• Clinical Research •

Microvascular alterations of the ocular surface and retina in connective tissue disease-related interstitial lung disease

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Abstract

● **AIM:** To examine the disparities in macular retinal vascular density between individuals with connective tissue disease-related interstitial lung disease (CTD-ILD) and healthy controls (HCs) by optical coherence tomography angiography (OCTA) and to investigate the changes in microvascular density in abnormal eyes.

● **METHODS:** For a retrospective case-control study, a total of 16 patients (32 eyes) diagnosed with CTD-ILD were selected as the ILD group. The 16 healthy volunteers with 32 eyes, matched in terms of age and sex with the patients, were recruited as control group. The macular retina's superficial retinal layer (SRL) and deep retinal layer (DRL) were examined and scanned using OCTA in each individual eye. The densities of retinal microvascular (MIR), macrovascular (MAR), and total microvascular (TMI) were calculated and compared. Changes in retinal vascular density in the macular region were analyzed using three different segmentation methods: central annuli segmentation method (C1-C6), hemispheric segmentation method [uperior right (SR), superior left (SL), inferior left (IL), and inferior right (IR)], and Early Treatment Diabetic Retinopathy Study (ETDRS) methods [superior (S), inferior (I), left (L), and right (R)]. The data were analyzed using Version 9.0 of GraphPad prism and Pearson analysis.

● **RESULTS:** The OCTA data demonstrated a statistically significant difference (P<0.05) in macular retinal microvessel density between the two groups. Specifically, in the SRL and DRL analyses, the ILD group exhibited significantly lower surface density of MIR and TMI compared to the HCs group (P<0.05). Furthermore, using the hemispheric segmentation method, the ILD group showed notable reductions in SL, SR, and IL in the superficial retina (P<0.05), as well as marked decreases in SL and IR in the deep retina (P<0.05). Similarly, when employing the ETDRS method, the ILD group displayed substantial drops in superficial retinal S and I (P<0.05), along with notable reductions in deep retinal L, I, and R (P<0.05). In the central annuli segmentation method, the ILD group exhibited a significant decrease in the superficial retinal C2-4 region (P<0.05), whereas the deep retina showed a notable reduction in the C3-5 region (P<0.05). Additionally, there was an observed higher positive likelihood ratio in the superficial SR region and deep MIR. Furthermore, there was a negative correlation between conjunctival vascular density and both deep and superficial retinal TMI (P<0.001).

● **CONCLUSION:** Patients with CTD-ILD exhibits a significantly higher conjunctival vascular density compared to the HCs group. Conversely, their fundus retinal microvascular density is significantly lower. Furthermore, CTD-ILD patients display notably lower superficial and deep retinal vascular density in comparison to the HCs group. The inverse correlation between conjunctival vascular density and both superficial and deep retinal TMI suggests that detecting subtle changes in ocular microcirculation could potentially

serve as an early diagnostic indicator for connective tissue diseases, thereby enhancing disease management.

● **KEYWORDS:** connective tissue disease-related interstitial lung disease; optical coherence tomography angiography; microvessel density; ocular surface; retina

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INTRODUCTION

Interstitial lung disease (ILD) is a heterogeneous group of non-neoplastic and non-infectious lung conditions, characterized by the presence of interstitial fibrosis and inflammation within the alveolar units[1]. Connective tissue diseases (CTD) refer to rheumatologic diseases that impact the proteins constituting the body's connective tissue framework. In individuals diagnosed with CTD, ILD affects a substantial proportion, with varying degrees of inflammation and fibrosis observed within the interstitial compartments of the lungs[2]. Connective tissue disease-related interstitial lung disease (CTD-ILD) progresses rapidly from incidental radiographic findings to respiratory failure and death, resulting in significant morbidity and mortality[3]. Various CTD, including systemic sclerosing disease (SSc), rheumatoid arthritis (RA), polymyositis (PM)/dermatomyositis (DM), systemic lupus erythematosus (SLE), and Sjögren's syndrome, may exhibit manifestations of CTD-ILD. Given that CTD-ILD serves as a leading cause of mortality, it exerts substantial detrimental effects on quality of life, underscoring the importance of accurate diagnosis and appropriate clinical management[4].

