

The Gut Microbial Metabolite Trimethylamine N-Oxide is Linked to Specific Complications of Systemic Sclerosis

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Background: Systemic sclerosis (SSc) is a rare immune-mediated connective tissue disease characterized by fibrosis of the skin and internal organs, whose pathogenesis is not fully understood. Recent studies have revealed dysbiosis in patients with systemic sclerosis and have indicated the possible role of the microbiota and its metabolites in the pathogenesis of the disease. Trimethylamine N-oxide (TMAO) is a compound produced by dysbiotic microbiota observed at higher concentrations in several autoimmune diseases.

Objective: To determine concentrations of the bacteria-derived metabolite TMAO in patients with systemic sclerosis and to assess possible correlation between TMAO and a specific manifestation of the disease.

Patients and Methods: The study included 63 patients with SSc and 47 matched control subjects. The concentration of TMAO was measured with high-performance liquid chromatography.

Results: Plasma TMAO level was significantly increased in patients with SSc (283.0 [188.5–367.5] ng/mL versus 205.5 [101.0–318.0] ng/mL; $p < 0.01$). An increased concentration of TMAO was observed in patients with concomitant interstitial lung disease (ILD) (302.0 ng/mL [212.0–385.5] ng/mL versus 204.0 [135.5–292.0] ng/mL; $p < 0.01$) and esophageal dysmotility (289.75 [213.75–387.5] ng/mL versus 209.5 ng/mL [141.5–315.0] ng/mL; $p < 0.05$) compared to patients without these complications. Furthermore, TMAO concentration exhibited significant correlation with markers of heart involvement (left ventricle ejection fraction, NT-proBNP), marker of ILD severity and Scleroderma Clinical Trials Consortium Damage Index.

Conclusion: The concentration of TMAO, gut microbiota-associated metabolite, is increased in systemic sclerosis, particularly in patients with advanced organ involvement. This is the first study evaluating plasma TMAO in systemic sclerosis. Bacterial metabolites may be a link between dysbiosis and organ involvement in the course of the disease. Modulation of gut bacterial-derived metabolites may represent a new therapeutic approach in the management of systemic sclerosis.

Keywords: gut microbiota, systemic sclerosis, trimethylamine N-oxide, bacterial metabolites, dysbiosis, damage index

Introduction

Systemic sclerosis (SSc) is an immune-mediated connective tissue disease. It is characterized by fibrosis of the skin, internal organs (eg, lungs, gastrointestinal tract) and vascular abnormalities. Based on the extent of skin involvement, SSc is commonly classified into two main subsets: diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc).^{1–3} To date, the pathology of systemic sclerosis remains not fully elucidated and there is a lack of established biomarkers of SSc severity and organ involvement.^{4,5} Early intervention with immunomodulators in patients with SSc may lead to better outcome.^{6,7}

Recently, there has been a growing number of studies presenting alteration in intestinal microbiota (dysbiosis) in patients with systemic sclerosis and its impact on the disease. Gut microbiota profiling in systemic sclerosis revealed significant changes in its biodiversity and composition compared to healthy controls. Patients with SSc are characterized

by an increased abundance of *Fusobacterium*, *Desulfovibrio*, *Ruminococcus*, and *Lactobacillus*, and a decreased abundance of *Faecalibacterium*.^{8–10} Furthermore, some of the SSc complications, such as interstitial lung disease (ILD) and esophageal dysmotility, are associated with more pronounced dysbiosis.^{11,12} Currently, research on modifying microbiota in SSc is emerging. Some studies have shown that probiotics or fecal microbiota transplantation may improve SSc, particularly gastrointestinal symptoms of the disease.^{13–16}

In systemic sclerosis, metabolic proficiencies of gut microbiota also differ from the control group;⁹ thus, it is highly probable that patients are exposed to a different spectrum of bacterial substances. This exposure can be magnified by vascular abnormalities and intestinal involvement¹⁷ which increase intestinal permeability.^{18,19} The pathways by which microbiota can regulate human homeostasis have recently been intensively investigated. Among them, the impact of microbiota metabolites is the most direct and has been the best described. Some bacterial metabolites present in increased concentrations in the state of dysbiosis are considered detrimental to homeostasis. These metabolites include trimethylamine N-oxide (TMAO).^{20,21} TMAO is known for its pro-inflammatory properties, which exacerbate several diseases, especially atherosclerosis, chronic kidney disease and type 2 diabetes.^{22–24}

