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Plasma TMAO Levels in Relation to Perioperative Venous Thromboembolism in Patients With Gynecological Malignancies: A Nested Case–Control Study

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Article

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Abstract

Background:

Venous thromboembolism (VTE) is a common and potentially fatal perioperative complication in patients with gynecological malignancies. Emerging evidence links gut microbiota metabolite trimethylamine N-oxide (TMAO) with thrombosis, but their roles in cancer-related VTE remain unclear.

Methods:

A nested case-control study was conducted among 981 patients who underwent gynecologic cancer surgery.Fifty-one VTE cases confirmed by vascular ultrasound or CTPA were matched 1:1 with controls by surgical method and age (± 5 years). Plasma TMAO concentrations were measured using ELISA.

Results:

Plasma TMAO levels varied widely (15-fold range), and the median level was slightly higher in the VTE group (5.32 [3.73–10.87] μmol/L) than controls (4.95 [3.32–11.55] μmol/L), though the difference was not statistically significant (P = 0.83).

Conclusion:

There was no correlation between the formation of VTE and TMAO levels in patients with gynecological malignant tumors during perioperative period.Although we found that plasma TMAO levels were higher in the VTE group, the lack of statistical significance may be due to the limited sample size. Larger, multicenter studies are needed to explore the role of gut microbiota and TMAO metabolism in tumor-associated thrombosis.

1. Introduction

Venous thromboembolism(VTE)[1], encompassing deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common and potentially life-threatening cardiovascular disorder. It is one of the most frequent perioperative complications in patients with gynecological malignancies. Surgery and malignancy are both strongly associated with VTE formation, and studies have shown that cancer-related VTE accounts for approximately 20% of all VTE cases[2]. The reported incidence of VTE after surgery for gynecological malignancies ranges from 12% to 38%[3], and remains as high as 13.2% even after standard preventive measures have been implemented.

Currently, three risk assessment models are commonly used for perioperative VTE prevention—the Caprini, Autar, and Padua scores. However, these models have limitations, including low sensitivity and

inability to predict VTE events in real time[4].Vascular ultrasound [5]remains the preferred imaging modality for the initial diagnosis of DVT, and computed tomography pulmonary angiography (CTPA)[6]is the gold standard for the diagnosis of PE. Nevertheless, both methods provide only point-in-time assessments and cannot enable continuous monitoring. The diagnostic utility of D-dimer is also limited in cancer patients. Although negative D-dimer results may help rule out acute VTE, positive results are nonspecific, as D-dimer levels can be elevated in malignancy, after surgery, and during inflammation[7]. Consequently, current diagnostic and preventive approaches fail to achieve precise and timely identification of high-risk patients. Novel biomarkers and mechanistic indicators are therefore urgently needed to enhance early detection and prevention of postoperative VTE in gynecologic oncology.

The formation of VTE is a multifactorial process involving the vasculature, coagulation system, and immune cells[8].Recent microbiome research has revealed that the intestinal microbiota plays an important role in thrombosis[9]. By modulating levels of trimethylamine N-oxide (TMAO) and bacterial lipopolysaccharides (LPS), gut microbes can activate endothelial cells, enhance platelet reactivity, and promote coagulation cascade activation, thereby contributing to thrombus formation.

The gut microbiota, comprising over 1,100 known species of commensal, neutral, and pathogenic microorganisms[10, 11] is a key regulator of host immunity and metabolism. Environmental and genetic factors that disrupt gut microbiota homeostasis can trigger inflammatory signaling in vascular endothelial cells, platelets, and innate immune cells, leading to increased expression of procoagulant proteins and a prothrombotic state[12, 13].An imbalance in gut microbiota—characterized by a reduction in beneficial anaerobes and an overgrowth of pathogenic bacteria—has been associated with increased VTE risk[9].

