RESEARCH PAPER

Gut-Derived TMAO as a Biomarker for Atherosclerosis and Cardiovascular Disease: A Systematic Review and Meta-Analysis.

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ABSTRACT

Background Gut microbial metabolites, particularly trimethylamine (TMA) and its oxidized form, trimethylamine N-oxide (TMAO), contribute significantly to the progression of atherosclerosis-driven cardiovascular disease (CVD). Elevated TMAO levels are associated with an increased risk of myocardial infarction and stroke, yet their potential as predictive biomarkers remain unclear. Understanding the mechanisms linking TMAO to CVD could provide insights into the gut-heart axis and support novel therapeutic strategies.

Aims : This systematic review and meta-analysis evaluate the association between serum or plasma concentrations of TMA and TMAO with the development and progression of atherosclerosis-driven CVD.

Methods : A comprehensive systematic literature review and meta-analysis were undertaken to investigate the association between TMAO, a gut microbial metabolite, and CVD in humans.The keywords "GUT MICROBIOTA" and "CVD" were used to do a systematic search of the PubMed and Scopus databases for publications published in English up until March 2023. The results were qualitatively summarised, and in order to guarantee openness and compliance with systematic review guidelines, the project was registered with PROSPERO (CRD42024547156). Overall effect sizes were quantified by meta-analysis, and the results' robustness was assessed using extra tests for heterogeneity and publication bias. IBM SPSS Statistics 29.0 (1-month trial version) was used for statistical analysis.

Results : The database search identified 722 studies, which were segregated based on predefined inclusion and exclusion criteria. After evaluation, six studies met the eligibility criteria and were included in the meta-analysis. These studies encompassed diverse study designs and patient populations. The pooled analysis (Cohen's d = 0.65, 95% CI) revealed a significant positive correlation between elevated TMAO levels and increased cardiovascular risk. This discovery emphasises TMAO's possible function as a cardiovascular disease biomarker.

Conclusion: This meta-analysis provides compelling evidence that elevated TMAO levels are significantly associated with increased CVD risk, reinforcing its potential role as a predictive biomarker for atherosclerosis-related cardiovascular events. However, due to substantial methodological heterogeneity and population variability, the prognostic value of TMAO remains uncertain. Future studies should focus on large-scale, long-term research with consistent TMAO measurement protocols to better define its clinical relevance.Additionally, therapeutic interventions targeting gut microbial TMAO metabolism should be explored to assess their potential in CVD prevention and management..

Keywords: TMA, TMAO, atherosclerosis, cardiovascular disease, systematic review, gut microbiota, biomarkers, serum, plasma

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INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading global cause of mortality, accounting for 32% of all deaths in 2019, with 17.9 million fatalities. According to the World Health Organization, heart attacks and strokes contribute to 85% of these deaths. Recent research highlights the role of gut microbiota in cardiovascular health, with trimethylamine N-oxide (TMAO) emerging as a key biomarker linking dietary intake, microbiome composition, and CVD risk (1,2).

This is the gut-heart Axis: a meta-organismal pathway that interlinks dietary intake, microbiota, and host hepatic metabolism. Choline, phosphatidylcholine, and L-carnitine are micronutrients abundant in eggs, poultry, red meat, dairy products, and fish that undergo conversion by the gut microbiome TMA (3).

TMA then enters the bloodstream and reaches the liver, where it is oxidized to TMAO via the isoenzyme flavin monooxygenase (FMO3) (2,4). TMAO is found to contribute to CVD pathogenesis through various mechanisms including promoting atherosclerosis, endothelial dysfunction, thrombosis and platelet activation, modulation of inflammation, inhibiting the reverse cholesterol transport etc. highlighting its potential as a therapeutic target and prognostic biomarker in cardiovascular medicine. It has also been associated with the pathogenesis of atherosclerosis through multiple mechanistic pathways, both in vivo and in vitro (4).

