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Prognostic value of gut microbiota-derived trimethylamine N-oxide in patients with ischemic stroke: A meta-analysis

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Abstract

Objective: This study aimed to evaluate the relationship between the intestinal microbial metabolite trimethylamine N-oxide (TMAO) level in the plasma and the outcomes of patients with ischemic stroke (IS). *Methods:* The PubMed, Web of Science, Cochrane Library, and Embase databases were systematically searched until July 1, 2022 to identify clinical studies on TMAO levels and prognosis of IS. The identified studies were then screened according to the inclusion and exclusion criteria, and a meta-analysis on the final included studies was performed using the Stata (version 17.0) software to investigate the effects of TMAO level on the prognosis of IS. *Results:* Four studies involving 1,026 participants from 2019 to 2022 were considered. The results indicated an association between high circulating TMAO concentration and poor functional outcomes (rate ratio [RR]: 2.76; 95% confidence interval [CI]:1.33,5.72; P = 0.006; I^2 = 72.7%; P-heterogeneity = 0.012; random-effects model), mortality (RR: 5.37; 95%CI: 2.63,10.97; P < 0.001; I^2 = 0.0%; P-heterogeneity = 0.704; fixed-effects model), and recursive ischemic events (RR: 2.71; 95%CI: 1.68,4.36; P < 0.001; I^2 = 0.0%; P heterogeneity = 0.779; fixed-effects model) 3 months after IS.
Conclusions: Elevated TMAO level may be an adverse prognostic indicator in patients with IS.

Keywords: trimethylamine-N-oxide, ischemic stroke, prognostic value, meta-analysis

INTRODUCTION

Stroke is a global health problem, the second leading cause of death, and the third most common cause of disability worldwide.[1] Ischemic stroke (IS) is the most common type of stroke, accounting for 85% of all stroke cases.[1] Although IS has the best treatment based on guidelines, it remains a heavy burden with high recurrence and mortality rates, indicating that current treatment does not adequately address important pathogenic mechanisms. Effective prognostic assessment and detection are not only an important means of prolonging the survival time of patients with IS, but also contributes to better nursing and allocation of medical resources.[2] However, because the disease often occurs in middle-aged and elderly individuals, it is often accompanied by complex underlying diseases, which increase the difficulty of medical decision-making and evaluating prognosis. Existing prediction models are complex and diverse; however, the evaluation efficiency for prognosis is not high.[3] Therefore, exploring new biomarkers to improve IS prognosis is important.

The intestinal flora acts on the homeostasis of the human environment through metabolism and immunity, which affects the occurrence and development of stroke.[4-6] Trimethylamine-N-oxide (TMAO), a gut-derived metabolite[7], is a circulating organic compound produced by the metabolism of dietary L-carnitine and choline. Both L-carnitine and choline are metabolized by intestinal bacteria trimethylamine, a metabolite which is absorbed from the intestine and subsequently oxidized via hepatic flavin monooxygenase enzymes to form TMAO.[8] Atherosclerosis is an important risk

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factor of IS. The TMAO level in blood circulation increases the risk of IS and poor prognosis by promoting thrombosis and atherosclerosis.[9] Studies have reported that platelet activation is significantly associated with target organ damage and poor prognosis.[10] Higher plasma TMAO levels suggest an increased risk of first-time IS and the possible deterioration of neurological function.[11-13] The TMAO level of patients with IS indicates dynamic changes in different periods, and some studies have explored the relationship between TMAO and poor prognosis of IS, although their findings are not completely consistent.[14-17] To address this problem, we conducted a meta-analysis to explore the prognostic value of TMAO in IS.

METHODS

Search strategy

A systematic search was conducted for studies published on July 1, 2022, using the following four English electronic databases: PubMed, Excerpta Medica Database (EMBASE), Cochrane Library, and Web of Science. We used a search strategy combining MeSH heading and text with the following terms: "trimethyloxamine", "trimethylammonium oxide", "trimethylamine N-oxide", "TMAO" or "trimethylamine oxide" and "ischemic stroke", "cryptogenic stroke" or "wake up stroke". The reference lists of the included papers were also screened.

Selection criteria

The original trials included in this meta-analysis met the following criteria: the participants were patients with IS; evaluating the relationship between circulating TMAO levels and prognosis of IS, including, 3-month modified Rankin scale (mRS), mortality at 3 months, and major ischemic events within 3 months; cohort study or case-control study; and reporting the multivariable-adjusted hazard ratio (RR) and corresponding 95% confidence interval (CI).

Meanwhile, we excluded studies that reported only unadjusted risk estimates; lack related data; animal trials; were not written in English; and were case reports, letters, conference abstracts, duplications, or unpublished studies.