Some of the symptoms associated with CTD-ILD include those related to organizing pneumonia and acute lung injury, as well as diffuse interstitial processes such as usual interstitial pneumonia, nonspecific interstitial pneumonia, and lymphocytic interstitial pneumonia[5]. However, during the initial stages of the disease, patients may exhibit either no apparent symptoms or mild non-specific symptoms of pulmonary disease (such as fatigue, coughing, or exertional dyspnea)[1-6]. Hence, the prognosis of the disease can be improved through the implementation of efficient early detection techniques. Additionally, the differentiation between interstitial lung illness associated with CTD and idiopathic lung disease poses a challenging yet clinically important task[5,7]. In fact, there is currently no universally accepted diagnostic framework for CTD-ILD[8-9]. The novel concept of detecting CTD-ILD has the potential to enhance the accuracy of lung disease diagnosis.

Apart from impacting the integumentary system, pulmonary system, renal system, and neurological system, CTD can also exert its influence on ocular structures[10]. Ocular involvement may serve as the primary indicator of a systemic ailment necessitating further investigation and coordinated management[11]. Individuals with CTD may display ocular manifestations including erythema, xerophthalmia, discomfort, and visual impairment. The occurrence of blindness can arise from eye involvement, indicating a broader systemic involvement. Numerous eye disorders can be attributed to CTD that adversely affect the microvessels of the organs[11].

Optical coherence tomography angiography (OCTA), a novel non-invasive imaging modality, has emerged as a promising technology capable of expeditiously providing high-resolution volumetric flow data in conjunction with angiographic images. This innovation represents a notable advancement in optical coherence tomography (OCT)[12-13]. Notably, OCTA exhibits commendable sensitivity and accuracy in eye diseases characterized by neovascularization, while surpassing fluorescein angiography in its ability to offer a comprehensive depiction of blood flow in the retina and choroids[14-15]. OCTA has been employed for imaging the fundus in various systemic illnesses, such as DM[16] and SSc[17], owing to its evident advantages. In terms of non-invasive assessment of microvascular alterations induced by CTD in the ocular region, OCTA emerges as the optimal choice. Given that ocular manifestations may serve as the initial indication of CTD, identification of vascular irregularities in the eye can aid in the early diagnosis of CTD-ILD.

The comprehensive evaluation of OCTA findings in patients with CTD-ILD remains limited in existing literature, with scarce reporting on identified ocular alterations. This study aimed to investigate the abnormal microvessel density in the conjunctiva, as well as the superficial and deep macular retinal microvessel density, among individuals with CTD-ILD who underwent OCTA examination.

SBUJECTS AND METHODS

Ethical Approval The study methods and protocols were approved by the Medical Ethics Committee of the First Affiliated Hospital of Nanchang University (Nanchang, China) and followed the principles of the Declaration of Helsinki. All subjects were notified of the objectives and content of the study and latent risks, and then provided written informed consent to participate (No.2021039).

Study Subjects From September 2022 to September 2023, a cohort of 16 patients (32 eyes) diagnosed with CTD-ILD were recruited from the Rheumatology Department at The Nanchang University First Affiliated Hospital to constitute the ILD group. In order to establish a comparison, 16 healthy

individuals (32 eyes) of similar age and gender were selected from the Ophthalmic Research Center to form the healthy controls (HCs) group. The control participants were devoid of any ocular trauma or autoimmune disorders known to induce abnormal ocular circulation. The researchers collected and recorded fundamental patient information, including gender, age, and medical history. Additionally, both groups underwent specialized examinations, such as tests for antinuclear antibodies, visual acuity, fundus examinations, and OCTA. An ophthalmologist evaluated the OCTA results of all patients using a blinded methodology.

