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ORIGINAL ARTICLE

Unveiling the Th17/Treg Imbalance: a New Lens on Macular Perfusion in Thyroid-Associated Ophthalmopathy

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ABSTRACT

Background: **The aim of this study was to investigate the association between Th17/Treg imbalance and macular blood flow abnormalities in patients with thyroid-associated ophthalmopathy (TAO).**
Methods: **A prospective cohort study was designed to include 71 patients with TAO and 50 healthy controls. Macular blood flow measurements were quantified by optical coherence tomography angiography, including foveal vessel density (FVD) and foveal perfusion density (FPD) in 1 mm from the central fovea, macular vessel density (MVD) and macular perfusion density (MPD) in 6 mm from the central fovea, and macular foveal avascular zone (FAZ) was also measured. Peripheral blood Th17/Treg ratio was measured by flow cytometry and its association with blood flow measurements was assessed by multiple linear regression modeling, correcting for confounding factors such as age and gender.**
Results: **MVD (16.38 ± 2.83 vs. 18.91 ± 2.20, $p < 0.001$) and MPD (44.5% vs. 48.1%, $p < 0.001$) in the TAO group were significantly lower than those of the control group. Th17/Treg ratio showed a significant positive correlation with MPD (multifactorial $\beta = 0.01$, $p = 0.002$) and negatively correlated with FAZ (multifactorial $\beta = -0.36$, $p = 0.018$). No significant correlations were found for other indicators (FVD, FPD, and MVD) and confounders (TAO activity, gender, and age).**
Conclusions: **Th17/Treg imbalance is directly involved in microcirculatory disorders in TAO by decreasing MPD and enlarging macular FAZ, suggesting that immunomodulation may be a therapeutic target to improve macular blood flow abnormalities in TAO patients.**
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KEYWORDS

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INTRODUCTION

Thyroid-associated ophthalmopathy (TAO) is the most common extra-thyroidal complication of Graves' disease, characterized by inflammatory infiltration of orbital soft tissues and extra-ocular muscles, fibrosis, and fatty hyperplasia. Clinical manifestations include proptosis (bulging of the eye), diplopia, eyelid recession and corneal exposure, which can lead to loss of vision in severe cases [1]. TAO develops through a complex process involving genetic susceptibility, autoimmune reac-

tions, and environmental factors, with immune imbalance as a primary driver [2,3]. Th17/Treg imbalance is essential in the onset of autoimmune conditions, potentially including a strong link to TAO. Direct research on Th17/Treg balance in TAO is limited, but its effects can be speculated based on studies of other autoimmune diseases. In a study of 26 patients with autoimmune thyroid disorders (16 Graves' disease and 10 Hashimoto's thyroiditis), increased levels of pathogenic Th17 lymphocytes in the peripheral blood of these patients are detected, while these levels are associated with disease activity and disease duration [4]. Moreover, the percentage of Treg cells is markedly lower in individuals with Hashimoto's thyroiditis than in healthy people [5]. Th17/Treg imbalance has been shown to cause localized immune cell infiltration in the orbit [6]. Thus, there is still a need for systematic exploration into whether Th17/Treg imbalance affects retinal microcirculation, particularly macular perfusion, through systemic immune modulation.

The macula is the most sensitive part of visual function in the retina. Its microcirculatory condition directly impacts central vision [7]. Optical coherence tomography angiography (OCTA) technology has enabled noninvasive evaluation of macular blood flow [8]. Studies have demonstrated that changes in macular vascular density (MVD), macular perfusion density (MPD), and foveal vessel density (FAZ) in ocular diseases are closely related to disease progression [9,10]. This is especially true when assessing the progression of retinal diseases and treatment outcomes. Measurements of blood flow taken 1 mm and 6 mm from the central fovea yield more precise and detailed data on retinal blood flow. The 1 mm measurements can accurately depict the blood flow in the central fovea. The central fovea, being the most visually sensitive area, experiences direct effects on visual function due to changes in its blood flow. For example, in diabetic retinopathy, enlargement of the macular FAZ is closely associated with vision loss [11]. The 6 mm measurements extend over a larger macular area, giving a more comprehensive view of the overall retinal blood flow. This measurement can help identify changes in the macular microcirculation, such as in patients with retinitis pigmentosa, where a reduction in macular blood flow is associated with reduced central vision sensitivity [12]. However, whether the immune imbalance in TAO, an autoimmune disease, leads to visual impairment by affecting macular microcirculation has not been reported. Notably, Th17 cells can act directly on vascular endothelial cells by secreting factors such as vascular endothelial growth factor (VEGF) [13], whereas Treg cells inhibit the release of inflammatory mediators through the secretion of IL-10 and TGF-β [14]. It is suggested that Th17/Treg imbalance may be indirectly involved in the pathologic process of macular blood flow abnormalities by modulating angiogenesis and inflammatory response.