Multiple Research has identified the CD36/MAPK/JNK pathway as critical in TMAO-induced foam cell formation, a key event in atherogenesis (5). Patients with severe atherosclerosis have consistently shown elevated plasma TMAO levels in comparison to individuals with moderate or mild forms of the disease, highlighting the biomarker's potential as an indicator of atherosclerotic burden (6).

It has also been found that TMAO promotes endothelial dysfunction, a harbinger to cardiovascular disease, through inflation in inflammation and oxidative stress of the vascular endothelium. It particularly reduces the bioavailability of NO, which is essential in maintaining endothelial function and vascular health. In expansion, TMAO has been shown to have caused platelet hyperreactivity, which promotes thrombi formation. It exerts this prothrombotic effect by modifying platelet signaling pathways, namely phosphoinositide 3-kinase and protein kinase C, and activation of thrombosis pathways, enhancing thrombin production and fibrinogen expression (7).

Moreover, TMAO promotes vascular inflammation and atherogenesis by activating inflammatory signaling pathways like mitogen-activated protein kinase and nuclear factor-kappa B. Besides, TMAO also inhibits LXRs, which is a key transcription factor required to regulate cholesterol efflux thus reducing cholesterol metabolism (8). As reported by Koeth et al., this leads to reduced cholesterol transporters like ABCA1 and ABCG1 reducing cholesterol efflux. peripheral tissues and promotes the formation of atherosclerotic plaque. Together, our results demonstrate complex role of TMAO in cardiovascular illness and point to using it as a novel diagnostic and therapeutic target for atherosclerosis and associated cardiovascular diseases (9).

One of the effective ways to reduce the risk of CVD associated with high levels of TMA is the inhibition of its synthesis and oxidation to TMAO. Dietary therapies include increasing intake of plant-based meals and reduction of intake of TMA precursors present in red meat and eggs, such as choline and L-carnitine (10). Prebiotics like inulin and probiotics with Lactobacillus and Bifidobacterium can alter gut flora to decrease the production of TMA (11). TMAO levels are efficiently lowered by the inhibition of TMA synthesis enzymes by pharmaceutical drugs such 3,3-dimethyl-1-butanol (DMB) (12,13). Naturally occurring substances that influence gut microbiota and prevent TMA synthesis include resveratrol and berberine (14). In addition to decreasing TMA development, faecal microbiota transplantation can reconstitute a healthy gut flora. While several studies have demonstrated a relationship between high TMAO levels and poor cardiovascular outcomes, the prognostic role of the measurement of TMAO in subjects with CVD remains controversial and an area of continuous investigation (15,16).

In this context, to assess the potential predictive significance of high blood levels of TMAO and the incidence of CVD events, we undertook a systematic review of published studies that investigated this relationship prospectively.

Thus, in this context, even after adjustment for traditional risk factors, such as age, blood pressure, sex, cholesterol, and lifestyle, the levels of TMAO remained a strong predictor of CVD risk. Patients with established atherosclerotic disease, older adults living in the community, and individuals with numerous cardiovascular risk factors were among the groups whose predictive significance of TMAO was investigated (17,18).

Despite variances in study design and populations, the link between elevated TMAO and increased cardiovascular risk is consistent. Understanding the predictive relevance of TMAO also opens the door to new treatment therapies. For instance, research on animal models has investigated the use of 3,3-dimethyl-1-butanol (DMB), a TMAO inhibitor, to lower TMAO levels and lessen the risk of

cardiovascular disease. TMAO can improve risk classification and perhaps direct preventive and therapeutic measures by offering added predictive power beyond conventional risk indicators (12,13).

METHODS

This systematic review and meta-analysis adhered to PRISMA guidelines and was registered with PROSPERO (CRD42024547156) to maintain methodological transparency.

LITERATURE SEARCH STRATEGY

This study adhered to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines **(Fig. 1)**. PubMed and SCOPUS were systematically searched for studies published up to April 2023 using the keywords 'gut microbiota' and 'CVD'. Only English-language studies were considered. Two authors independently reviewed titles and abstracts, and discrepancies were resolved through discussion. In addition, the researchers retrieved the full texts of all potential articles to look for any additional relevant studies. Abstracts, meeting proceedings, personal communications, conference papers, book chapters were not used for this study. This systematic review was registered on PROSPERO with CRD42024547156 an international database of prospectively registered systematic reviews in social care and Health, public health, justice, international development, crime and Education, where there is a health-related outcomes.