Inclusion and Exclusion Criteria Patients diagnosed with CTD-ILD are required to fulfill the diagnostic criteria for autoimmune-like interstitial pneumonia as established by the European and American Respiratory Societies in 2015. Additionally, these patients must be within the age range of 16 to 63, exhibit no ocular neuritis, choroiditis, or retinal vasculitis, be devoid of any other immune-related illnesses such as Sjögren's syndrome or other corneal and eye conditions, and have not undergone any previous immune system medication therapy. The normal control group should meet the following criteria: age between 16 and 63y, absence of any systemic diseases that may impact ocular circulation, no history of eye disease surgery, and absence of any systemic immune system disorders have a refractive error of <6.00 D and intraocular pressure (IOP) of 10–21 mm Hg in each eye. The exclusion criteria encompass the following: 1) the presence of autoimmune illnesses other than CTD; 2) the existence of systemic issues, such as severe hypertension, diabetes, and diseases affecting the eye and neurological system; 3) the occurrence of retinopathy or choroidopathy, which encompasses glaucoma and arterio-venous disease; 4) a history of eye trauma or surgery; 5) the presence of other disorders that hinder fundus imaging; 6) the inclusion of pregnant or nursing women.

Clinical Examinations All participants were subjected to a series of assessments, including: 1) an immunological assay utilizing immunofluorescence to quantify complement and antibodies, thereby evaluating the individuals' immune capacity; 2) a blood analysis to determine the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels, providing insights into the patients' inflammatory status; 3) a comprehensive evaluation of pulmonary health through lung CT imaging and pulmonary function testing; 4) the utilization of the Hospital Anxiety and Depression Scale (HADS) to assess the participants' mental well-being; 5) the implementation of OCTA for further examination; 6) various ocular indicators were measured, such as spherical equivalent ametropia, visual acuity (VA) using the Snellen chart, and IOP assessed with the Goldmann tonometer.

Optical Coherence Tomography Angiography The RTVue Avanti XR system (Optovue, CA) is utilized for OCTA imaging and the simultaneous display of retinal cross sections and microvessels. The scan parameters are established based on the parameters outlined by Ye *et al*[18]. With an axial resolution of 5 mm and a vertical resolution of 22 mm, along with scan rates of 7000 A scan/s for each, we captured images for a duration of 3.9s using the 840 nm wavelength and the 45 nm bandwidth. A total of five angiographic images were obtained in a scanning mode of 3 mm ×3 mm. After conducting two horizontal and two vertical volume scans, resulting in a combined total of a 3 mm ×3 mm OCTA frontal angiography image was acquired for each eye. Subsequently, a specialized software program is employed to partition the image into smaller regions.

The bed of retinal capillaries was categorized into two distinct physiological layers, namely the deep retinal layer (DRL) and the superficial retinal layer (SRL), based on its location. The DRL is situated between the anterior border of the ganglion cell layer and the vitreous retinal interface, while the SRL occupies the space between the outer boundary of the outer plexiform layer and the inner border of the inner plexiform layer. In order to segment blood vessels, the image size of 245×245 pixels should be changed to 1024×1024 pixels. The blood vessels prior to segmentation are commonly referred to as total microvascular (TMI). Segmentation techniques in image processing, such as equalization, inversion, and the elimination of background noise and non-vascular objects, are employed to remove microvessels and separate major blood arteries from the microvascular network, resulting in the generation of a binary image. Blood vessels with a diameter exceeding 25 μm are classified as major arteries (macrovascular, MAR), distinguished from other blood vessels referred to as minor arteries (microvascular, MIR) based on contrast. Additionally, this program effectively eliminates the graphical projection artifact of large blood vessel shadows in the SRL onto the DRL. In accordance with customary practice, all participants utilized their right eye. In order to acquire the mirrored representation and corresponding data, the data from the left eye was horizontally inverted. Subsequently, these numerical values were averaged and scrutinized in conjunction with the data obtained from the right eye. Macular retinal segmentation method: 1) The hemispherical segmentation method involves dividing the image into four sections, namely superior right (SR), superior left (SL), inferior left (IL), and inferior right (IR), using the horizontal and vertical diagonals as reference points (Figure 1K, 1O). 2) The Early Treatment Diabetic Retinopathy Study (EDTRS) utilizes a division of the image into four quadrants, namely superior (S), inferior (I), left (L), and right (R), based on the diagonal of the two