Given the context, this study hypothesized that Th17/Treg imbalance in TAO patients might cause retinal microcirculation by reducing MPD and enlarging FAZ area. To test this hypothesis, the present study was designed as a prospective cohort study, using OCTA to accurately measure the blood flow parameters in 1 mm and 6 mm from the central fovea and FAZ area in TAO patients combined with flow cytometry to measure the peripheral blood Th17/Treg ratio. It is hypothesized that the elevated Th17/Treg ratio may lead to decreased MPD and FAZ enlargement by promoting inflammatory response and microvascular injury. By combining immune cell subpopulation ratios with macular microcirculation parameters, this study offers a novel perspective on the 'immune-vascular' interaction to comprehend the pathological process of TAO.

MATERIALS AND METHODS

Subject inclusion

Participants for the study were untreated TAO patients at Shanghai Pudong Hospital from January 2023 to January 2025, as well as age-matched healthy volunteers from the same period.

Inclusion criteria: 1) patients with a clinical diagnosis of TAO based on the criteria of the European Group of Graves' Orbitopathy (EUGOGO) Consensus Statement [15]; 2) patients with no hormonal therapy, immunosuppressive therapy, or orbital radiotherapy within three months. Exclusion criteria: 1) the presence of circulatory and metabolic disorders (e.g., diabetes, hypertension, and cardiovascular disease); 2) comorbid ocular diseases that affect vision other than TAO, such as high myopia, diabetic retinopathy, glaucoma, macular degeneration, optic neuritis, ischemic optic neuropathy, etc.; 3) refractive interstitial turbidity that makes it difficult to perform fundus-related examinations, such as severe corneal ulcers, cataracts, and severe vitreous opacity, etc.; 4) history of ocular trauma and eye decompression surgeries; (5) comorbidities such as hypertension, diabetes mellitus, and carotid stenosis that may affect hemodynamics.

The control group, consisting of age- and gender-matched healthy volunteers, had no eye diseases, maintained a visual acuity of 20/20 (corrected or uncorrected). This group cohort had no surgical trauma or ocular operations, nor any high myopia, retinopathy, or other conditions affecting hemodynamics.

Finally, 71 patients (142 eyes) with TAO were obtained, of which 55 (77.46%) were first diagnosed with hyperthyroidism, 9 (12.68%) with hypothyroidism, and 7 with normal thyroid function (9.86%). Another 50 healthy controls were enrolled. Written informed consent was obtained from all subjects. The research received approval from the Shanghai Pudong Hospital Ethics Committee and adhered to the principles outlined in the Declaration of Helsinki.

Basic information

The study applied a systematic process to collect information and perform a detailed examination for all involved participants. Demographic characteristics (name, gender, and age) and medical history data (smoking, thyroid dysfunction, treatment, and previous illnesses) were recorded, followed by testing of physiological indicators, including systolic blood pressure (SBP), diastolic blood pressure (DBP), and body mass index (BMI). Intraocular pressure (IOP) was assessed using a tonometer, with three readings averaged for each eye. Eye proptosis was measured with a Hertel proptometer, refraction was determined using TOPCON optometry, and best-corrected visual acuity (BCV) was converted to LogMAR values. Funduscopy and optic nerve OCT scan were completed. Thyroid function was also assessed. Disease activity was finally assessed using the CAS scale recommended by EUGOGO [15] by 7 indicators (spontaneous retrobulbar pain, oculomotor pain, eyelid congestion, eyelid edema, conjunctival congestion, conjunctival edema, and swollen lacrimal caruncle), with a total score of ≥ 3 determined to be active.

