2	1.09 (0.51–2.30)	0.720	1.02 (0.59–1.98)	0.84
Quartile 3	2.94 (1.44–6.03)	0.003	1.86 (1.09–4.66)	0.02
Quartile 4	5.49 (2.64–11.43)	<0.001	3.85 (1.37–7.56)	<0.001
Continuous TMAO level				
<4.29 μmol/L	1		1	
≥4.29 μmol/L	4.76 (2.82–8.03)	<0.001	3.21 (1.28–5.35)	<0.001

Abbreviations: CI = confidence interval; TMAO = trimethylamine *N*-oxide.
[a] Odds ratios were adjusted for age, sex, proportion of symptomatic carotid artery stenosis, coronary artery disease, systolic blood pressure, serum glucose, low-density lipoprotein, high-density lipoprotein, homocysteine, proportion of type III aortic arch.

Figure 2 Receiver operating characteristic curve shows an area under the curve = 0.706 and $p < 0.001$



use of TMAO may be reasonable to help in the selection of patients for either CAS or CEA in clinical decision-making, although the mechanism of TMAO and new lesions on DWI remains unclear.

The intestinal microbiota has attracted considerable interest in the past years as a potential contributor to systemic disease processes, such as obesity,[24] diabetes,[25] and atherosclerosis.[26] Certain dietary nutrients, such as phosphatidylcholine, choline, and carnitine, are processed specifically by the gut microbiota to produce trimethylamine, which is absorbed in the gut and converted in the liver to TMAO by hepatic flavin-containing monooxygenases.[27] Platelet activation and aggregation enhance potential for thrombotic events and are associated with cardiovascular and cerebrovascular diseases. Meanwhile, the intestinal microbiota directly contributes to platelet hyperreactivity and enhances thrombosis potential by generating TMAO.[13] A previous study found that in patients with coronary artery disease, the circulating TMAO level may reveal coronary plaque vulnerability and progression assessed by optical coherence tomography.[14] Theoretically, TMAO may be associated with ischemic brain lesions after CAS.

In humans, dietary phosphatidylcholine is associated with increased levels of TMAO, which in turn are associated with an increased risk of incident major adverse cardiovascular events.[12] However, little is known about the involvement of TMAO and ischemic stroke. In a previous study, patients with stroke and TIA showed significant dysbiosis of the gut microbiota, and decreased blood TMAO levels.[28] This is contradictory to the increased TMAO involved in atherosclerosis[26] and the increased cardiovascular events.[12] Yin et al.[28] explained that they examined TMAO in patients who already had stroke or TIA, and the treatment of stroke or TIA may reduce the levels of TMAO. Further studies are necessary to establish the association between TMAO and the risk of cerebrovascular disease.

This study has several limitations. First, some of the baseline characteristics were not well matched between the new DWI lesion group and the without DWI lesion group. However, such bias is unlikely to explain the large difference in plasma TMAO levels between the 2 groups. In addition, after adjusting for all other significant predictors and other traditional risk factors, elevated plasma levels of TMAO remained a significant predictor of the risk of new ischemic brain lesions. Second, the follow-up period was 30 days, which may fail to detect the long-term difference in cerebrovascular diseases. Further studies are warranted to establish whether TMAO can predict poor prognosis after CAS. Third, a previous study showed that the risk of cerebral ischemia may be higher among patients undergoing stenting with cerebral protection devices than without.[8] At present, EPDs are routinely used during CAS in China. Only 3 patients in this cohort did not use any EPDs. Thus, we could not analyze whether the use of filter-type devices or other types of devices (e.g., with distal or proximal balloon occlusion) influenced the proportion of new lesions on DWI.

Our study provides preliminary data showing that increased plasma TMAO levels before CAS treatment are independently associated with increased risk of new ischemic lesions on DWI after CAS among patients with severe carotid artery stenosis. Future directions should focus on the mechanism between TMAO and new ischemic lesions after CAS. If these pathophysiologic mechanisms are elucidated, they may be used as potential preventive targets for ischemic injury after CAS and CEA or even ischemic stroke. Furthermore, the obtained results of the present study should be confirmed in larger-sample studies and in real-world settings involving different ethnic groups.

Author contributions

Chuanjie Wu: analysis of the data, drafting the manuscript for intellectual content. Chuanhui Li, Wenbo Zhao, Nanchang Xie, Feng Yan, Yajun Lian, Li Zhou, Xiaoya Xu, Yong Liang, and Lu Wang: acquisition of data. Ming Ren, Sijie Li, and Xuan Cheng: acquisition of data, interpretation of the data. Ran Meng, Qingfeng Ma, and Lu Zhang: analysis and interpretation of data, revising the manuscript for intellectual content. Haiqing Song and Xunming Ji: design of the study, revising the manuscript for intellectual content.

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Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

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