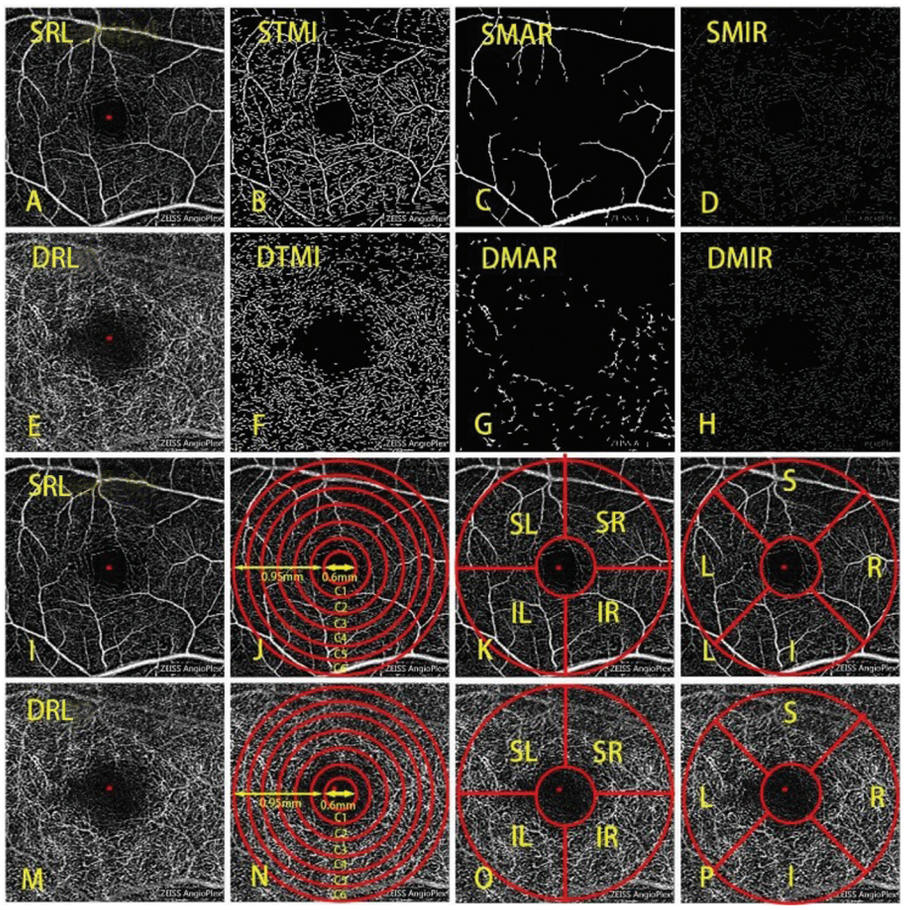

Figure 1 The 3×3-mm^2 OCTA image of the retina and three division methods A, I: Superficial retinal layer (SRL); E, M: Deep retinal layer (DRL); B: Superficial total microvascular (STMI); C: Superficial macrovascular (SMAR); D: Superficial retinal microvascular (SMIR); F: Deep total microvascular (DTMI); G: Deep macrovascular (DMAR); H: Deep retinal microvascular (DMIR); J, N: Central annuli segmentation method; K, O: Hemispherical segmentation method; L, P: Early Treatment Diabetic Retinopathy Study (EDTRS); L: Left; R: Right; S: Superior; I: Inferior; IL: Inferior left; IR: Inferior right; SL: Superior left; SR: Superior right; C1–C6: Central annuli segmentation method; OCTA: Optical coherence tomography angiography.

quadrants (Figure 1L, 1P). 3) The central annuli segmentation method involves the exclusion of the non-vascular middle area, measuring 0.6 mm in diameter, from a circular region with a diameter ranging from 0.6 to 2.5 mm. The remaining area is then divided into six thin rings, each having a bandwidth of 0.16 mm. These rings are sequentially labeled as C1 through C6, starting from the innermost ring. This process is illustrated in Figure 1J and 1N.