Blood flow testing

OCTA was performed on all subjects. The macular area was scanned using a Cirrus HDOCT5000 (Carl Zeiss, Germany) with a scanning area of 6 mm × 6 mm. Participants were instructed to position their chin and forehead on the respective rests and focus on the green stationary cursor on the lens for over 3 seconds. Image tracking with FastTrac was enabled for every scan. Images with a quality score less than 8 were not included in the study, as image quality was measured quantitatively using OCTA. OCTA data were automatically imported into the FORUM system (version 4.0) and quantitatively evaluated by Vascular Analysis. The superficial retinal layer (SRL) was calculated from the inner limiting membrane to the inner plexiform layer. The superficial macular capillary plexus was measured using a 6 × 6 mm macular scan. The macula was divided into a 1-mm center circle, a 3-mm inner ring, a 6-mm outer ring, and a 6-mm circle according to the Early Treatment Diabetic Retinopathy Study [16]. Because the 1-mm center circle and the 6-mm circle can better represent macular blood flow, only these two areas were analyzed in this study, i.e., foveal vessel density (FVD) and foveal perfusion density (FPD) in 1 mm from the central fovea, and MVD and MPD 6 mm from the central fovea. Vascular density was defined as the linear length of retinal microvessels per unit area of perfusion within the measurement area. Perfusion density was defined as the total area of retinal microvessels perfused per unit area within the measurement area. FAZ boundaries were manually checked and automatically drawn by the angiographic software. All tests were conducted by the same physician and analyzed by two independent physicians. In the event of a disagreement, a third physician provided the final judgment. In TAO patients, the eye with the worse condition was chosen for analysis, whereas for controls, the average data from both eyes was analyzed.

Flow cytometry analysis of Th17/Treg

Peripheral blood single nucleated cells (PBMCs) were fractionated at 2 - 6 × 10^6/mL, followed by cellular phenotyping through a two-step immunolabeling method. For Treg cell assay, PBMCs were labeled with monoclonal anti-human CD4-FITC, followed by anti-human Foxp3-PE for transcription factor staining. While for Th17 cell detection, PBMCs were stimulated with Fobol Myristate Acetate in combination with ionomycin at 37°C and 5% CO_2. Monensin was added to block intracellular protein transport. After fixation/permeabilization and resuspension with BD Perm/Wash™ buffer, anti-human IL-17A-PE was added for intracellular cytokine staining. Throughout the experiment, tubes with isotype control and fluorescence subtraction were established. Data compensation and circle-gate analysis were performed using BD FACSDiva software (BD Biosciences, Heidelberg, Germany), with three replicates established for each sample (coefficient of variation < 5%).

Statistical analysis

Statistical analyses were performed using SPSS 26.0 software. Data normality was determined using the Shapiro-Wilk test. Continuous variables data were shown as mean ± standard deviation. For normally distributed data, comparisons were made using Student's t-test between two groups, whereas skewed data were analyzed using the Mann-Whitney U-test. Correlations between continuous variables were performed using Pearson's correlation test (normal distribution) or Spearman's correlation test. Multivariate linear analyses were employed to associate blood flow indices with Th17/Treg. Figures were prepared using the online platform https://hiplot.cn/. $p < 0.05$ was considered statistically significant.

RESULTS

Patient demographic and clinical characteristics

A total of 71 TAO patients and 50 healthy controls were included in this study. There were no statistically significant differences in baseline demographic characteristics between the two groups (Table 1) in terms of age, gender, smoking history, SBP, DBP, and BMI (all $p > 0.05$). IOP ($p = 0.001$), proptosis ($p = 0.001$), and BCV ($p < 0.001$) were significantly higher in the TAO group than in the control group. In the TAO group, 53.32% (38/71) of the patients had a CAS score ≥ 3, suggesting active disease. Notably, thyroid function in TAO patients was heterogeneous: 77.46% were hyperthyroid, 12.68% were hypothyroid, and 9.86% were normal.

Table 1. Demographic and clinical characteristics of the TAO and control groups.

Data	TAO (n = 71)	Control (n = 50)	p-value
Demographic characteristics			
Age, years	45.6 ± 11.2	44.6 ± 10.5	0.662
Gender			
Male	18 (25.35%)	13 (26.0%)	1
Female	53 (74.65%)	37 (74.0%)	
Smoking history	16 (22.54%)	13 (26.0%)	0.66
Physiological indicators			
SBP, mmHg	122.5 ± 8.7	118.3 ± 7.9	0.067
DBP, mmHg	76.4 ± 6.2	74.9 ± 5.8	0.215
BMI	23.1 ± 3.5	22.8 ± 3.1	0.623
Specialized indicators			
Intraocular pressure, mmHg	16.8 ± 2.5	15.2 ± 2.1	0.001
Proptosis	19.3 ± 3.3	15.7 ± 2.0	0.001
Refraction	-1.2 ± 2.5	-0.8 ± 1.6	0.312
BCV (LogMAR)	0.12 ± 0.08	0.05 ± 0.03	< 0.001
CAS score ≥ 3	38 (53.32%)	N/A	N/A

Continuous variables are expressed as (X ± S), and categorical variables are expressed as n (%). SBP systolic blood pressure, DBP diastolic blood pressure, BCV (LogMAR) best-corrected visual acuity in LogMAR units, CAS score clinical activity score, ≥ 3 suggests that the disease is in an active phase. Comparisons between groups of continuous variables were performed using the independent samples *t*-test, comparisons between groups of categorical variables were performed using the chi-squared test. p < 0.05 was significantly different.