Data extraction

Publications that met the inclusion criteria were independently searched by two reviewers (Y.L. and S.L.). Subsequently, the two investigators collected data of the eligible trials and recorded them in the information extraction table. The extracted information included the year of publication, first author's surname, study locations, type of study, sample size, sex distribution and age, TMAO mean level, outcome measures, adjusted RR (95% CI), and corresponding 95% confidence interval. Any disagreements were resolved by a third author.

Quality assessment

The Newcastle–Ottawa Scale (NOS) was used to assess the methodological quality of the studies.[18] The domains of NOS cover selection, comparability, and outcome, which offer a maximum of nine points. Studies with a score≥7 were rated as high quality, and those with scores 0 to 3 were considered of low quality.[18]

Statistical analysis

Data were analyzed using Stata 17.0 (StataCorp, College Station, TX, USA). RRs and 95% CIs were used to describe the overall effects. The overall effect was calculated using a Z-test, and statistical significance was set at $P < 0.05$ (two-tailed). Potential heterogeneity was evaluated using Cochran's Q and I^2 statistic; when heterogeneity was low ($P \geq 0.05$, $I^2 \leq 50\%$), a fixed-effects model was applied. If high heterogeneity occurred ($P < 0.05$, $I^2 > 50\%$)[19,20], we further analyzed its potential sources and applied corresponding measures. First, a subgroup analysis was performed if clinical heterogeneity was evident, and a random-effect model was used if statistical heterogeneity was considered.[21] Our meta-analysis eventually included <10 articles without testing for publication bias.

RESULTS

Literature search and screening

In total, 273 articles requiring further evaluation were retrieved from four foreign databases (43 in PubMed, 3 in Cochrane Library, 171 in Embase, and 56 in Web of Science). Of the articles, 64 were deleted because of duplicate records. After screening the titles and abstract, 176 papers were excluded for several reasons. Finally, after screening the full text, only four studies met the inclusion criteria. Figure 1 presents the detailed flow of the research-selection process.

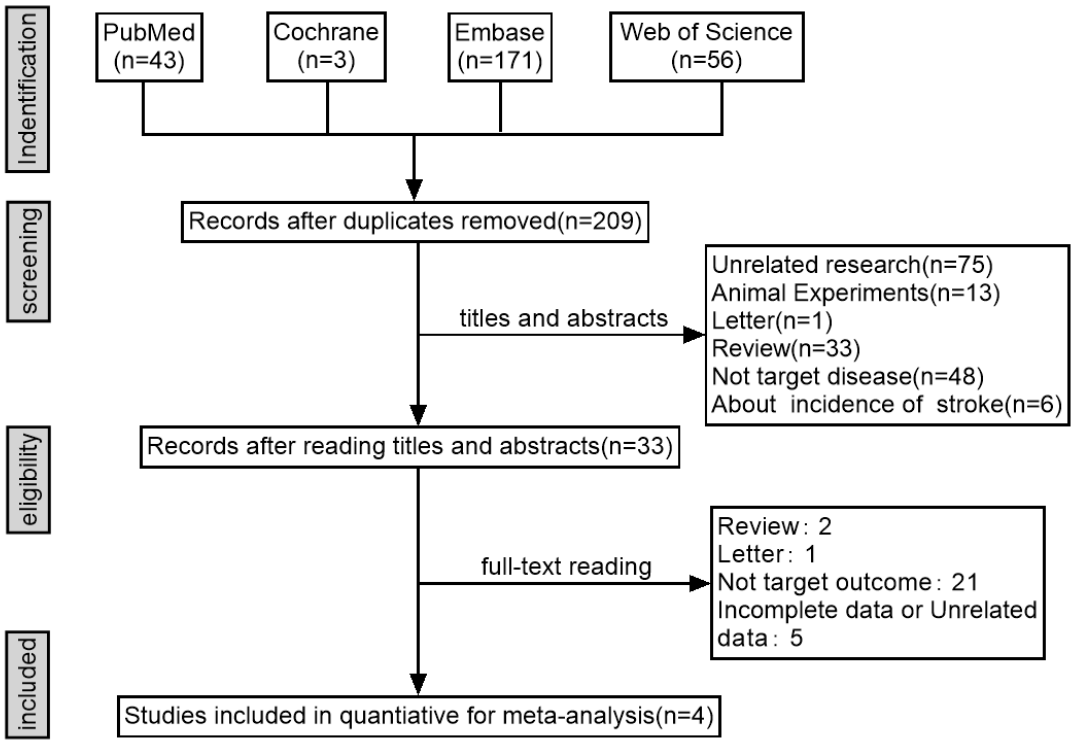

Figure 1. Flowchart of study selection

Study characteristics and quality assessment

Finally, four studies involving 1026 participants from 2019 to 2022 were included. Four studies were conducted in China, one of which was a case–control study, and the others were cohort studies. The average age of the study participants ranged from 59 to 66.9 years, and there were more men than women. TMAO levels and mRS scores at 3 months were reported in all the studies, and mortality at 3 months was reported in two of the studies. The major ischemic events within 3 months was also reported in two of the studies. All the included studies were adjusted for various potential influencing factors, and three studies were rated as high-quality. The detailed information of those studies were summarized in Table 1.