Statistical Analysis SPSS 22.0 (IBM, Armonk, NY, USA) was utilized for the purpose of data analysis, and the results were reported as mean±standard deviation (SD). The two-sample *t* test was employed to examine the outcomes for the ILD and HCs groups, while ANOVA procedures and Bonferroni correction for multiple comparisons were utilized to evaluate the data across groups. The density of the superficial and deep retinal microvessels, along with the conjunctival vessels, were determined using receiver operating characteristic (ROC) curves. The relationship between the density of conjunctival blood vessels and macular retinal blood vessels was evaluated through Pearson correlation analysis in GraphPad Prism (Version 9.0; GraphPad Software). Additionally, a linear correlation analysis was conducted to investigate the association between the density of superficial total microvascular (STMI) and deep total microvascular (DTMI) and the conjunctival capillary plexus. A significance level of $P<0.05$ was employed to indicate statistical significance, and the false discovery rate was utilized to adjust the *P*-values for multiple comparisons.

RESULTS

Subjects The study comprised a total of 16 patients diagnosed with CTD-ILD with a corresponding total of 32 eyes, as well as 16 HCs with a total of 32 eyes. Alongside their demographic data, clinical markers potentially associated with CTD were recorded for each participant. Notably, there were no significant differences observed in age, gender, IOP, or blood pressure among the groups (Table 1).

Analysis of Superficial Retinal Microvessel Density Figure 2 presents the comparative findings regarding the retinal density

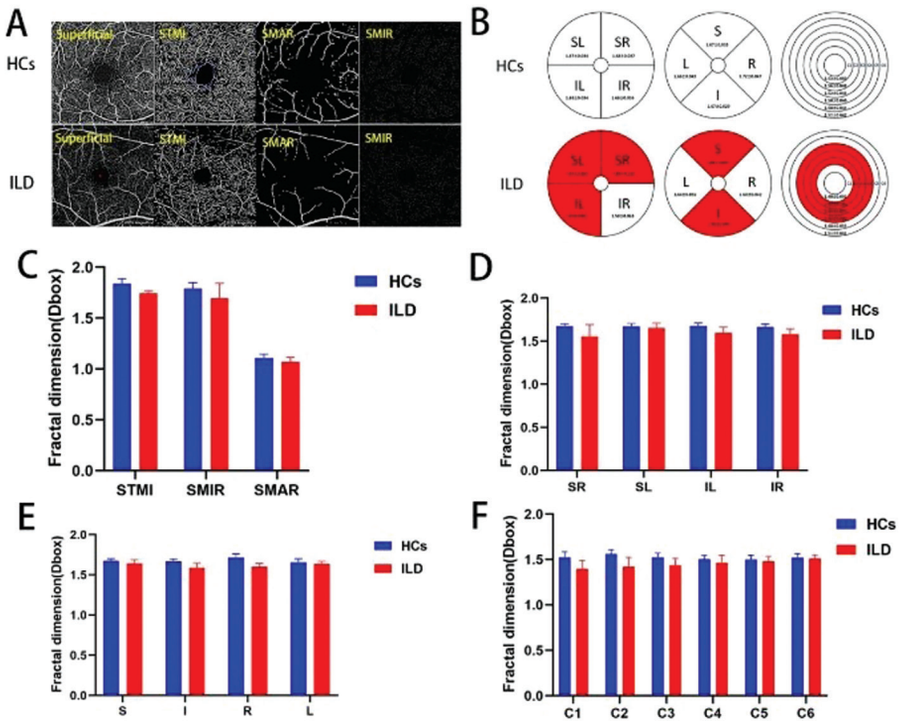

Figure 2 Analysis of the superficial retinal layer's macular retinal microvessel density in the ILD and HCs A: The superficial retinal vascular density as seen in OCTA pictures; B: A comparison of the retinal vascular density using three different techniques of grouping. C–F: Results of an examination of the superficial retinal vascular density in every subregion across groups. ILD: Interstitial lung disease; HC: Healthy control; STMI: Superficial total microvascular; SMAR: Superficial macrovascular; SMIR: Superficial retinal microvascular; L: Left; R: Right; S: Superior; I: Inferior; IL: Inferior left; IR: Inferior right; SL: Superior left; SR: Superior right; C1–C6: Central annuli segmentation method; OCTA: Optical coherence tomography angiography.