Table 2. Differences in macular blood flow between TAO and controls.

Macular blood flow	TAO (n = 71)	Control (n = 50)	p-value
1 mm from the central fovea			
FVD, mm^{-1}	17.58 ± 2.78	17.15 ± 2.95	0.418
FPD, %	26.8 (21.8, 30.4)	27.5 ± 3.6	0.229
6 mm from the central fovea			
MVD, mm^{-1}	16.38 ± 2.83	18.91 ± 2.20	< 0.001
MPD, %	44.5 (40.0, 47.4)	48.1 (45.0, 50.6)	< 0.001
FAZ area (mm^2)	0.25 (0.21, 0.33)	0.23 ± 0.07	0.072

Continuous variables X ± S or median (M1, M3) are indicated. VD vascular density, PD perfusion density, FAZ Foveal Avascular Zone. p < 0.05 was significantly different.

Macular blood flow

Quantitative analysis of macular blood flow showed (Table 2) that both MVD ($p < 0.001$) and MPD ($p < 0.001$) were reduced in the TAO group and the healthy control group, suggesting impaired microvessel density and perfusion capacity. In contrast, the difference in FVD ($p = 0.418$) and FPD ($p = 0.229$) between the two groups was not statistically significant. The FAZ area in the TAO group (0.25 vs. 0.23, $p = 0.072$) was slightly larger than that in the control group, but did not reach significance ($p = 0.072$).

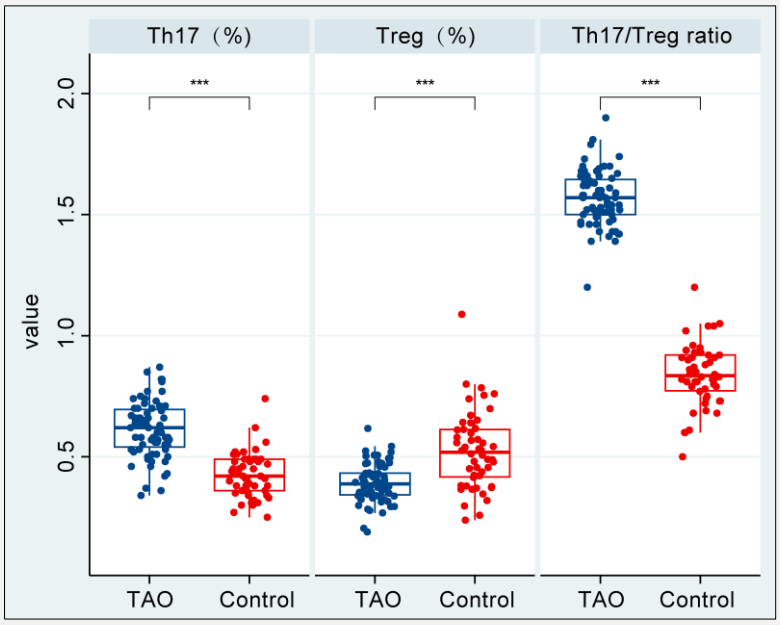
Th17/Treg ratio in TAO patients

Box plot analysis showed (Figure 1) that Th17 levels were significantly higher ($p < 0.001$) and Treg levels were significantly lower ($p < 0.001$) in TAO patients compared with healthy controls, resulting in Th17/Treg imbalance ($p < 0.001$). This result suggests that there is an immune dysregulation of over-activation of Th17 cells and suppression of Treg cell function in patients with TAO and that Th17/Treg imbalance may be involved in the pathologic progression of TAO by mediating inflammatory responses and immune disorders.

Table 3. Multiple linear analysis of the correlation between the levels of Th17/Treg and macular blood flow.