TMAO is a small molecule that is transformed by the liver from bacterial metabolite – trimethylamine (TMA).^{22,25} The literature reports that the concentration of plasma TMAO is increased in conditions such as cardiovascular diseases,^{26–28} and renal insufficiency,²⁹ also in rheumatic diseases such as psoriatic arthritis,³⁰ and rheumatoid arthritis.³¹ Furthermore, a recent pre-clinical study has found that TMAO significantly increases the expression of extracellular matrix proteins associated with fibrosis and the major profibrotic cytokine TGF- β 1, which are the key elements of SSc pathomechanism.³²

The study aimed to determine concentrations of the bacteria-derived metabolite TMAO in plasma of SSc patients as well as assess potential correlation between plasma TMAO concentration and various clinical (age, disease subtype, concomitant lung, gastrointestinal, or cardiac involvement) and metabolic factors in SSc.

Materials and Methods

Subject

The study included 63 adult patients diagnosed with systemic sclerosis based on the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2013 classification criteria. Patients were recruited from the Department of Dermatology at the Medical University of Warsaw between February 2021 and January 2022.

Exclusion criteria included gastrointestinal infection during the last 3 months before the study, concomitant chronic gastrointestinal disorder (eg, Crohn's disease, celiac disease), any gastrointestinal surgical procedures or unexplained weight loss during the last 6 months prior to the study, dietary restrictions or intake of medications modulating gut microbiota (antibiotics, probiotics or prebiotics) within the previous 3 months, chronic liver and pancreatic disease, other autoimmune diseases, estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m², pregnancy, and breastfeeding.

The control group consisted of 47 individuals who were matched for age, body mass index (BMI), and gender. Control group subjects fulfilled the same exclusion criteria.

Clinical Assessment

Detailed medical history was obtained from all participants. The extent of skin involvement was analyzed with a modified Rodnan skin score (mRSS). Microvascular abnormalities assessed in capillaroscopy were classified into early, active, or late scleroderma patterns according to Cutolo et al.³³ To evaluate the gastrointestinal manifestation of systemic sclerosis a barium swallow X-ray was performed. To determine lung involvement, high-resolution computed tomography (HRCT) and pulmonary function tests, including diffusing capacity for carbon monoxide (DLCO), were performed. Cardiac involvement was assessed in echocardiography and by measuring N-terminal pro-B-type natriuretic peptide (NT-proBNP). To quantify organ damage in the course of SSc, we used Scleroderma Clinical Trials Consortium Damage Index (SCTC-DI).

Laboratory Assessment

Patients have the following tests performed: complete blood count, erythrocyte sedimentation rate glucose, lipid, kidney and liver profiles. Venous blood samples were taken for analysis following a 12-hour fast. Estimated glomerular filtration

rate (eGFR) was calculated using the CDK-EPI equation. Antinuclear antibodies were detected and identified with immunoblot assay.

Plasma TMAO concentration was measured using high-performance liquid chromatography (HPLC) coupled with triple-quadrupole mass spectrometry as previously described.^{34,35}

Statistical Analysis

The statistical software STATISTICA 13.3 was used for all calculations (TIBCO, Palo Alto, CA, USA). The Shapiro–Wilk test was used to determine if the distribution of the data was normal. The mean with standard deviation were used to represent normally distributed data, while the median and interquartile range (IQR) were used to express non-normally distributed variables. A chi-squared test was used to compare categorical data that were presented as counts and percentages. For two independent samples, a Student's *t*-test after assessment of the equality of variances by Levene's test, or Mann–Whitney *U*-test was used to assess the continuous variables that were parametric and nonparametric, respectively. To evaluate potential linear correlations between two continuous variables, the Spearman rank test with correlation coefficient was applied. P-values <0.05 were considered to be statistically significant.