Table 1 Demographic characteristics and clinical data of subjects in the two groups

Parameters	ILD group (*n*=16, 32 eyes)	HC group (*n*=16, 32 eyes)	*P*	*t*
Age (y)	51.1±6.423	49.38±6.032	0.440	0.783
Gender (M/F)	12/4	12/4	-	1.000
IOP (mm Hg)	15.98±1.73	15.23±1.69	0.764	1.764
VA (logMAR)	0.43±0.13	0.9±0.13	<0.001	14.396
Duriation of CDT-ILD	27.25±38.01	N/A	-	-
ESR (mm)	35.75±25.01	N/A	-	-
CRP (10 mg/L)	6.24±3.59	N/A	-	-
CK-MB	32.32±20.49	N/A	-	-
MDA-5, *n* (%)	11 (68.75)	N/A	-	-
ANA, *n* (%)	11 (68.75)	N/A	-	-
FVC (L), % 72.41±15.83	2.12±0.46	N/A	-	-
RV (L), % 118.94±25.64	2.15±0.44	N/A	-	-
TLC (L), % 83.93±9.5	4.14±0.45	N/A	-	-
DLCO (mol/min•kPa), % 63.29±7.78	4.88±0.52	N/A	-	-
SBP (mm Hg)	125.69±5.71	123.86±5.6	0.487	0.915
DBP (mm Hg)	83.9±8.5	82.94±6.29	0.257	0.362
HADS	8.63±2.37	2.72±1.1	<0.001	-

ILD: Interstitial lung disease; CTD-ILD: Connective tissue disease-related interstitial lung disease; HC: Healthy control; SD: Standard deviation; IOP: Intraocular pressure; VA: Visual acuity; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; CK-MB: Creatine kinase isoenzymes; MDA-5: Melanoma differentiation-associated protein-5; ANA: Antinuclear antibody; FVC: Forced vital capacity; RV: Residual volume; TLC: Total lung capacity; DLCO: Diffusion capacity for carbon monoxide of lung; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HADS: Hospital Anxiety and Depression Scale. N/A: Not applicable.

in the superficial macular region across various areas. The results indicate a significant decrease in both superficial retinal microvascular (SMIR) and TMI in comparison to the HCs group. However, there was no significant difference observed in the superficial macrovascular (SMAR) between the patients with CTD-ILD and the HCs group (P>0.05; Figure 2B and 2C). Furthermore, employing the hemispheric segmentation approach revealed a considerably lower capillary density in the superficial retinal capillaries of the SL, SR, IL regions compared to the HCs (P<0.05; Figure 2B and 2D). In the ETDRS segmentation method, the MIR in the S and I regions exhibited a significant reduction when compared to the HCs (P<0.05; Figure 2B, 2E). Similarly, in the central annuli segmentation method, there was a significant decrease in the superficial regions of C2-C4 (P<0.05; Figure 2B, 2F).

The comparative results for retinal density in the deep macular region among different regions were presented in Figure 3. Contrary to the HCs group, it was observed that there were no significant differences in the superficial MAR of patients with CTD-ILD (P>0.05; Figure 3B, 3C). However, their superficial MIR and TMI exhibited a significant decrease (P<0.05) compared to the HCs group. Furthermore, the capillary density of the SL, IR in deep retinal capillaries was significantly lower than that of the HCs group, as determined by our hemispherical segmentation method (P<0.05; Figure 3B, 3D). When employing the ETDRS segmentation approach, a significant decrease in the MIR was observed in the I, L, and R regions compared to HCs (P<0.05; Figure 3B, 3E). Furthermore, the utilization of the central annuli segmentation method revealed a noteworthy reduction in vascular density (P<0.05; Figure 3B, 3F) within the deep regions of C3, C4, and C5.