Variables	Univariate					Multivariate				
	β	SE	t	p	β (95% CI)	β	SE	t	p	β (95% CI)
FVD	0	0	0.51	0.609	0.00 (-0.01 - 0.01)	0	0.01	-0.52	0.603	-0.00 (-0.01 - 0.01)
FPD	0	0	0.6	0.548	0.00 (-0.00 - 0.01)	0	0	0.12	0.907	0.00 (-0.00 - 0.01)
MVD	0	0	-0.68	0.501	-0.00 (-0.01 - 0.01)	0	0	0.17	0.867	0.00 (-0.01 - 0.01)
MPD	0.01	0	3.44	< 0.001	0.01 (0.01 - 0.01)	0.01	0	3.21	0.002	0.01 (0.01 - 0.01)
FAZ	-0.37	0.15	-2.45	0.017	-0.37 (-0.67 - -0.07)	-0.36	0.15	-2.42	0.018	-0.36 (-0.65 - -0.07)
TAO status										
Active										
Inactive	0.03	0.03	0.94	0.348	0.03 (-0.03 - 0.08)					
Gender										
Male										
Female	0.01	0.03	0.45	0.652	0.01 (-0.05 - 0.07)					
Age median										
1										
2	0.02	0.03	0.8	0.425	0.02 (-0.03 - 0.07)					

CI Confidence Interval. Age (less than or greater than the median of 45.3) and gender (male/female) were included as confounders for correction.



***** p < 0.001.**

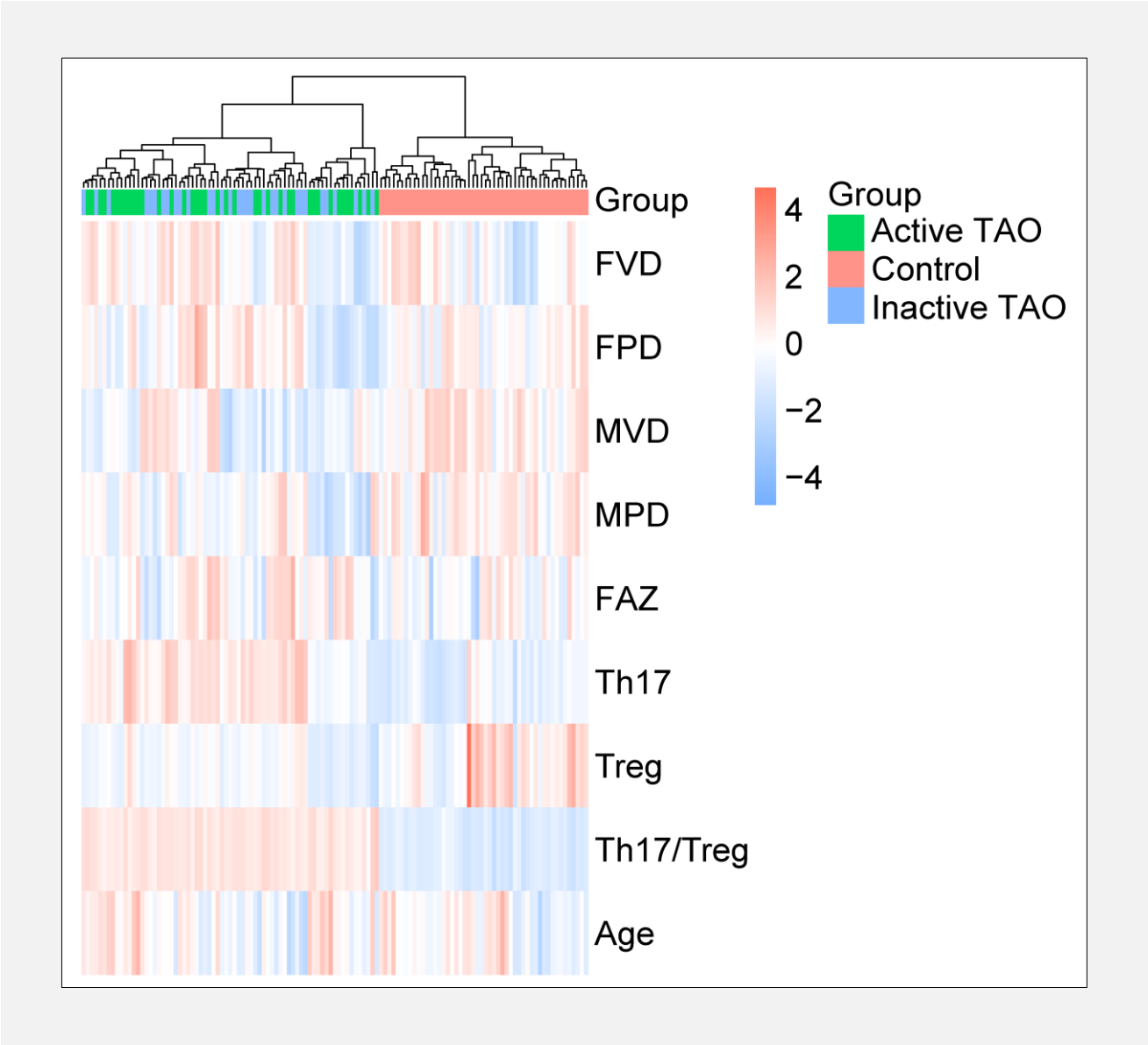


Figure 2. Heat map demonstrating the correlation between the levels of Th17/Treg and macular blood flow in both groups.