Ethics

The study was approved by the Regional Bioethical Committee at the Medical University of Warsaw (KB/136/2021). Written informed consent was obtained from all participants. The study has been carried out in accordance with the Declaration of Helsinki.

Results

Characteristics of the Study Group

The study included 63 SSc patients (57 women and 6 men) and 47 controls (42 women and 5 men) matched for age, sex and BMI. The mean age of patients and volunteers was 59.9 and 55.5 years, respectively. The SSc cohort consisted of 34 (54.0%) individuals with dcSSc and 29 (54.0%) individuals with lcSSc based on LeRoy's criteria.

Detailed characteristics of SSc patients including demographical, clinical, and serological features are presented in Table 1.

TMAO in Systemic Sclerosis

The median concentration of TMAO in the group of patients with systemic sclerosis was 283.0 ng/mL (IQR 188.5–367.5) and it was significantly higher compared to the control group (205.5 ng/mL, IQR 101.0–318.0, $p = 0.006$, Figure 1).

Furthermore, we found a significant moderate correlation between TMAO concentration and the age of patients ($\rho = 0.446$; $p = 0.0002$; Figure 2).

When patients were categorized according to antinuclear antibodies, there were no significant differences between patients with positive anti-RNA polymerase III (298.5 ng/mL, IQR 230.5–368.0), antitopoisomerase I (262.5 ng/mL, IQR 169.0–340.0) and anticentromere antibodies (284.7 ng/mL, IQR 189.0–345.0).

TMAO and Skin Involvement

There was no significant difference between patients with lcSSc (280.7 ng/mL; IQR 183.5–330.7) and dcSSc (287.7 ng/mL; IQR 185.2–375.7). No significant correlation was found between TMAO concentration and skin involvement measured by mRSS.

TMAO level did not differ between SSc patients presenting early (284.7 ng/mL; IQR 206.2–632.0), active (253.5 ng/mL; IQR 178.0–315.0), and late (308.7 ng/mL; IQR 204.0–366.0) patterns in NVC.

Table I The Main Characteristics of SSc Patients and the Control Group

	SSc Patients (n = 63)	Control Group (n = 47)	p-value
Age, years	59.9±12.7	55.5±14.4	0.09
Sex			0.85
Men, n (%)	6 (5.4%)	5 (4.5%)	
Women, n (%)	57 (51.8%)	42 (38.2%)	
Body mass index, kg/m ²	24.1±3.4	24.8±3.2	0.11
Disease subset, n (%)			
Limited cutaneous SSc	29 (46%)	-	-
Diffuse cutaneous SSc	34 (54%)	-	-
Modified Rodnan skin score	5 [2–9]	-	-
Disease duration, years	8.5 [4–14]	-	-
Autoantibody positivity, n (%)			
Antinuclear autoantibodies (ANA)	62 (98.4%)	-	-
Anticentromere autoantibodies (ACA)	27 (42.9%)	-	-
Antitopoisomerase I autoantibodies (ATA)	29 (46.0%)	-	-
Anti-RNA polymerase III autoantibodies (ARA)	4 (6.3%)	-	-
Interstitial lung disease, n (%)	47 (74.6%)	-	-
Pulmonary hypertension, n (%)	2 (3.2%)		
Esophageal dysmotility, n (%)	37 (58.7%)	-	-
Current Immunosuppressive therapy, n (%)			
Methotrexate	13 (20.6%)	-	-
Mycophenolate mofetil	24 (28.0%)	-	-
Current vasoactive therapy, n (%)			
Alprostadil	42 (66.7%)	-	-
Calcium channel antagonist	15 (23.8%)	-	-
Phosphodiesterase 5 inhibitors	26 (41.3%)	-	-
Sulodexide	48 (76.1%)	-	-

TMAO and Interstitial Lung Disease

A comparison of TMAO concentration in the group of patients showed significantly higher TMAO levels in SSc patients with interstitial lung disease (Figure 3). The median concentration of TMAO in the subgroup with ILD was 302.0 ng/mL (IQR 212.0–385.5) compared to 204.0 ng/mL (IQR 135.5–292.0) in the subgroup without this complication ($p = 0.001$). Furthermore, DLCO, a marker of the restriction caused by ILD, showed a significant negative correlation with TMAO level ($\rho = -0.53$; $p = 0.013$ (Figure 4).