ROC Evaluation of Retinal Microvessel Density in the Superficial and Deep Layers The OCTA measurement of macular retinal microvascular density demonstrated a high level of sensitivity and specificity in distinguishing between individuals with ILD and HCs. Specifically, the subregions of MIR, TMI, SL, SR, IL, S, I, and C2-C4 in the superficial retina exhibited significant statistical differences between the two groups. Notably, the area under the curve (AUC) for the SR region of the SRL was calculated to be 0.9641 (95%CI: 0.9179–1) when compared to the HCs group, indicating a superior diagnostic sensitivity for detecting retinal microangiopathy associated with CTD-ILD (Figure 4A). The group with ILD displayed significant changes in various regions of the DRL, including MIR, TMI, SL, IL, S, I, R, and C3-C5. Among these regions, the MIR model exhibited the highest positive likelihood ratio and achieved an AUC of 0.9583 (95%CI: 0.9260–0.9906). These findings suggest that the use of CTD-ILD diagnostics may be more effective in detecting DMIR (Figure 4B).

Connection Between the Density of Superficial and Deep Retinal Microvessel and the Density of Conjunctival Capillaries The present study observed a significant negative correlation (correlation coefficient of -0.1184, P<0.001) between the superficial retinal STMI area in the ILD group and conjunctival vascular density when examining the relationship between conjunctival capillary density and retinal vascular density (Figure 5A). Similarly, a negative association of -0.1885 (P<0.001) was found between conjunctival vascular density and the DTMI area (Figure 5B).

DISCUSSION

Our study provides a comprehensive analysis of the microcirculation of the macular retina in individuals with CTD-ILD. To assess the retinal capillary bed, we employed software to divide it into distinct physiological layers: the superficial layer and the deep layer. Simultaneously, we employed multiple segmentation techniques to segment the retina. The results indicated that individuals with ILD exhibited significantly lower densities of MIR, TMI, SL, SR, IL, S, I, R, and C3-C5 in their SRL compared to HCs (P<0.05). Similarly, in the DRL, the densities of MIR, TMI, SL, IL, S, I, R, and C3-C5 were found to be statistically lower than those observed in the HCs group (P<0.05). Previous research suggests that changes in ocular microcirculation may serve as an early indicator of CTD. Hence, it is imperative to detect retinal microcirculation changes through OCTA technology in order to facilitate early diagnosis and potentially improve the prognosis of patients with CTD-ILD. Our study contributes to the limited body of research utilizing OCTA to investigate the retinal microvascular alterations in CTD-ILD.

Vascular abnormalities in microcirculation structure and function play a significant role in the pathogenesis of CTD. Among the various CTD, alterations affecting the retina are particularly noteworthy. Individuals with SLE often encounter symptoms such as pain, visual impairment, and dryness in the eyes. Retinal vasculitis emerges as the most severe ocular complication for SLE patients. Furthermore, retinopathy not only serves as an unfavorable prognostic indicator of SLE but also demonstrates associations with central nervous system involvement and ongoing systemic illness[19]. Retinal damage poses a significant risk as a side effect of hydroxychloroquine, the prescribed treatment for Sjögren's syndrome, an autoimmune condition known to induce retinopathy[20]. The initial manifestation of SSc often involves the microvascular system, attributed to vascular lesions that lead to extensive fibrosis due to immune activation[21]. Examination of capillary microscopy reveals enlarged and distorted capillary rings of SSc patients with areas of reduced vascularity, alongside hypertensive alterations in the retina[22]. Furthermore, microangiopathy can lead to pathological changes such as