TMAO is an intestinal metabolite that has attracted increasing attention due to its strong association with cardiovascular and thrombotic diseases[14, 15]. Its production depends on two key components: trimethylamine (TMA) and flavin-containing monooxygenases (FMO)[16]. Dietary precursors such as choline, phosphatidylcholine, and L-carnitine—abundant in red meat, eggs, and dairy—are metabolized by gut bacteria into TMA, which is subsequently absorbed into the circulation and converted to TMAO by hepatic FMO3[17]. Increasing evidence supports a link between the gut microbiota–TMAO axis and thrombosis: elevated TMAO levels have been identified as an independent risk factor for cardiovascular events such as myocardial infarction, stroke, and other thrombotic diseases[18, 19]. Zhu et al. [14]demonstrated that TMAO enhances platelet responsiveness to multiple agonists, thereby increasing platelet aggregation and thrombosis potential.

At present, no studies have investigated the relationship between TMAO levels and VTE risk specifically in patients with gynecological malignancies. We hypothesize that higher circulating TMAO levels are associated with an increased risk of VTE. Therefore, the purpose of this study was to evaluate the changes of TMAO concentration in patients with and without VTE history to explore the relationship between intestinal flora metabolite TMAO and VTE formation.

2.Methods

2.1. Study cohort

This is a Nested Case-Control study (NCC) conducted in the Department of Gynecologic, a tertiary hospital in Chengdu, Sichuan Province.We confirm that all methods complied with relevant guidelines and regulations, and that the reporting followed the recommendations of the STROBE Statement.Patients with gynecological malignant tumors who were scheduled to undergo surgery from July 2021 to December 2022 were included by convenience sampling as the study population. A follow-up cohort was established and blood samples were collected.

2.2 Inclusion and exclusion criteria

(1)Inclusion criteria:patients who were diagnosed with gynecological malignant tumor or gynecological malignant tumor waiting for diagnosis and planned to be hospitalized for surgical treatment ; age ≥ 18 years ; the patient is conscious, can communicate normally, and cooperate with the specimens required for the study.

(2)Exclusion criteria : pregnancy ; combined with blood system diseases ; anticoagulant or antiplatelet drugs, such as aspirin, warfarin, heparin or low molecular weight heparin, are being used on admission.

(3) Rejection criteria: patients whose postoperative pathological examination results were non-malignant tumors;unqualified blood samples (insufficient sample retention, hemolysis,etc.);patients who did not undergo vascular color Doppler ultrasound or CTPA examination during the perioperative period.

2.3Research Participants

According to the results of vascular ultrasound or CTPA examination, we took the patients with VTE as the experimental group and the patients without VTE as the control group.In the selection of the control group and the experimental group, the matching principle of ' the same surgical method ' and ' similar age (difference ± 5 years) ' was used, and a 1:1 matching was performed in the follow-up cohort.

Studies have shown that there is a significant correlation between the incidence of VTE and factors such as age and surgical methods (laparoscopic or open). There was a positive correlation between age and the incidence of VTE. For every 1 year increase in age, the incidence of VTE increased by 1.07 times[20];Fang F et al[21] reported that compared with the laparoscopic surgery group, the incidence of lower extremity DVT after surgery was higher in the open surgery group. Therefore, this study adopted the surgical method to match the age and surgical method .

2.4 Sample Collection

Fasting venous blood samples were collected within 72 hours after surgery. Venous blood was collected using EDTA anticoagulant tubes.Serum was isolated from blood samples by centrifugation at 3000 rpm

and 4℃ for 10 min and stored at − 80℃ until further analysis.

2.5 TMAO detection

Plasma TMAO concentrations were determined using a double-antibody one-step sandwich enzyme-linked immunosorbent assay (ELISA) kit (Jiangsu Jingmei Technology Co., Ltd., China).