TMAO and Gastrointestinal Manifestations of the Disease

In SSc patients with concomitant esophageal dysmotility (confirmed in barium swallow) TMAO concentration was significantly higher (289.75 ng/mL [IQR 213.75–387.5]) than in subgroup with normal esophageal passage (209.5 ng/mL [IQR 141.5–315.0] $p = 0.026$, Figure 5).

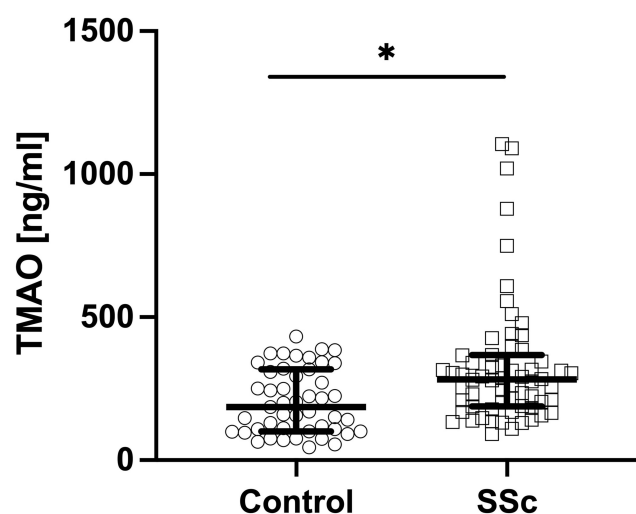


Figure 1 Plasma concentration of trimethylamine N-oxide (TMAO) in patients with systemic sclerosis compared to controls (* $p < 0.05$).

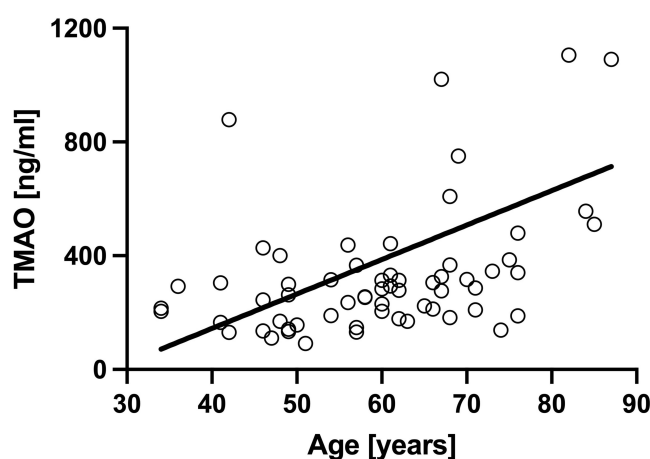


Figure 2 Correlation of TMAO with the age of patients with SSc ($\rho = 0.446$; $p < 0.001$).

TMAO and Cardiac Involvement of SSc

Spearman correlation coefficient revealed a significant negative correlation between TMAO plasma concentration and left ventricle (LV) ejection fraction in echocardiography ($\rho = -0.39$; $p = 0.009$, Figure 6), and a significant positive correlation between TMAO and NT-proBNP concentration ($\rho = 0.41$; $p = 0.0009$, Figure 7).

TMAO and Organ Damage in SSc

There was a significant correlation ($\rho = 0.78$; $p = 0.0001$) between TMAO concentration and the Scleroderma Clinical Trials Consortium Damage Index (SCTC-DI) as shown in Figure 8.