3-month mRS score

Four studies with a total of 1026 participants have reported an association of functional outcomes after 3 months. The mRS was used to assess functional outcomes. Random effects model analyses indicated that higher TMAO levels were associated with a higher 3-month mRS (RR: 2.76, 95%CI: 1.33–5.72, P = 0.006, I^2 = 72.7%, P-heterogeneity=0.012, random-effects model, Figure 2). Thus, a high circulating TMAO concentration was associated with poor prognosis of patients 3 months after IS.

3-month mortality

Two studies with a total of 576 participants have reported an association between 3-month mortality and TMAO levels (RR: 5.37, 95%CI: 2.63–10.97, $P < 0.001$, $I^2 = 0.0\%$, P-heterogeneity = 0.704, fixed-effects model, Figure 3). This result indicated that a high circulating TMAO concentration was associated with mortality in patients 3 months after IS.

3-month major ischemic events

Two studies with a total of 450 participants have reported an association between recurrent ischemic events and TMAO levels (RR, 2.71; 95%CI: 1.68–4.36; P < 0.001; I^2 = 0.0%; P-heterogeneity = 0.779; fixed-effects model; Figure 4). This result indicates that a high circulating TMAO concentration was associated with major ischemic events within 3 months in patients with IS.

Publication bias analysis and sensitivity analysis

We finally included no more than 10 papers, so no

Table 1: Summary of clinical studies included in the meta-analysis

	Location	Sample size (%male)	Age (years)	TMAO level	Outcome	Adjusted risk factors	Study quality
Zhang *et al.*(17) 2020	China	351 50.4%	66 (55,74)	6.1umol/L (3.7–9.9)	mRS scale, mortality	Age,sex, BMI, traditional risk factors, NIHSS and Infarct volume at admission, stroke subtype, pre-stroke treatment, acute stroke treatment, eGFR, plasma levels of glucose, CRP, IL-6, Choline, and Betaine	7
Zhai *et al.*(16) 2019	China	225 58.9%	66.5 (55.3,77.7)	3.8umol/L (1.9–4.8)	mRS scale, mortality	Demographic characteristics, hypertension, coronary heart disease,body mass index, NIHSS score,DWI-ASPECT score 0–7, stroke subtypes, and homocysteine ,and low-density lipoprotein levels	7
Tan *et al.*(15) 2020	China	204 66.7%	59	2.5umol/L	mRS scale, major ischemic events	Age,sex,history of smoking,hypertension, diabetes, atrialfibrillation, coronary heart disease,eGFR,dyslipidemia,re vascularization therapies, stroke etiologies, dysphagia, NIHSS score ,antiplatelet agents, NEU, NT-proBNP ,and TC levels	6
Chen *et al.*(14) 2022	China	246 54.6%	61.68 (54.4,70)	129.7pg/ml (83.4-175.9)	mRS scale, major ischemic events	Age, sex, hypertension, diabetes mellitus, smoking, and creatinine	7

†NIHSS, National Institutes of Health Stroke Scale; CRP, C-reactive protein; IL, interleukin; DWI-ASPECT, Diffusion-Weighted Imaging–Alberta Stroke Program Early Computed Tomography Scores; NEU, neutrophil; NT-proBNP, N-terminal-pro hormone B-type natriuretic peptide; TC, triglyceride; eGFR, estimated glomerular filtration rate.