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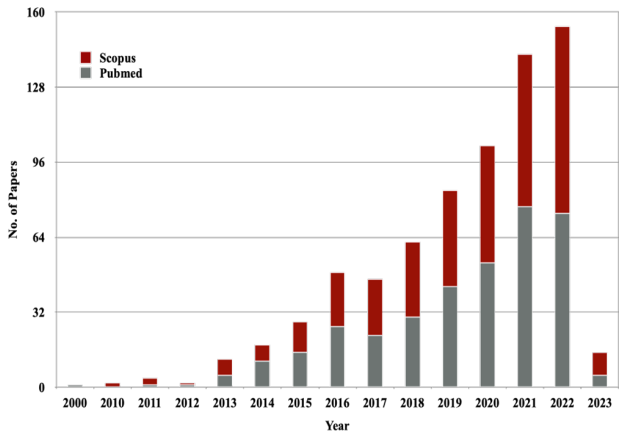

Figure 1: Number of studies related to "Gut Microbiota" and "CVD" from 2000 to 2023 included in the study. The stacked bars show the number of studies from PUBMED (gray) and SCOPUS (maroon). The chart highlights a growing research interest over time, with a notable increase in recent years.

Eligibility Criteria

Studies were incorporated if they estimate the association of plasma/serum TMAO levels and mortality, any cardiovascular events listed as either categorical or continuous variables.

Studies were omitted if any of the following conditions applied

Duplicates. Reviews, notes, book chapters, systematic reviews and meta-analysis,

Lack of TMAO serum and Plasma conc data.

That did not involve any CVDs or that only had data about CKD

That had a combination of different diseases like obesity, diabetes etc

The number of patients included was <100 6. That did not have control data.

Data Extraction

Two researchers (AN and KCP) independently assessed papers to determine if they met the inclusion criteria and extracted the relevant characteristics for each study (author, year, country, study design), population specifics (number of participants, age, gender distribution, health status, duration of the follow-up,), and methodology (study objectives, TMAO measurement methods, and definitions of cardiovascular outcomes). Additionally, results were meticulously recorded, focusing on baseline TMAO levels, statistical associations between TMAO and cardiovascular outcomes, and adjustments for confounding variables.

Conclusions from each study were summarized, highlighting key findings, clinical implications, and potential preventive measures. This extraction process was performed independently by two reviewers to minimize discrepancies, with any disagreements resolved through discussion or consultation with other authors.

Estimates and their 95% confidence intervals (CI) for plasma /serum TMAO concentration in relation to CVD were extracted exactly as presented in the original reports, including 10μM or per 1- SD increment, median and interquartile range (IQR) for the highest vs the lowest categories (eg. tertiles, quartiles or quintiles) of TMAO, as reported in supplementary material.

After screening of abstracts, six studies were included for meta-analysis consisting of 2897 CVD patients and 6858 healthy controls. Plasma/ serum TMAO conc was considered as the main variable for the study. Some of whose data formats were in median and IQR were converted to mean and Standard deviation (SD) using an online tool (https://www.math.hkbu.edu.hk/~tongt/papers/median2mean.html) that calculates the mean and SD based on Shi et al. 2023. Data for the Meta-analysis was prepared in accordance with the SPSS meta-analysis package requirement (20,21,22,23,24).

Identification and selection of studies

722 possibly pertinent studies were found using PUBMED and SCOPUS searches. 29 were thought to be possibly eligible based on the abstract and/or title. Following this, twenty-three studies were eliminated: fourteen of them did not fit the predetermined inclusion criteria. 5 included dietary intervention and CVD. Two studies involved small molecule inhibition studies. One included only fCAD. One was a duplicate of a study already included in the study.