2.6 Statistical analysis

IBM SPSS 25.0 was used for statistical analysis, and all data were statistically described : count data (such as demographic data) were statistically described by the number of cases (percentage). Measurement data, the first normality test, in line with the normal distribution of data (such as laboratory tests) : describe the concentration and dispersion trend of the data using mean ± standard deviation (± S), paired t test for comparison between groups ; if it does not conform to the normal distribution, the median (quartile) method is used to describe the centralized trend and discrete trend of the data, and the Wilcoxon signed rank sum test is used to compare the differences between groups. The classification data were described by relative number (rate or composition ratio) and the difference between groups was compared by chi-square test.A two-tailed p-value of < 0.05 was considered statistically significant.

3. Results

3.1 General Information

A total of 981 patients with gynecological malignant tumor surgery were included in this study. A total of 56 patients developed VTE in the study cohort, of which 5 patients with VTE were excluded due to unqualified blood samples. Therefore, this study finally included 51 patients diagnosed with VTE by vascular ultrasound or CTPA screening as a case group (VTE group), and matched 51 patients without VTE as a control group.

The results showed that the age of 102 patients with gynecological malignant tumors ranged from 39 to 88 years, with an average age of 59.71 ± 10.09 years. The average age of the VTE group (60.02 ± 10.45 years) was similar to the average age of the control group (59.47 ± 10.03 years). The mean admission body mass index (BMI) was 23.39 ± 3.24 kg/m² (range: 17.45–32.88 kg/m²). Similarly, the average BMI in the VTE group (24.39 ± 3.85 kg/m²) was not significantly different from that in the control group (23.50 ± 3.07 kg/m²). No statistically significant differences were observed between the two groups in age, surgical method, BMI, ethnicity, PICC implantation, prior stroke, prothrombin time (PT), thrombin time (TT), activated partial thromboplastin time (APTT), fibrinogen (FIB), or albumin levels (P > 0.05) (Table 3 − 1).

Table 3
− 1 Basic characteristics of VTE group and control group(n,%)

Parameter	VTE (n = 51)	Control(n = 51)	P
Age(year)	60.02 ± 10.45	59.47 ± 10.03	0.06
surgical approaches			1.00
laparoscopic	21(41.20)	21(41.20)	
laparotomy	30(58.80)	30(58.80)	
nationality			0.37
han	47(92.20)	50(98.00)	
other	4(7.80)	1(2.00)	
PICC			0.58
no	43(84.30)	46(90.20)	
yes	8(15.70)	5(9.80)	
Previous stroke			0.62
no	48(94.10)	50(98.00)	
yes	3(5.90)	1(2.00)	
BMI(kg/m^2)	24.39 ± 3.85	23.50 ± 3.07	0.18
PT(s)	11.72 ± 0.59	11.73 ± 0.53	0.87
APTT(s)	24.64 ± 2.15	25.39 ± 1.82	0.05
TT(s)	16.55 ± 0.76	16.55 ± 0.97	0.97
FIB(g/L)	370.14 ± 97.38	372.71 ± 114.83	0.90
Albumin(g/L)	44.21 ± 4.26	45.92 ± 5.91	0.07

BMI, body mass index.PT is prothrombin time; APTT is partial activated prothrombin time; TT is thrombin time; FIB is fibrinogen content.The tables are all data at admission.

3.2 Alterations in TMAO concentration

A total of 102 patients with gynecological malignant tumors were included in this study for TMAO detection, and the median TMAO level was 5.24 (3.39,10.96) μmol / L. The results showed that the overall TMAO concentration was widely distributed, with the lowest level of 1.47 μmol / L and the highest level of 23.32 μmol / L (Fig. 3 − 1).The normality K-S test result was P = 0.000, which did not obey the

normal distribution. The overall TMAO level of the population was skewed, and nearly half (49%) of the population had a TMAO level between 1.00 ~ 4.00μmol/L.

The median plasma TMAO level in the VTE group was slightly higher than that in the control group, but the difference was not statistically significant [median (interquartile range): 5.32 (3.73–10.87) μmol/L vs. 4.95 (3.32–11.55) μmol/L, P = 0.833] (Fig. 3 – 2).