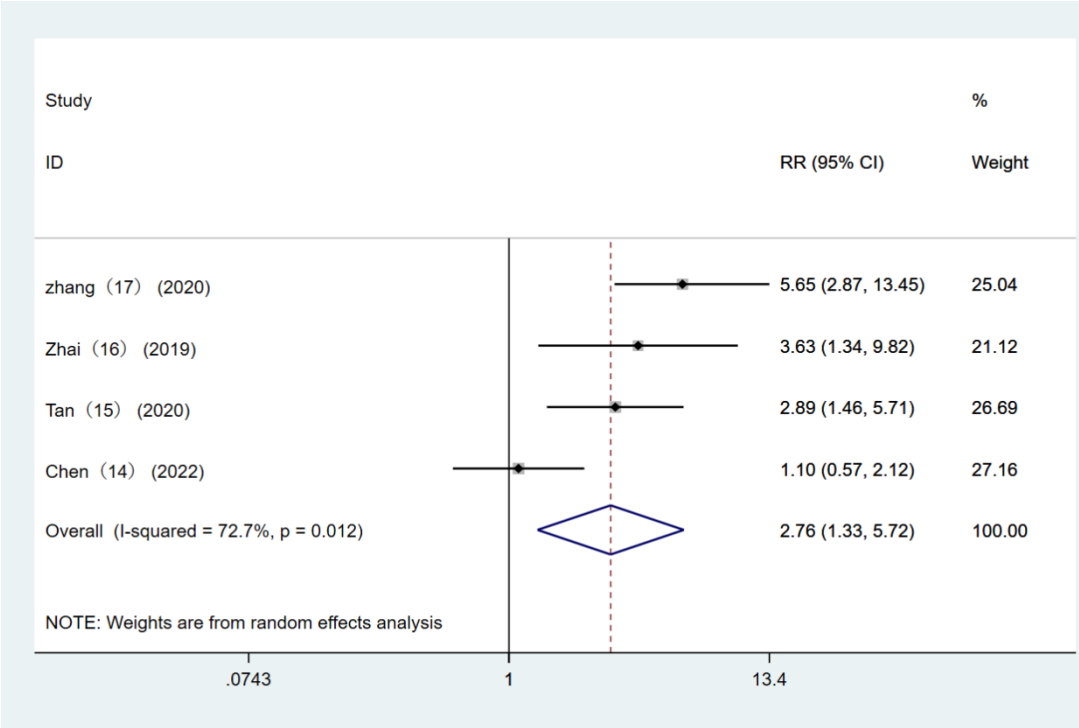

Figure 2. Forest plot for the association between TMAO level and functional outcome at 3 months

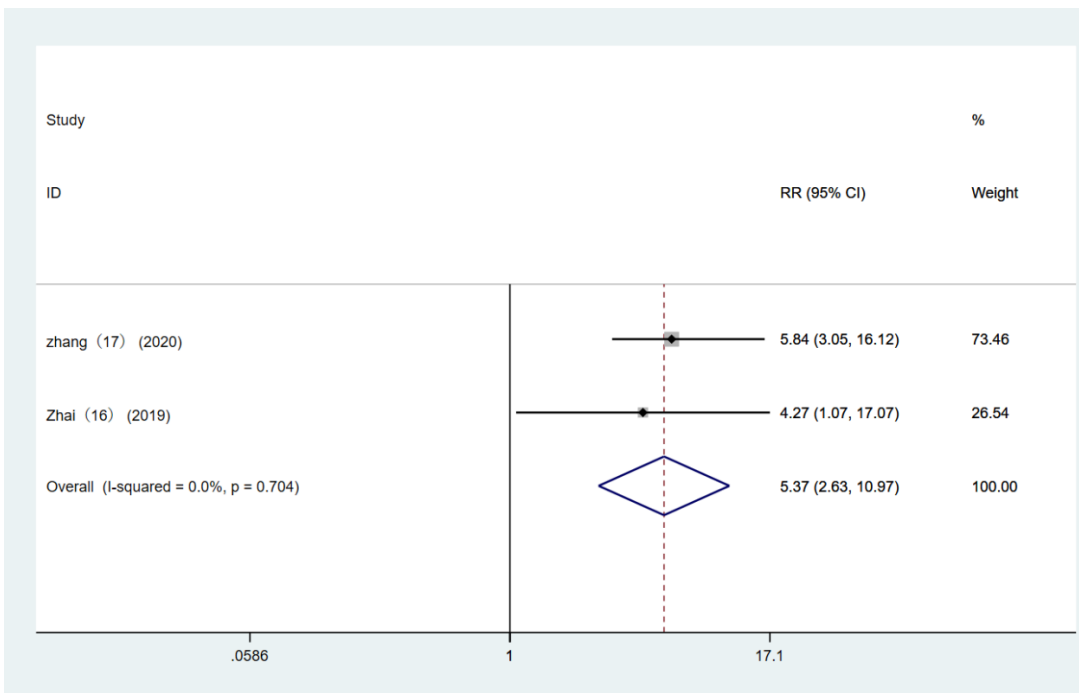

Figure 3. Forest plot for the association between TMAO level and 3-month mortality