Discussion

This work provides evidence that intestinal microbiota metabolite TMAO is associated with some specific complications of systemic sclerosis. To the best of our knowledge, this is the first human study investigating microbiota metabolite TMAO in SSc. We found that this compound is present in a greater concentration in patients with SSc compared to the age-, sex-, and BMI-matched control subjects. The possible explanation for this could be that bacterial genera which are more abundant in the dysbiotic microbiota of SSc patients (ie, *Fusobacterium*, *Desulfovibrio*, *Ruminococcus*) have been observed to be linked with

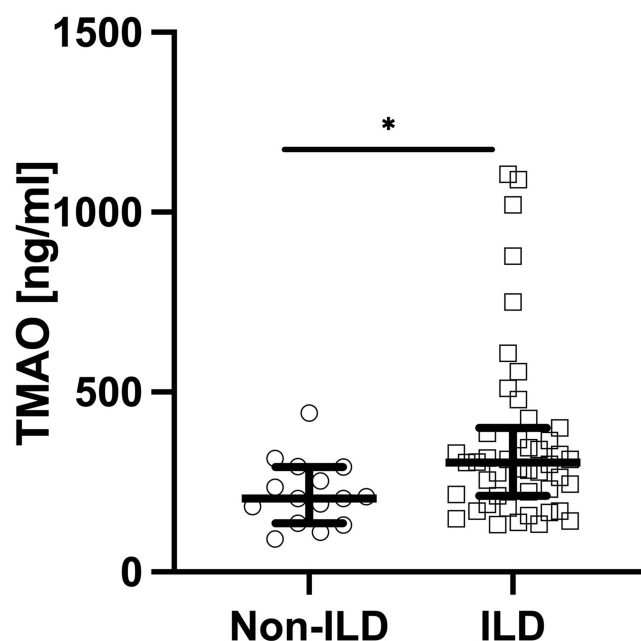


Figure 3 Plasma concentration of trimethylamine N-oxide (TMAO) in SSc patients with interstitial lung disease (ILD) and without this complication (* $p < 0.05$).

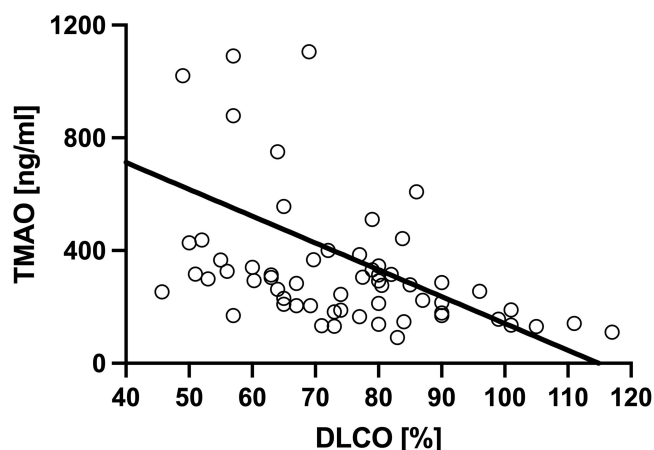


Figure 4 Correlation of trimethylamine N-oxide (TMAO) with diffusing capacity of lung for carbon monoxide (DLCO; $\rho = -0.53$; $p < 0.05$).

greater plasma TMAO concentrations.³⁶ Moreover, dysbiosis with an abundance of these bacteria has been observed in conditions such as end-stage renal disease,³⁷ pulmonary arterial hypertension,³⁸ and cardiac failure.³⁹

Additionally, SSc patients with concomitant interstitial lung disease and reflux disease were characterized by an increased level of TMAO compared to patients without mentioned comorbidities. Significant correlations between the TMAO level and markers of cardiac involvement were also revealed. The observed results are corresponding to the previous preclinical studies. Endothelial injury is an early feature of systemic sclerosis and plays a key role in pathogenesis.⁴⁰ Recent preclinical studies have shown that TMAO can potentially impact the pathogenesis of systemic sclerosis by inducing endothelial injury, promoting profibrotic cytokine signaling and collagen deposition, suppressing endothelium-dependent dilation of arteries, and inhibiting nitric oxide release in the epithelium.^{32,41,42} It was observed that TMAO can induce the differentiation of fibroblasts into myofibroblasts and endothelial-mesenchymal transition.³² The presence of myofibroblasts in involved organs is one of the histopathological hallmarks of systemic sclerosis.³³

The available literature reveals that the incidence and increased severity of SSc-associated complications may be linked to an increased level of TMAO. An elevated level of this metabolite has been observed in pulmonary arterial hypertension