Finally, six studies were included in our systematic review and meta-analysis **(Fig. 2).** The final six studies were included and represented in **Table 1.**

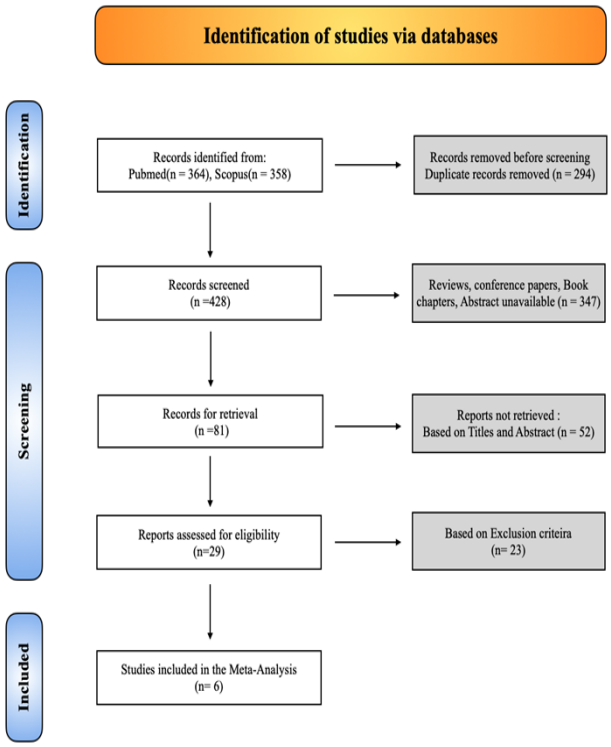

Figure 2: PRISMA workflow of the study illustrating the systematic process of identifying studies for inclusion in a meta-analysis

Table 1: Final six studies included in the Meta-analysis

First Author	Year	Follow up Length (Years)	Number of participants	Outcome of the study
Amrein et al. 2022	2022	5	1726	In patients with suspected fCAD, TMAO was a powerful predictor of incident mortality and CVD.
Tang et al. 2021	2021	8	2181	The incident development of coronary artery disease is linked to increased TMAO levels.
Lee et al. 2021	2021	7	1287	An increased risk of acute ASCVD, with an apparent change attributable to impaired renal function, and an increased risk of recurrent ASCVD were linked to serial measures of TMAO.
Zheng et al. 2018	2018	4.8	172	Positive correlation exists between TMAO and CVD risk in the future.
Sentheng et al. 2021	2021	-	134	Patients at high risk of atherosclerosis can predict SMD independently using fasting plasma TMAO.
Yang et al. 2022	2022	1	124	elevated plasma level In PAH, TMAO was linked to a poor prognosis and severity of the disease, suggesting that it may have a bright future as a biomarker.

Included studies

TMAO and fCAD

Melissa Amrein et al. conducted on 1,726 Individuals with suspected actively relevant coronary artery disease (fCAD), aimed to evaluate the diagnostic and prognostic value of serum TMAO and its precursors (Carnitine, Choline, and betaine). Patients suspected to have fCAD were recruited from the University Hospital of Basel, Switzerland. TMAO was quantified using LC-MS/MS and performed fCAD diagnosis. The study followed up on myocardial infarction (MI), cardiovascular disease (CVD), and all-cause death over a period of five years. They concluded that TMAO and its precursors have limited diagnostic value for fCAD but are strong predictors of death and CVD, with TMAO showing robust prognostic accuracy. Therefore, stated that TMAO is a significant prognostic marker for adverse outcomes in fCAD patients.

TMAO in Community-Based Populations

Tang et al. conducted a community-based study in Norfolk, UK, involving a middle-aged, apparently healthy European population. The investigation aimed to evaluate the relationship between the onset of cardiovascular disease (CVD) and the baseline fasting levels of TMAO and its nutritional precursors, choline and betaine. Utilizing a case-control design, the study compared 908 participants who developed CVD with 1,273 controls over an average follow-up period of 8 years. Results showed that participants who developed CVD had significantly higher plasma levels of TMAO (3.70 µM vs. 3.25 µM, $p<0.001$) and choline (9.09 µM vs. 8.89 µM, $p=0.001$). The prognostic utility of TMAO was robust across various cutoff levels. The study concludes that elevated plasma TMAO and choline levels are significant predictors of

CVD risk in apparently healthy individuals, independent of traditional risk factors.