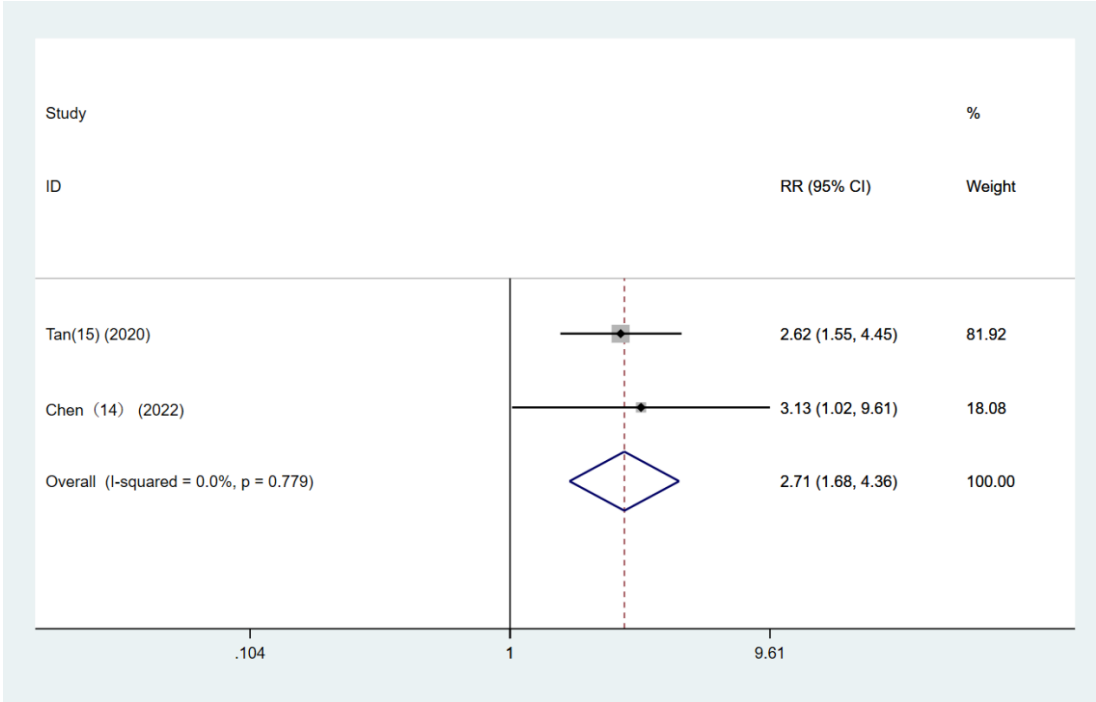

Figure 4. Forest plot for the association between TMAO level and major ischemic events within three month

relevant test of publication bias was conducted. To evaluate the stability of the meta-analysis, these studies were deleted individually, and the effect values (RRs) were recalculated. Thus, the research results are reliable (Figure 5).

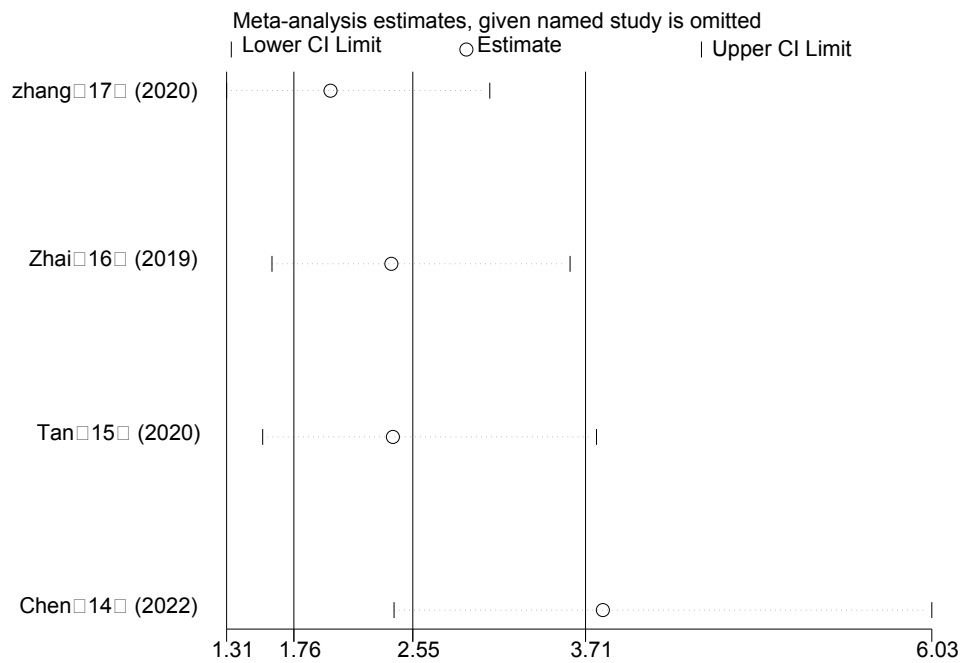

Figure 5. The sensitivity analysis of the study

DISCUSSION

To the best of our knowledge, this was the first systematic review and meta-analysis to explore whether high levels of TMAO are related to the poor prognosis of patients with IS. Therefore, this meta-analysis demonstrated that circulating TMAO was independently correlated with the increased risk of 3-month mRS score, mortality at 3 months, and major ischemic events at 3 months in patients with IS.