Heatmap showed (Figure 2) that the active TAO group showed a significant clustering trend with the inactive TAO group in the cluster analysis, while no significant clustering was observed between the TAO group (both active and inactive) and the control group. In terms of hemodynamic analysis, the results showed that most of the TAO patients (both active and inactive) showed a trend toward lower MVD and MPD. Of particular interest, Th17/Treg ratio was significantly different between the two groups, with a significantly higher Th17/Treg ratio in patients with TAO compared to controls. This trend was not significantly different in patients with active and inactive TAO.

Correlation analysis of Th17/Treg ratio with macular blood flow

Correlation analysis showed that in patients with TAO, Th17/Treg imbalance showed a significant positive correlation with MPD ($r = 0.32$, $p < 0.001$, Figure 3D), but no statistical correlations were observed with FVD, FPD, and MVD (Figure 3A-C, all $p < 0.05$). Notably, the ratio showed a moderate positive correlation with FAZ area ($r = 0.63$, $p < 0.001$, Figure 3E), suggesting that immunoregulatory abnormalities may exacerbate the pathological process of TAO by inducing expansion of the FAZ area, which in turn exacerbates TAO. Although the FAZ area in the TAO group showed mild expansion compared with the healthy control group, the difference between the groups had not yet reached the

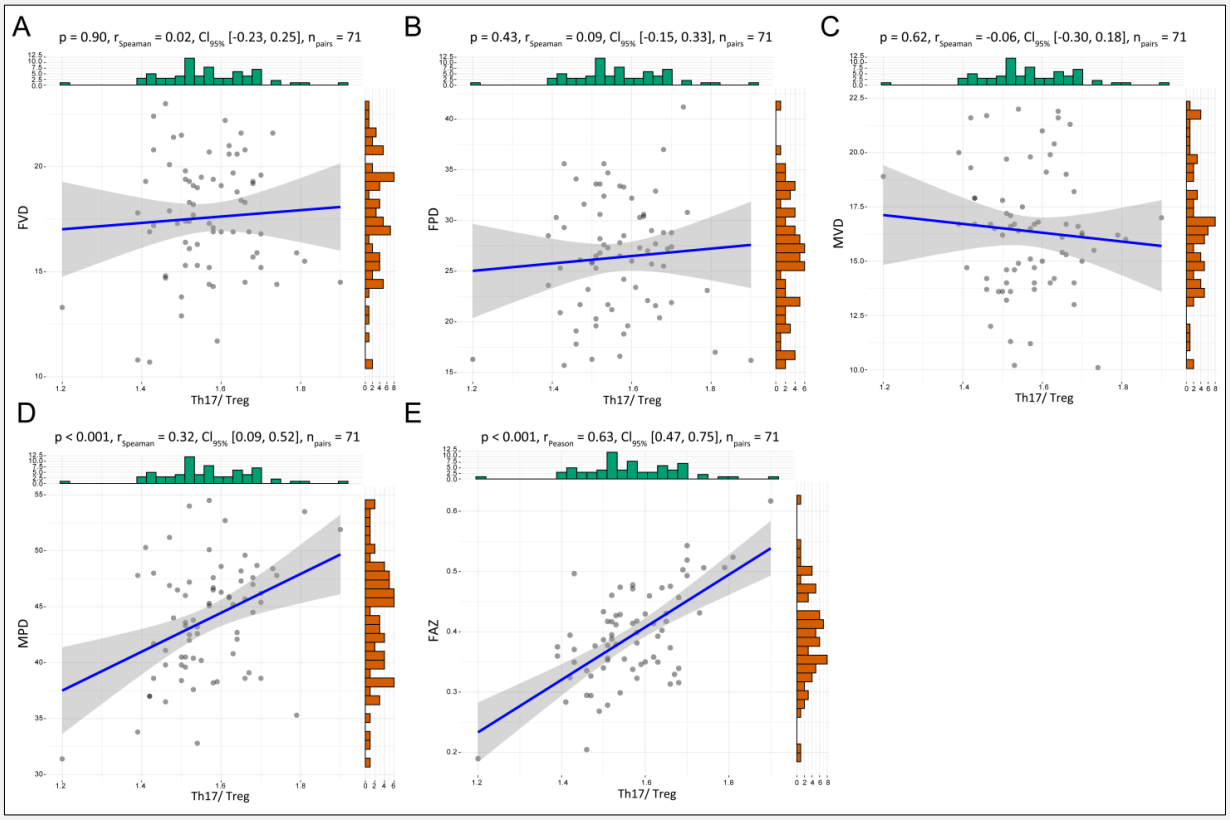


Figure 3. Scatter plot demonstrating the correlation between the levels of Th17/Treg and macular blood flow in patients with TAO.