In the cardiovascular health study reported by Yujin Lee et al., they conducted a community-based cohort study of older adults in the United States, involving 4,131 participants for incident atherosclerotic cardiovascular disease (ASCVD) and 1,449 participants for recurrent ASCVD. The objective was to investigate the relationship between serum plasma TMAO levels, renal function, and the risk of incident and recurrent ASCVD. TMAO levels were measured at baseline and after 7 years using the LC-MS/MS.

ASCVD events were independently reviewed from medical records, and risk was analyzed using multivariable Cox proportional hazards regression, adjusting for demographics, lifestyle, medical history, lab measures, and diet. The study found that higher TMAO levels were associated with an increased risk of incident ASCVD (HR for extreme quintile: 1.21, p-trend=0.029), particularly in those with impaired renal function (eGFR <60 mL/min/1.73 m^2: HR 1.56, p-trend=0.007). TMAO levels also predicted recurrent ASCVD (HR 1.25, p-trend=0.009) without significant modification by renal function. Their study concludes that elevated TMAO levels are associated with higher risks of both incident and recurrent ASCVD, with renal function acting as a modifying factor.

"In a nested case-control study by Liqiang Zheng et al., participants were recruited from a rural community-based prospective cohort study conducted between June and September 2012. The study involved 4,157 individuals aged 35 and above from nineteen rural villages and two towns, Anmin and Helong, located in Xifeng County, Liaoning Province, China." After inclusion and exclusion, a total of 86 newly diagnosed CVD cases were included with a median follow-up period of 4.83 years, including 86 controls. Their goal was to assess the association of baseline TMAO levels with future risks of CVD events. The TMAO levels were measured using liquid chromatography-tandem mass spectrometry; at baseline, the new CVD cases expressed significantly higher median levels of TMAO in comparison with the controls: 1.57 versus 0.68 μmol/L, $P < 0.001$. Those with higher TMAO levels, ≥1.05 μmol/L, had higher significant odds to develop CVD after adjustment for multiple variables. Moreover, the predictive model with TMAO showed better risk discrimination for CVD in comparison to a model without TMAO. Indeed, the study concluded that the TMAO level is positively related to future risk of CVD and TMAO may be considered as a novel preventive target for the management of low-risk CVD individuals.

TMAO and Subclinical Myocardial Damage

The study conducted by Vichai Senthong et al. in Thailand as part of the Cohort of Patients at a High Risk of Cardiovascular Events (CORE-Thailand) registry, involved 134 patients, predominantly with established atherosclerotic disease. Their aim was to study the connection between plasma TMAO levels and early signs of heart muscle damage, as measured by high-sensitivity cardiac troponin-T (hs-cTnT). The levels of Plasma TMAO were measured using NMR spectroscopy. The cohort had a mean age of 64 ± 8.9 years, and 61% were men. Results revealed a significant correlation between TMAO and hs-cTnT levels ($r = 0.54$; $p < 0.0001$), with higher TMAO levels in patients exhibiting subclinical myocardial damage (hs-cTnT ≥ 14 ng/L; 4.48 μM vs. 2.98 μM, $p < 0.0001$). The study found that plasma TMAO functions as an independent predictor of subclinical myocardial damage in this population, even after controlling conventional risk factors. Increased TMAO levels were independently linked to subclinical myocardial damage (adjusted OR: 1.58; 95% CI 1.24–2.08; $p = 0.0007$).

TMAO and Pulmonary Arterial Hypertension (PAH)

Yang et al. evaluated in research carried out at Fuwai Hospital whether levels of circulating TMAO could turn into a biomarker of PAH and if the TMAO inhibitor, 3,3-dimethyl-1-butanol, might exert protective effects in rats with MCT-induced PAH.