TMAO is an intestinal microbial-dependent metabolite of dietary trimethylamine. TMAO production can be divided into two steps. First, intestinal microbes produce trimethylamine from dietary ingredients such as choline or carnitine.[22] TMA then enters the cycle and is oxidized to TMAO in the liver by the monooxygenase-containing rispero.[8] At present, TMAO is considered an important mediator in the pathogenesis of atherosclerotic disease and the occurrence of major atherosclerotic complications.[8,23-25] Studies have demonstrated that higher TMAO levels may indicate an increased risk of stroke in patients with high blood pressure, even after adjusting for choline, L-carnitine, and other important covariables, including baseline systolic blood pressure (SBP) and time-averaged SBP.[12] Recently, another study has reported that increased TMAO levels were associated with an increased risk of new ischemic brain damage on magnetic resonance imaging after carotid artery stenting.[26] These studies add increasing amounts of data, suggesting that TMAO levels may be used as clinically useful and potentially changeable prognostic marker for IS, even exceeding the risk factors and laboratory test results currently used.[16] Moreover, this study was designed to evaluate the relationship between TMAO levels and short-term functional prognosis in patients with IS. Importantly, this preliminary result leads to an interesting conclusion that elevated TMAO levels at admission are associated with poor functional outcomes, mortality, and major ischemic events at 3 months, indicating that this biomarker disorder is detrimental to prognosis.

The underlying mechanism between elevated TMAO levels and the poor prognosis of IS can be speculated as follows. These results may be partly explained by activation of the inflammatory state, which plays a key role in the development of IS.[16,27] Previous studies have suggested that physiological levels of TMAO can induce the expression of inflammatory cytokines and adhesion molecules and activate NLRP3 inflammatory bodies, which are involved in the destruction of the blood–brain barrier and neuronal regeneration.[27-30] Therefore, higher levels of circulating TMAO may stimulate persistent inflammation and lead to poor prognosis in patients with IS. Furthermore, >40% of IS cases are caused by major atherosclerosis, which can serve as a pathological basis for IS. TMAO affects cholesterol and sterol metabolism, enhances cholesterol accumulation and foam cell formation in macrophages, and promotes vascular inflammation and endothelial dysfunction.[9,30] TMAO may also promote the development of atherosclerosis by impairing the reverse transport of cholesterol.[32] TMAO may also increase oxidative stress, enhance mitochondrial damage, and inhibit the mTOR signaling pathway, all of which impair neural function.[33] Moreover, some investigators have revealed through cerebral IS–reperfusion animal models that TMAO promotes reactive astrocytosis and glial scarring formation through the Smurf2/ALK5 axis, thus exacerbating neurological damage.[31] Furthermore, hypertension and diabetes are known risk factors for stroke recurrence and have been reported to be facilitated by TMAO. Specifically, TMAO increases plasma osmolarity and triggers regulation of the TMAOAVP–AQP–2 axis, thus causing greater water reabsorption.[34] TMAO inhibits hepatic insulin signaling and exacerbates impaired glucose tolerance.[35] Furthermore, TMAO has been reported to directly promote platelet aggregation and lead to multiple agonist-dependent enhanced platelet activation capacity as well as increased thrombosis risk by activating the intracellular storage risk of Ca^{2+} release.[9]

This meta-analysis had some limitations. First, since all of the included studies were conducted in China, whether the relationship between TMAO levels and the outcomes of patients with IS is similar in populations with different dietary patterns (e.g., European or American populations) is unclear. Second, as plasma TMAO concentrations are affected not only by carnitine intake but also by gut microbiota disorders and renal dysfunction, the estimated increased risk may not be fully elucidated by considering diet.[36] Finally, as plasma TMAO concentrations are affected by diet, whether the effects of TMAO levels on functional outcome and mortality risk in patients with IS are attributable to L-carnitine-rich foods (e.g., red meat) is unclear[37], as the investigators did not assess dietary intake in detail, and the results should be interpreted cautiously because of the insufficient number of included studies.

In conclusion, elevated TMAO plasma levels were associated with a poorer prognosis for patients with IS, although the underlying mechanism requires further investigation. Future therapies targeting TMAO may be a new way to prevent and treat cerebrovascular diseases; however, further research is required to confirm these findings.