4.Discussion

This study investigated the relationship between intestinal microbiota metabolite TMAO and VTE in patients undergoing surgery for gynecological malignancies. Plasma TMAO concentrations were measured in 102 patients, but no statistically significant difference was observed between the VTE and control groups.

In recent years, a newly discovered important risk factor affecting the occurrence and development of thrombosis is TMAO, a metabolite of intestinal flora. Studies have shown that[22], there is a correlation between TMAO and thrombosis. Elevated TMAO levels are associated with an increased risk of arterial thrombosis events such as myocardial infarction and stroke.By increasing the cholesterol uptake of macrophages, TMAO[23] leads to the formation of foam cells, which increases the size of atherosclerotic lesions and leads to arterial wall thrombosis. In addition, TMAO can stimulate platelet hyperresponsiveness and increase platelet adhesion and aggregation[14].VTE and arterial thrombosis were considered to be two independent diseases in the past, with different pathology. Arterial thrombosis is mainly composed of platelets, while venous thrombosis is usually composed of red blood cells and fibrin[24]. However, more and more epidemiological evidence suggests that there may be a link between arterial thrombosis and VTE, and platelets also play a crucial role in the occurrence of venous thrombosis. Platelet adhesion to the endothelium enhances leukocyte recruitment and stimulates neutrophil-dependent coagulation to promote VTE progression [25]; the risk of atherosclerotic disease is increased in patients with VTE [26] the risk of venous thrombosis in patients with previous cardiovascular disease will increase by 1.31 times in the short term. The strong lipid-lowering drugs (statins) commonly used in patients with atherosclerosis are negatively correlated with the risk of VTE[27].In addition, these two vascular complications have common risk factors such as advanced age, obesity,hypertension, etc.[28]. In the pathogenesis, both of them are based on the formation of inflammation and hypercoagulability, and the formation of both is related to tissue factors[29].

In contrast to the above-mentioned works, no differences were found in our study regarding TMAO concentrations between the control and VTE groups.In our cohort, plasma TMAO levels varied widely, with a 15-fold difference between the lowest and highest values, consistent with previous population-based studies. Similar to the study of Wang et al[30], the distribution of TMAO in community population was detected by stable isotope internal standard liquid chromatography-mass spectrometry. Although the median TMAO concentration in the VTE group was slightly higher than that in the control group, the difference was not statistically significant (P = 0.83). These results are in line with the findings of Reiner

et al. [31], who reported no significant association between TMAO levels and VTE recurrence risk, although they did observe a U-shaped association with all-cause mortality.This negative result also agrees with the recent study of Canyelles M et al. in which no differences were found regarding TMAO, γBB, and TML concentrations between the control and VTE groups[32].

The lack of statistical significance in our study may be attributable to the relatively small sample size, as well as inter-individual variability influenced by diet, renal function, age, and perioperative status. Previous studies have shown that acute inflammatory responses can alter TMAO levels. For example, Matsuzawa et al[33] observed that plasma TMAO concentrations were lower during the acute phase of myocardial infarction compared to the chronic phase, suggesting that acute events may transiently affect TMAO metabolism.

Although we did not detect a statistically significant difference in TMAO levels between groups, the observed upward trend in the VTE group is consistent with prior studies on thrombotic diseases.The incidence of arterial thrombotic diseases is different in people with different TMAO levels, and high TMAO levels are associated with an increased risk of arterial thrombotic diseases[19].Hu et al. also found the same trend after measuring the level of TMAO in VTE patients, and the level of TMAO in the case group was higher than that in the control group[34]. These results underscore the need for larger, multicenter studies with longitudinal follow-up to validate the potential role of TMAO and gut microbiota composition in perioperative VTE risk stratification.

This study employed matched case-control design and used standardized methods for sample collection and laboratory testing, reducing potential confounding. However, several limitations must be acknowledged. First, the study was conducted at a single tertiary hospital, which may limit generalizability. Second, the sample size for TMAO analysis was relatively small, which may have limited statistical power. Third, acute metabolic changes associated with surgery and VTE events may have influenced TMAO concentrations, potentially underestimating the true association.