In this study, they included 124 patients with PAH, and the research team collected the fasting blood samples at the first and second hospitalization for measuring TMAO content. In addition, an MCT-induced PAH rat model was fed a regular diet or a diet containing 1% DMB for 4 weeks. The results indicated a correlation between high TMAO levels and increased disease severity and poor prognosis in PAH patients, even after adjusting for confounding factors. "Supplementation with DMB in the animal model resulted in reduced TMAO levels, improved hemodynamic parameters, reduced right ventricular hypertrophy, and ameliorated pulmonary vascular remodeling. In addition, DMB treatment reduced abnormal apoptosis, excessive cell proliferation, and the expression of transforming growth factor-β, while restoring endothelial nitric oxide synthase. Their study ultimately concluded that DMB has protective benefits in the MCT-induced PAH rat model and that increased levels of TMAO are linked to poor prognoses in PAH patients.

Meta-analysis

The meta-analysis was performed to analyze the relationship between serum TMAO concentrations and CVD by combining data from the final six studies using random-effects models to account for the variability in effect sizes across studies. The DerSimonian and Laird method was used to estimate between-study variance, allowing for a weighted average of effect sizes to be calculated. Statistical heterogeneity was calculated using the I^2 statistic, τ^2 H^2. I^2 statistic, which quantitates the percentage of total variation across studies attributable to heterogeneity rather than chance. Publication bias was assessed using funnel plots and Egger's test. All analyses were conducted using SPSS 29.0 software, with a significance level of $p < 0.05$ applied for hypothesis testing.

RESULTS

Meta-analysis

The final analysis was performed for the selected six studies and the overall effect size was found to be a Cohen's d of 0.65, indicating a moderately strong effect size, i.e diseased has a significant effect on the blood /plasma TMAO concentration in a way that is statistically and practically significant. The I^2 value of 99% indicates that there is considerable heterogeneity, meaning that almost all the observed differences in effect sizes are due to actual differences between the studies. The τ^2 value of 0.29 confirms the presence of moderate between-study variance. The H^2 value of 72.96 further supports the presence of substantial heterogeneity, emphasising that the variability among the study results exceeds what would be expected by chance **(Table 2).**

Table 2: Model summary

Model SUMMARY	
Heterogeneity	Tau squared = 0.29 H-squared = 72.96 I-squared = 0.99
Test of Overall effect size	z= 2.86 p-value= 0.00

The forest plot **(Fig. 3)** highlights the variability in effect sizes across studies, ranging from 0.21 (Yujin Lee et al.) to 1.71 (Yicheng Yang et al.). Larger blue squares in the plot reflect studies with greater weight in the meta-analysis due to larger sample sizes or lower variance, while narrower confidence intervals (CIs) suggest more precise estimates. The overall effect size, depicted by the diamond at the bottom of the plot, confirms a moderate positive association between elevated TMAO levels and increased CVD risk (95% CI: 0.20 to 1.09, p = 0.00).

This significant heterogeneity implies that the effects of TMAO on CVD may change among populations or research conditions, necessitating cautious interpretation and more inquiry into the causes of this variability. Baseline characteristics of participants included in the studies are summarized in **Table 3.**

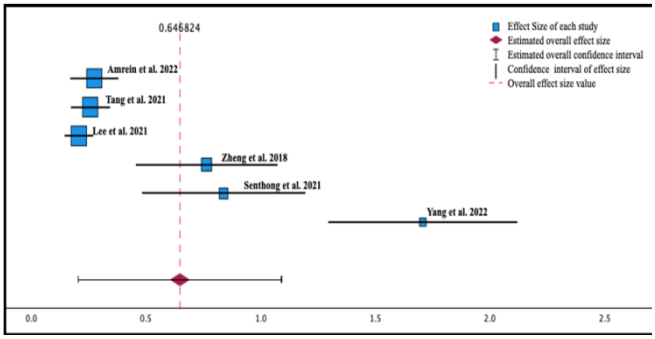

Figure 3: Forest plot showing the meta-analysis of TMAO and atherosclerosis-driven CVD. Each blue square represents a study's effect size, with size indicating weight; horizontal lines denote confidence intervals. The red diamond represents the pooled effect size and its confidence interval. The dashed red line marks the overall effect size for comparison.