DISCLOSURE

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Conflict of interest: None

REFERENCES

1. Feigin VL, Stark BA, Johnson CO, *et al*. Global, regional, and national burden of stroke and its risk factors, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol* 2021;20(10):795-820. doi:10.1016/S1474-4422(21)00252-0.
2. De Marchis GM, Dankowski T, König IR, *et al*. A novel biomarker-based prognostic score in acute ischemic stroke: The CoRisk score. *Neurology* 2019;92(13):e1517-e1525. doi:10.1212/WNL.0000000000007177.
3. Makris K, Haliassos A, Chondrogianni M, Tsivgoulis G. Blood biomarkers in ischemic stroke: potential role and challenges in clinical practice and research. *Crit Rev Clin Lab Sci* 2018;55(5):294-328. doi:10.1080/10408363.2018.1461190.
4. Marques FZ, Nelson E, Chu PY, *et al*. High-fiber diet and acetate supplementation change the gut microbiota and prevent the development of hypertension and heart failure in hypertensive mice. *Circulation* 2017;135(10):964-77. doi:10.1161/CIRCULATIONAHA.116.024545.
5. Eckburg PB, Bik EM, Bernstein CN, *et al*. Diversity of the human intestinal microbial flora. *Science* 2005;308(5728):1635-8.doi:10.1126/science.1110591.
6. Yin J, Liao SX, He Y, *et al*. Dysbiosis of gut microbiota with reduced trimethylamine-N-oxide level in patients with large-artery atherosclerotic stroke or transient ischemic attack. *J Am Heart Assoc* 2015;4(11):e002699. doi: 10.1161/JAHA.115.002699.
7. Cho Clara E,Caudill Marie A. Trimethylamine-N-oxide: Friend, foe, or simply caught in the cross-fire?. *Trends Endocrinol Metab* 2017;28: 121-30. doi: 10.1016/j.tem.2016.10.005.
8. Hoyles L, Jiménez-Pranteda ML, Chilloux J, *et al*. Metabolic retroconversion of trimethylamine N-oxide and the gut microbiota. *Microbiome* 2018;6(1):73. doi: 10.1186/s40168-018-0461-0.
9. Zhu W, Gregory JC, Org E, *et al*. Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. *Cell* 2016;165(1):111-24. doi: 10.1016/j.cell.2016.02.011.
10. Yang JJ, Lipworth LP, Shu XO, *et al*. Associations of choline-related nutrients with cardiometabolic and all-cause mortality: results from 3 prospective cohort studies of blacks, whites, and Chinese. *Am J Clin Nutr* 2020;111(3):644-56. doi: 10.1093/ajcn/nqz318.
11. Haghikia A, Li XS, Liman TG, *et al*. Gut microbiota-dependent trimethylamine N-oxide predicts risk of cardiovascular events in patients with stroke and is related to proinflammatory monocytes. *Arterioscler Thromb Vasc Biol* 2018;38(9):2225-35. doi:10.1161/ATVBAHA.118.311023.
12. Nie J, Xie L, Zhao BX, *et al*. Serum trimethylamine N-oxide concentration is positively associated with first stroke in hypertensive patients [published correction appears in *Stroke* 2019;50(6):e175]. *Stroke* 2018;49(9):2021-8. doi: 10.1161/STROKEAHA.118.021997.
13. Rexidamu M, Li H, Jin H, Huang J. Serum levels of trimethylamine-N-oxide in patients with ischemic stroke. *Biosci Rep* 2019;39(6):BSR20190515. doi: 10.1042/BSR20190515.
14. Chen YY, Ye ZS, Xia NG, Xu Y. TMAO as a novel predictor of major adverse vascular events and recurrence in patients with large artery atherosclerotic ischemic stroke. *Clin Appl Thromb Hemost* 2022;28:10760296221090503. doi:10.1177/10760296221090503.
15. Tan C, Wang H, Gao X, *et al*. Dynamic changes and prognostic value of gut microbiota-dependent trimethylamine-N-oxide in acute ischemic stroke. *Front Neurol* 2020;11:29. doi:10.3389/fneur.2020.00029.
16. Zhai Q, Wang X, Chen C, *et al*. Prognostic value of plasma trimethylamine N-oxide levels in patients with acute ischemic stroke. *Cell Mol Neurobiol* 2019;39(8):1201-6. doi:10.1007/s10571-019-00714-3.
17. Zhang J, Wang L, Cai J, *et al*. Gut microbial metabolite TMAO portends prognosis in acute ischemic stroke. *J Neuroimmunol* 2021;354:577526. doi: 10.1016/j.jneuroim.2021.577526.
18. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010;25(9):603-5. doi:10.1007/s10654-010-9491-z.
19. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;327(7414):557-60. doi:10.1136/bmj.327.7414.557.
20. Larsson SC, Mantzoros CS, Wolk A. Diabetes mellitus and risk of breast cancer: a meta-analysis. *Int J Cancer* 2007;121(4):856-62. doi:10.1002/ijc.22717.
21. Borenstein M, Hedges LV, Higgins JP, Rothstein HR. A basic introduction to fixed-effect and random-