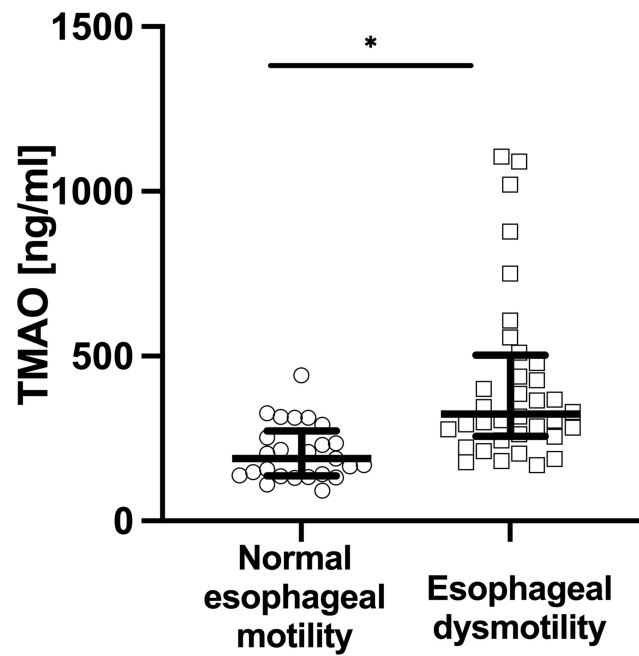


Figure 5 Plasma concentration of trimethylamine N-oxide (TMAO) in SSC patients subgroup with esophageal dysmotility and without this complication (* $p < 0.05$).

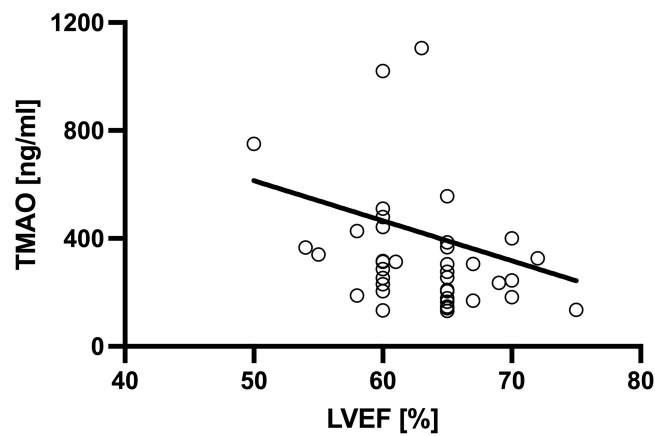


Figure 6 Correlation of trimethylamine N-oxide (TMAO) with left ventricular ejection fraction (LVEF; $\rho = -0.39$; $p < 0.01$).

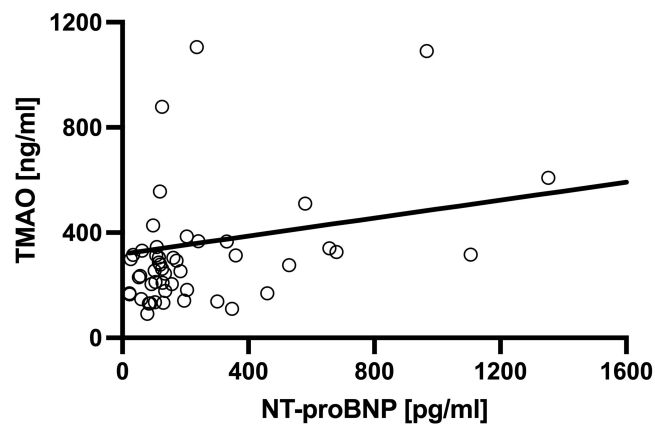


Figure 7 Correlation of trimethylamine N-oxide (TMAO) with N-terminal pro B-type natriuretic peptide (NT-proBNP; $\rho = 0.41$; $p < 0.001$).