Table 3: Baseline characters of included in the studies

ID	**Cohen's d**	**Std. Error**	**Lower**	**Upper**	**p-value**	**Weight (%)**
Amrein et al. 2022	0.27	0.05	0.17	0.38	0.00	3.42
Tang et al. 2021	0.26	0.04	0.17	0.34	0.00	3.43
Lee et al. 2021	0.21	0.03	0.14	0.27	0.00	3.44
Zheng et al 2018	0.76	0.16	0.45	1.07	0.00	3.18
Senthong et al. 2021	0.84	0.18	0.48	1.19	0.00	3.10
Yang et al. 2022	1.71	0.21	1.30	2.12	0.00	3.00
Overall	0.65	0.23	0.20	1.09	0.00	

Although the findings generally point to TMAO as a possible biomarker for CVD, the significant variation among studies suggests that more research is needed to completely comprehend the variables behind these variations and to improve the potential of TMAO as a cardiovascular risk predictor.

Publication bias

A p-value of 0.873 was obtained when the Egger's regression test was used to estimate possible publication bias. This finding implies that there isn't any significant evidence of publication bias. This is further supported by the funnel plot, which displays no discernible asymmetry. However, the test's ability to identify bias may be diminished due to the small number of papers included in this study, so publication bias cannot be completely ruled out.

Particularly in the study by Yang et al., the funnel plot showed some asymmetry, which could be a sign of bias or heterogeneity. Outliers and skewness indicate that additional research is necessary to rule out any underlying biases or study-specific variables contributing to the reported effects, even if most studies were symmetrically distributed around the overall impact size **(Fig. 4).**

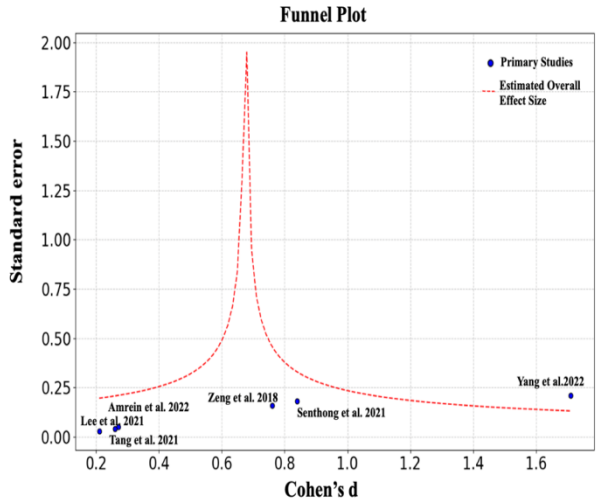

Figure 4: Funnel plot of the meta-analysis assessing publication bias in the studies. Each point represents a primary study included in the meta-analysis, with Cohen's d effect size on the x-axis and the standard error on the y-axis. The solid blue dots denote individual studies, while the red dashed line indicates the estimated overall effect size. The symmetry of the plot suggests no significant publication bias.

Studies outside of the 95% pseudo-confidence intervals are indicated by dotted lines, which suggest possible bias or heterogeneity in the meta-analysis. Although there was no discernible publishing bias overall, the existence of outliers calls for a careful interpretation of the results.

DISCUSSION

The outcomes of this systematic review and meta-analysis support the notion that TMAO is a reliable and independent indicator of the risk of CVD in a variety of clinical contexts and demographics. In addition to more severe signs of subclinical myocardial damage and pulmonary arterial hypertension (PAH), elevated TMAO levels were consistently linked to an increased risk of incidents and recurring cardiovascular events. Crucially, these correlations remained after controlling traditional cardiovascular risk variables, highlighting the potential of TMAO as a reliable biomarker for CVD risk assessment (3).