effects models for meta-analysis. *Res Synth Methods* 2010;1(2):97-111. doi:10.1002/jrsm.12.
22. Craciun S, Balskus EP. Microbial conversion of choline to trimethylamine requires a glycyl radical enzyme. *Proc Natl Acad Sci U S A* 2012;109(52):21307-12. doi:10.1073/pnas.1215689109.
23. Bennett BJ, de Aguiar Vallim TQ, Wang Z, *et al*. Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metab* 2013;17(1):49-60. doi:10.1016/j.cmet.2012.12.011.
24. Tang WH, Wang Z, Levison BS, *et al*. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med* 2013;368(17):1575-84. doi:10.1056/NEJMoa1109400.
25. Wilson A, McLean C, Kim RB. Trimethylamine-N-oxide: a link between the gut microbiome, bile acid metabolism, and atherosclerosis. *Curr Opin Lipidol* 2016;27(2):148-54. doi:10.1097/MOL.0000000000000274.
26. Wu C, Li C, Zhao W, *et al*. Elevated trimethylamine N-oxide related to ischemic brain lesions after carotid artery stenting. *Neurology* 2018;90(15):e1283-e1290. doi: 10.1212/WNL.0000000000005298.
27. Seldin MM, Meng Y, Qi H, *et al*. Trimethylamine N-oxide promotes vascular inflammation through signaling of mitogen-activated protein kinase and nuclear factor-κB. *J Am Heart Assoc* 2016;5(2):e002767. doi: 10.1161/JAHA.115.002767.
28. Boini KM, Hussain T, Li PL, Koka S. Trimethylamine-N-oxide instigates NLRP3 inflammasome activation and endothelial dysfunction. *Cell Physiol Biochem* 2017;44(1):152-62. doi:10.1159/000484623.
29. Chen ML, Zhu XH, Ran L, *et al*. Trimethylamine-N-oxide induces vascular inflammation by activating the NLRP3 inflammasome through the SIRT3-SOD2-mtROS signaling pathway. *J Am Heart Assoc* 2017;6(11):e002238. doi:10.1161/JAHA.117.006347.
30. Yang C, Hawkins KE, Doré S, Candelario-Jalil E. Neuroinflammatory mechanisms of blood-brain barrier damage in ischemic stroke. *Am J Physiol Cell Physiol* 2019;316(2):C135-C153. doi:10.1152/ajpcell.00136.2018.
31. Su H, Fan S, Zhang L, Qi H. TMAO aggregates neurological damage following ischemic stroke by promoting reactive astrocytosis and glial scar formation via the Smurf2/ALK5 axis. *Front Cell Neurosci* 2021;15:569424. doi:10.3389/fncel.2021.569424.
32. Sun X, Jiao X, Ma Y, *et al*. Trimethylamine N-oxide induces inflammation and endothelial dysfunction in human umbilical vein endothelial cells via activating ROS-TXNIP-NLRP3 inflammasome. *Biochem Biophys Res Commun* 2016;481(1-2):63-70. doi:10.1016/j.bbrc.2016.11.017.
33. Li D, Ke Y, Zhan R, *et al*. Trimethylamine-N-oxide promotes brain aging and cognitive impairment in mice. *Aging Cell* 2018;17(4):e12768. doi: 10.1111/acel.12768
34. Liu M, Han Q, Yang J. Trimethylamine-N-oxide (TMAO) increased aquaporin-2 expression in spontaneously hypertensive rats. *Clin Exp Hypertens* 2019;41(4):312-22. doi:10.1080/10641963.2018.1481420.
35. Gao X, Liu X, Xu J, Xue C, Xue Y, Wang Y. Dietary trimethylamine N-oxide exacerbates impaired glucose tolerance in mice fed a high fat diet. *J Biosci Bioeng* 2014;118(4):476-81. doi:10.1016/j.jbiosc.2014.03.001.
36. Cui X, Ye L, Li J, *et al*. Metagenomic and metabolomic analyses unveil dysbiosis of gut microbiota in chronic heart failure patients. *Sci Rep* 2018;8(1):635. doi:10.1038/s41598-017-18756-2.
37. Abbasi J. TMAO and heart disease: The new red meat risk? *JAMA* 2019;321(22):2149-51. doi:10.1001/jama.2019.3910.