Future research should expand sample size and include multi-center cohorts to enhance representativeness. Longitudinal sampling before and after surgery, as well as during acute and recovery phases of VTE, would help clarify the temporal relationship between gut microbiota, TMAO, and thrombus formation. Multi-omics approaches integrating metagenomics, metabolomics, and immune profiling may provide deeper mechanistic insights into the gut microbiota–thrombosis axis.

5. Conclusion

Although plasma TMAO levels were higher in the VTE group than in the control group, the difference did not reach statistical significance—likely due to the limited sample size and potential perioperative metabolic variability. Nevertheless, this trend supports the hypothesis that TMAO may contribute to the development of VTE in patients with gynecological malignancies.

In the future, more studies are needed to explore these associations and to explore whether the regulation of gut microbiota or TMAO metabolism can be used as a new strategy to prevent VTE in high-risk surgical populations.

Declarations

Ethics approval and consent to participate

This study has been approved by the Medical Ethics Committee of West China Second Hospital of Sichuan University. The ethical approval number is HLBKJ202027. All patients signed written informed consent after participation.

Declaration of competing interest

No conflicts of interest are declared.

Data availability

All the data utilized in the study are publicly available and/or contained within the manuscript or appendix.

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Clinical trial number: not applicable.

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Figures

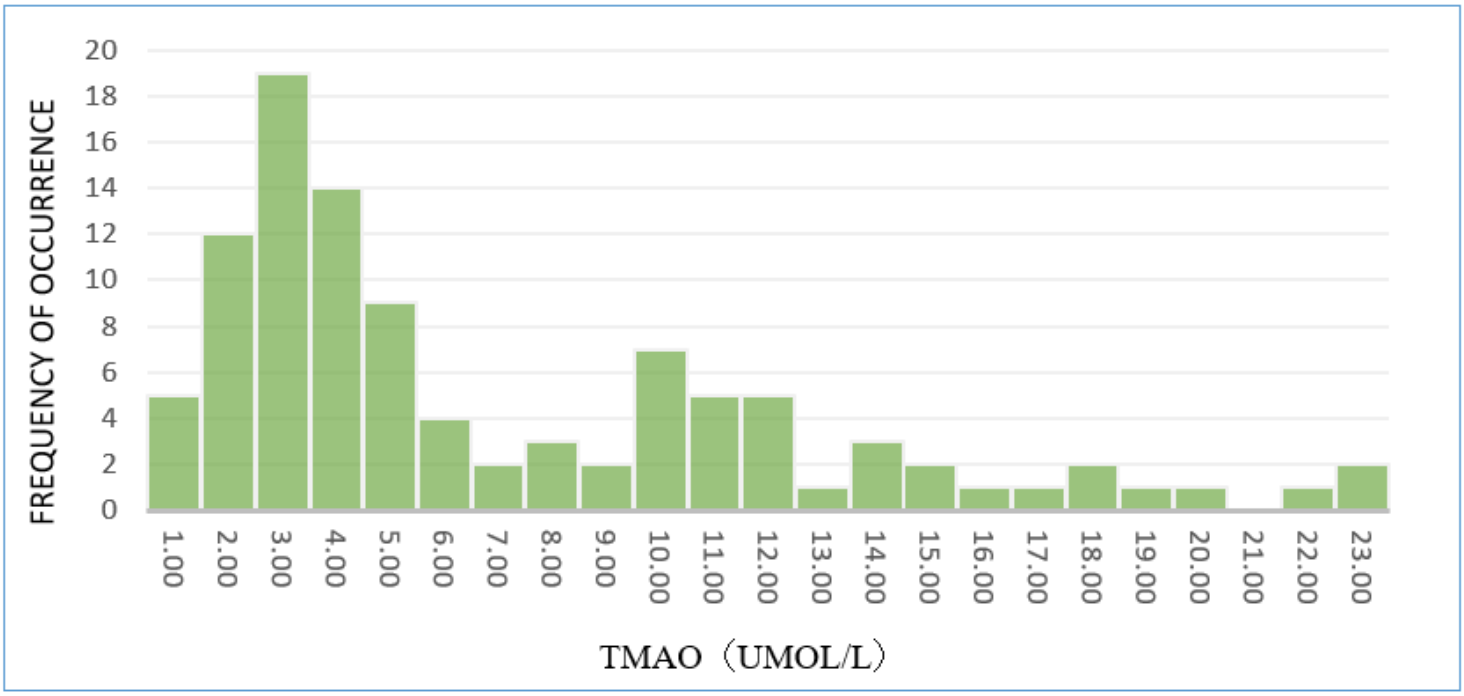


Figure 1

Fig. 3-1 The overall distribution trend of plasma TMAO

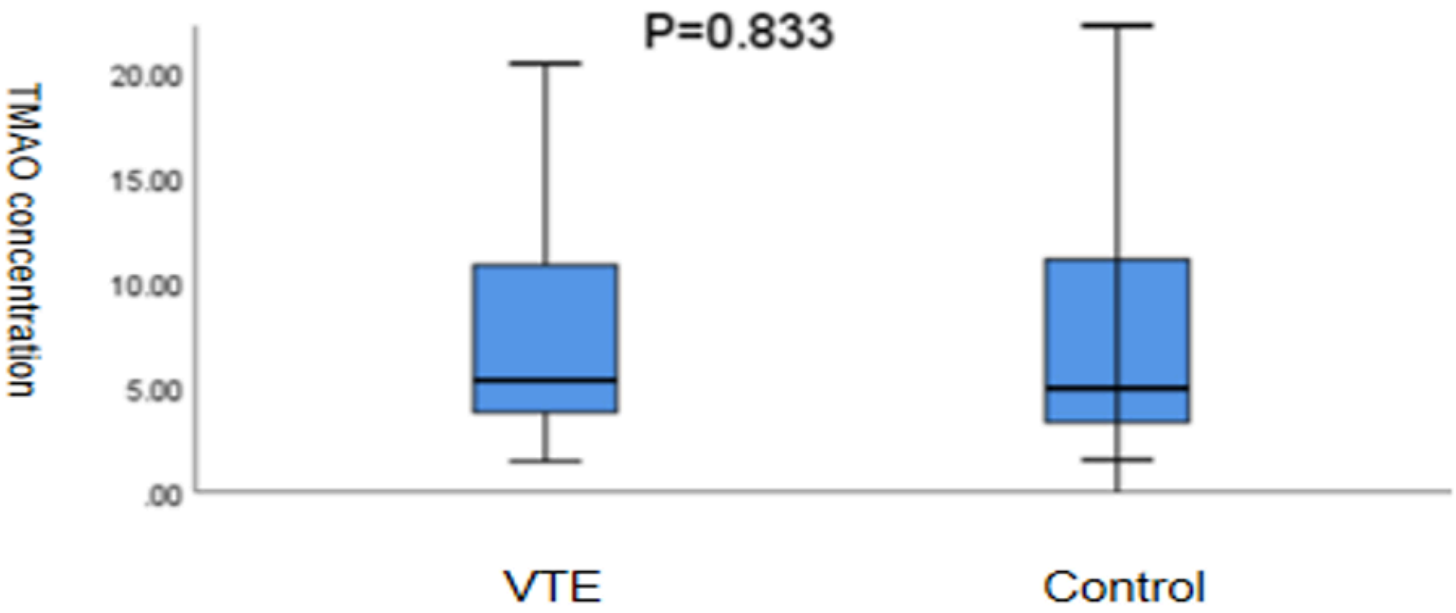


Figure 2

Fig 3-2 The difference of plasma TMAO concentration between the two groups was analyzed.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TheconcentrationofTMAOinthetwogroups.xlsx
- floatimage1.png