The significant heterogeneity identified in this meta-analysis (I^2 = 99%) suggests considerable variation in effect sizes. This variability may be attributed to differences in study design, population demographics, dietary habits, genetic factors, and methods of TMAO measurement. Variations in dietary intake of TMAO precursors, such as choline and carnitine, likely contribute to regional differences in TMAO-associated CVD risk. Additionally, the comparability of results may be impacted by the additional variability introduced using various analytical techniques for TMAO quantification among investigations (9).

Further quantifying the heterogeneity, the high H^2 value and between-study variance (τ^2) show that the variation in effect sizes reflects actual differences in study outcomes rather than being a result of sampling error. This emphasises the need for standardising study techniques in future research and the necessity for caution when extrapolating the findings to various populations. Significant variation also implies that although TMAO is a useful biomarker, population-specific characteristics may affect its predictive potential, requiring customised risk assessment techniques (25).

Despite these difficulties, Egger's test confirms that there is no publication bias, which strengthens the meta-analytic findings. The validity of TMAO as a predictive biomarker is further supported by this observation, which implies that the observed relationships between TMAO levels and CVD risk are unlikely to be caused by selective reporting of positive results.

These results highlight the necessity for more research into the molecular mechanisms connecting TMAO to CVD in the larger framework of cardiovascular studies. Although the precise biological processes are still unclear, new research indicates that TMAO may contribute to atherosclerosis and other cardiovascular disorders by means of pathways involving inflammation, endothelial dysfunction, and cholesterol metabolism. Comprehending these processes will be essential for creating focused treatment plans meant to alter TMAO levels to reduce the risk of CVD (7).

Future research should prioritize longitudinal studies and clinical trials with standardized protocols to validate the prognostic value of TMAO and to explore its potential as a therapeutic target. Given the significant heterogeneity observed, it will be essential to investigate the interplay between TMAO and other risk factors in diverse populations to develop more precise and individualized risk prediction models. Additionally, exploring the potential benefits of dietary interventions or pharmacological agents that reduce TMAO levels could provide new avenues for CVD prevention and treatment (25, 26).

This meta-analysis concludes by bringing forth strong evidence for TMAO's role as a fundamental biomarker that determines cardiovascular risk. However, the high amount of heterogeneity observed underlines the need to standardize study methodologies and also to investigate these biological mechanisms underlying the results more deeply. Only then will full potential of TMAO, both as a prognostic tool and therapeutic target in the area of cardiovascular medicine, be realized.

CONCLUSION

In summary, estimates of TMAO levels have been consistently related to increased risk and poor prognosis in cardiovascular and pulmonary diseases. Although TMAO has some limited diagnostic applications, its potent prognostic capabilities and as a therapeutic target make it very important in the current medical sciences. The incorporation of TMAO measurement in clinical practice will improve risk stratification and inform preventive and

therapeutic strategies for cardiovascular and pulmonary diseases.

Study Limitations: Diversity in TMAO measurement procedures, as well as demographic heterogeneity, might limit the generalizability of findings. These relationships need to be confirmed, and the mechanisms underlying them explored, through longitudinal research using standardized methodology and diverse populations. More clinical trial-based research is also needed to find out if the inhibition of TMAO possesses any therapeutic benefit, as postulated by the preclinical studies. Subgroup analysis was not conducted because there were fewer studies that were included in the meta-analysis.

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Conflict of interest statement
The authors declare that there is no conflict of interest.

Data availability statement
The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions
AN conducted the experimental studies, performed data acquisition and analysis, and prepared the manuscript. AN, KCP defined the intellectual content of the study and conducted a literature search. BH was involved in defining the intellectual content and contributed to editing and reviewing the manuscript. AN, SKS carried out both data analysis and statistical analysis. CS, RGA, and SK led the conceptualization and study design, supervised manuscript editing and review, and served as the guarantor of the study. All authors have read and approved the final manuscript.

Data Availability Statement
All the data originated from this research is available from the authors upon request.

Competing Interest statement
The authors declare that there is no competing interest.

Conflict of Interest
The authors declare that the study was not conducted under any conflict of interest.

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