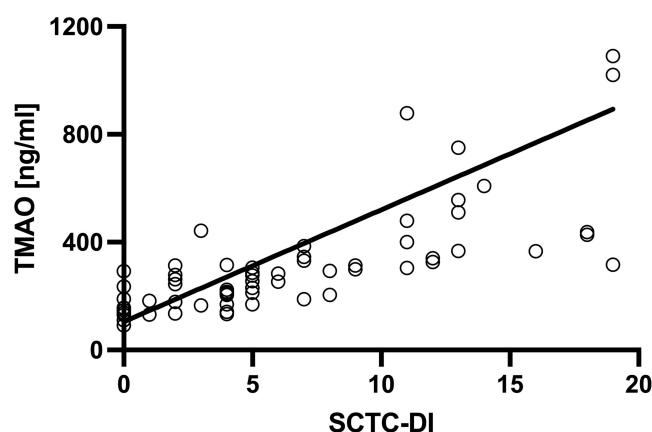


Figure 8 Correlation of trimethylamine N-oxide (TMAO) with the Scleroderma Clinical Trials Consortium Damage Index (SCTC-DI; $\rho = 0.78$; $p < 0.001$).

(PAH)^{43,44} which is a common comorbidity in systemic sclerosis and affects about 15% of patients.¹ Additionally, higher TMAO level was related to the worse course of PAH.^{44,45} In heart involvement, microbiota metabolites could be also an important factor in prognosis. Literature reports that TMAO can aggravate heart fibrosis and therefore decrease ejection fraction and contractility of the myocardium.⁴⁶ More severe gastrointestinal symptoms, including esophageal reflux, were observed in patients with higher circulating TMAO.⁴⁷ Despite these encouraging findings, it is still unknown whether a rise in TMAO is a causative factor of the mentioned conditions or just an effect of the disease. To accurately evaluate the pathogenic effect of TMAO on the complications of systemic sclerosis more studies are needed, especially prospective and multi-centered, that would evaluate the long-term exposition to TMAO and its potential effects. However, several studies reported the advantages of an approach based on reducing the TMAO level. Lowering TMAO by 3,3-dimethyl-1-butanol (DMB), an inhibitor of the bacterial synthesis of TMA, improved hemodynamic parameters and pulmonary vascular remodeling in mouse and rat models of PAH.^{44,45} In a mouse model of cardiac fibrosis lowering TMAO was associated with the decreased gene expression level of collagen I and TGF- β , and decreased fibrosis in a microscopic examination of cardiomyocytes.⁴⁸ According to the mentioned studies, patients with systemic sclerosis may benefit from adjuvant medication intended to reduce harmful bacteria metabolites and possibly experience reduced disease activity. A growing number of evidences suggest that TMAO concentration can be modulated by several bioactive compounds or dietary patterns. The low-fat diet, resveratrol, capsanthin and allicin decrease TMAO concentrations, whereas high-fat and high protein diet promote overproduction of TMAO. Diet can affect TMAO in several pathways, including the metabolism of its precursors, modulating intestinal permeability as well as shifting gut microbiota.^{49,50}

Understanding the mechanisms of how dysbiotic microbiota and its metabolites may influence systemic sclerosis seems to be valuable in clinical practice. Our study suggests that TMAO may serve as a useful marker for identifying the severity of multi-organ involvement in SSc due to exhibiting a strong positive correlation with SCTC-DI. Therefore, measurement of TMAO may help to stratify patients into different disease severity categories, which could help to optimize the treatment of the disease.

Limitations of our study include small sample size, single-center type, and cross-sectional character. To assess the causative effects of prolonged exposure to increased plasma levels of bacterial metabolites on the development of complications of SSc prospective, multicenter studies are needed.

Conclusion

Our cross-sectional study showed that increased plasma TMAO level was associated with increased prevalence and severity of interstitial lung disease and heart involvement in systemic sclerosis. Also, a higher concentration of TMAO was observed in patients with concomitant gastroesophageal dysmotility. This is the first study evaluating plasma TMAO in systemic sclerosis. The mentioned findings indicate the possible usefulness of TMAO as a biomarker of incidence, and severity of complications of the disease. A more profound knowledge of the causal link between microbiota metabolites and SSc may point to pathophysiological pathways that may be addressed for targeted therapeutics to improve disease severity and complications.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Disclosure

The authors report no conflicts of interest in this work.

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