A - D Correlations of Th17/Treg with FVD, FPD, MVD, MPD, and FAZ. r indicates correlation, with 0.3 to 0.5 being a weak correlation, 0.5 to 0.7 being a moderately strong correlation, and 0.7 to 1.0 being a strong correlation.

threshold of statistical significance (p = 0.072).

MPD showed a significant positive correlation with Th17/Treg ratio in univariate analysis (β = 0.01, 95% CI: 0.01 - 0.01, p < 0.001), which remained significant after correction for age and gender (multifactorial β = 0.01, p = 0.002). FAZ area showed a negative correlation with Th17/Treg ratio in univariate analysis (β = - 0.37, 95% CI: -0.67 - -0.07, p = 0.017), and the correlation was further enhanced after correction for confounders (multifactorial β = -0.36, 95% CI: -0.65 - -0.07, p = 0.018), suggesting that immune imbalance may exacerbate microcirculatory disturbances by expanding the FAZ area. The remaining blood flow indices (FVD, FPD, and MVD) and confounding variables (TAO activity, gender, and age) did not show significant correlations (all p > 0.05) (Table 3).

DISCUSSION

This study was the first to systematically investigate the association between Th17/Treg imbalance and macular perfusion abnormality in patients with TAO. In patients with TAO, a decrease in vascular and perfusion densities was detected 6 mm from the macula central fovea, with a significant correlation between Th17/Treg ratio and the MPD and FAZ area.

The study showed that MVD and MPD in the 6-mm macular zone of TAO patients were significantly lower than those of healthy controls, whereas there was no significant difference in the blood flow parameters in the 1-mm zone. This may be related to the hemodynamic characteristics and immunoinflammatory distribution in different zones of the macula. In terms of anatomy and physiology, the 6-mm macular zone covers an extensive area of the retinal SRL, noted for its dense microvascular network, substantial metabolic demands,

and complex interactions with the choroidal circulation [17]. In the immunoinflammatory context of TAO, Th17 cells may be able to directly damage retinal endothelial cells through secreted pro-inflammatory factors such as IL-17 and TNF-α, either through the blood circulation or local infiltration pathway, leading to endothelial dysfunction, vasoconstriction or occlusion, which in turn triggers a reduction in local blood perfusion. In contrast, the 1-mm zone is more resistant to systemic inflammation due to the special protective mechanism of the blood-retinal barrier (BRB) [18]. The BRB consists of tight junctions between endothelial cells, pericytes, and glial cells, which can effectively limit the infiltration of inflammatory cells and macromolecular substances, thus maintaining the stability of the microenvironment of macula central fovea. In the early stages of TAO, the immune-inflammatory response may not yet have breached the defense threshold of the BRB, and thus the alteration of blood flow parameters in the macula central fovea is not yet significant. As the disease advances, the buildup of inflammatory factors or impairment of the BRB function may gradually impact the perfusion of the macula central fovea, potentially resulting in reduced central vision [19].

The present study confirmed that patients with TAO had significantly increased levels of Th17 cells, decreased levels of Treg cells, and Th17/Treg imbalance, a phenomenon that is highly consistent with the findings of autoimmune thyroid disorders such as Graves' disease and Hashimoto's thyroiditis. For example, the proportion of Th17 cells in the peripheral blood of patients with Graves' disease is increased and positively correlated with thyroid-stimulating antibody levels, whereas a reduced proportion of Treg cells is strongly associated with disease activity [20]. Together, Th17/Treg imbalance may be a common immune feature of TAO [21]. Thus, in TAO, the balance between Th17 cells and Treg cells may be disrupted, resulting in an uncontrolled inflammatory reaction that impacts the macular microvasculature and function. Notably, the effect of Th17/Treg imbalance on microcirculation in TAO may be tissue-specific compared with other autoimmune diseases. For example, in rheumatoid arthritis, Th17 cells exacerbate joint destruction mainly by promoting synovial vascular opacification formation [22], whereas in multiple sclerosis, Th17 cell infiltration of the central nervous system leads to BBB disruption [23]. The present study demonstrated that Th17/Treg imbalance in TAO tended to affect the peripheral macular area (within 6 mm) of the retina more than the macula central fovea, which may be related to the vascular structural heterogeneity or local immune microenvironmental differences in different zones of the macula. This finding provides a new perspective for understanding the tissue specificity of “immune-vascular” interactions in autoimmune diseases.

Notably, the present study found that Th17/Treg ratio was significantly positively correlated with MPD and negatively correlated with FAZ area, a seemingly paradoxical correlation that may reveal the bi-directional complexity of immune imbalance-regulated microcirculation. Although Th17 cells theoretically increase perfusion by secreting pro-angiogenic factors such as VEGF [24], in the chronic inflammatory microenvironment of TAO, Th17-driven excessive inflammatory responses may lead to vascular endothelial damage and basement membrane remodeling, which may in turn trigger functional microvascular degeneration [25]. A similar mechanism has been reported in diabetic retinopathy. High VEGF levels in the early stages promote vascular proliferation but are accompanied by a sustained release of inflammatory factors (e.g., TNF-α, IL-6), which ultimately leads to vascular leakage and occlusion [26,27]. The reduction of MPD in this study may reflect functional dysfunction of the macular microvasculature in TAO patients rather than simple structural destruction. On the other hand, inhibition of Treg cell function may impair its negative regulation of pathologic angiogenesis. Research indicates that Treg cells can suppress the VEGF signaling pathway by releasing IL-10 and TGF-β, which aids in perivascular cell coverage and improves vascular stability [28]. In patients with TAO, the reduction of Treg cells may lead to the failure of this protective mechanism, contributing to the enlargement of the FAZ area due to degenerative vascular changes. This phenomenon contrasts with studies of age-related macular degeneration. Whereas FAZ enlargement in AMD is mainly associated with choroidal capillary atrophy [29], FAZ enlargement in TAO may be driven more by immune-mediated vascular endothelial damage. Further expansion of the FAZ area may lead to a reduced blood supply to the macula central fovea, aggravating retinal hypoxia and creating a vicious cycle, which might be a potential cause of visual function impairment in TAO patients.

While previous studies have mostly focused on periorbital inflammatory mechanisms in TAO, this study is the first to combine immune imbalance with macular microcirculatory parameters. Just like the enlargement of the FAZ in diabetic retinopathy, possible alterations in the FAZ of TAO patients indicate that immune-related microvascular damage might share pathways across different diseases. However, unlike VEGF-dominated vascular leakage in diabetic retinopathy, blood flow abnormalities in TAO may be more mediated by local inflammatory responses driven by Th17 cells. In addition, the present study found that Th17/Treg imbalance was specifically associated with blood flow abnormalities in the 6-mm macular zone, whereas FVD and FPD were not affected in the 1-mm zone, suggesting that the peripheral region of the macula may be more sensitive to immune disorders or due to the higher stability of the macula central fovea.

However, this study also has some limitations. First, the study was a single-center cross-sectional study with a relatively small sample size (71 cases in the TAO group) and a lack of longitudinal data to clarify the causal relationship between Th17/Treg imbalance and

blood flow abnormalities. Also, Th17/Treg cell infiltration or inflammatory factors in the local orbit were not detected, making it difficult to explain the spatial transmission mechanism of systemic immune imbalance and ocular microcirculatory dysfunction. In the future, it will be necessary to track the temporal association of Th17/Treg dynamic changes with blood flow parameters through multicenter longitudinal cohorts and to resolve the direct interaction between the orbital immune microenvironment and vascular endothelial cells by combining with single-cell sequencing or spatial transcriptome technology. Further validation of the effects of factors such as IL-17 and VEGF secreted by Th17 on microvessel density and FAZ using animal models is needed, as well as exploration of the potential of targeted immunomodulation (e.g., IL-17 inhibitors or Treg amplification therapies) to intervene in the macular blood flow abnormalities of patients with TAO.

CONCLUSION

In summary, Th17/Treg imbalance shapes the complex phenotype of macular microcirculation in TAO patients through a dynamic game of pro-inflammatory and anti-inflammatory mechanisms. This finding not only echoes the common pathological mechanisms of autoimmune diseases (e.g., Th17-driven vascular injury), but also reveals the unique "vascular-immune" interaction pattern of TAO, which provides a theoretical basis for the development of immunomodulatory therapies targeting microcirculation protection.

Availability of Data and Materials:
The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate:
The present study was approved by the Ethics Committee of Shanghai Pudong Hospital and written informed consent was provided by all patients prior to the study start. All procedures were performed in accordance with the ethical standards of the Institutional Review Board and The Declaration of Helsinki and its later amendments or comparable ethical standards.

Declaration of Interest:
The authors have no conflicts of interest to declare.

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