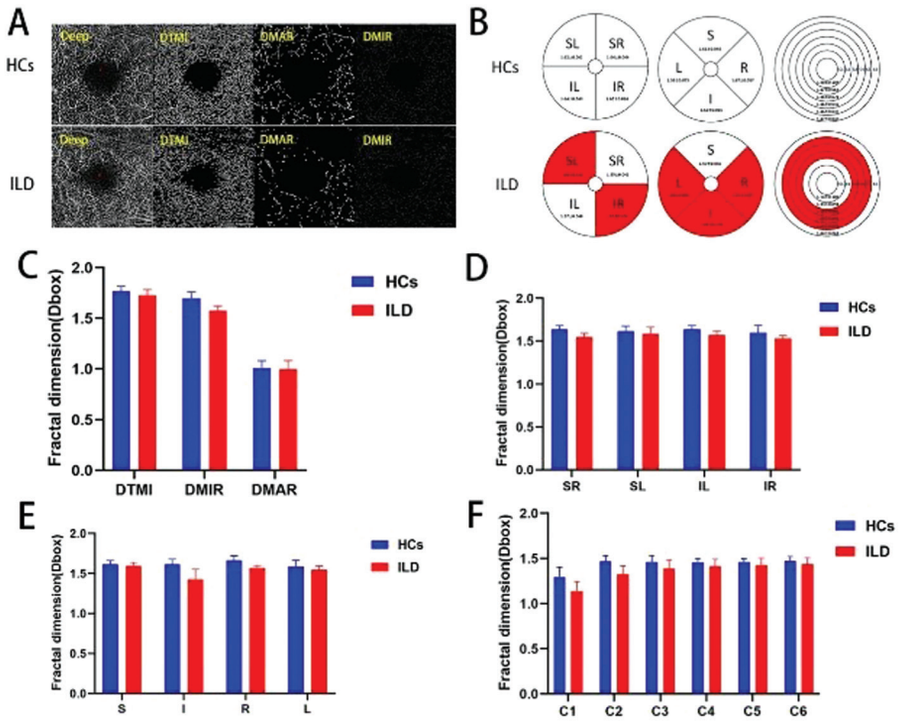

Figure 3 Analysis of the deep retinal layer's macular retinal microvessel density in the ILD and HCs A: The deep retinal vascular density in OCTA pictures; B: A comparison of the retinal vascular density using three different techniques of grouping; C–F: Results of an examination of the deep retinal vascular density in each subregion across groups. ILD: Interstitial lung disease; HC: Healthy control; DMAR: Deep macrovascular; DMIR: Deep retinal microvascular; DTMI: Deep total microvascular; L: Left; R: Right; S: Superior; I: Inferior; IL: Inferior left; IR: Inferior right; SL: Superior left; SR: Superior right; C1–C6: Central annuli segmentation method; OCTA: Optical coherence tomography angiography.

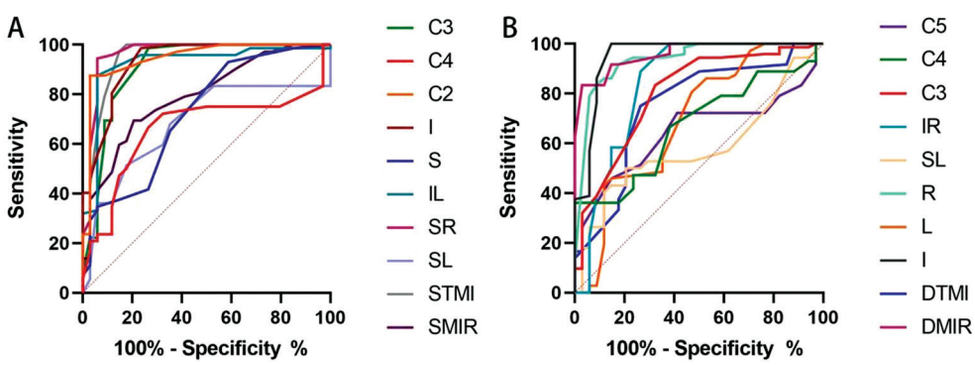

Figure 4 Microvessel density in various retinal zones of the fundus was analyzed using receiver operating characteristic curves ILD: Interstitial lung disease; HC: Healthy control; STMI: Superficial total microvascular; SMIR: Superficial retinal microvascular; DMIR: Deep retinal microvascular; DTMI: Deep total microvascular; L: Left; R: Right; S: Superior; I: Inferior; IL: Inferior left; IR: Inferior right; SL: Superior left; SR: Superior right; C1–C6: Central annuli segmentation method.

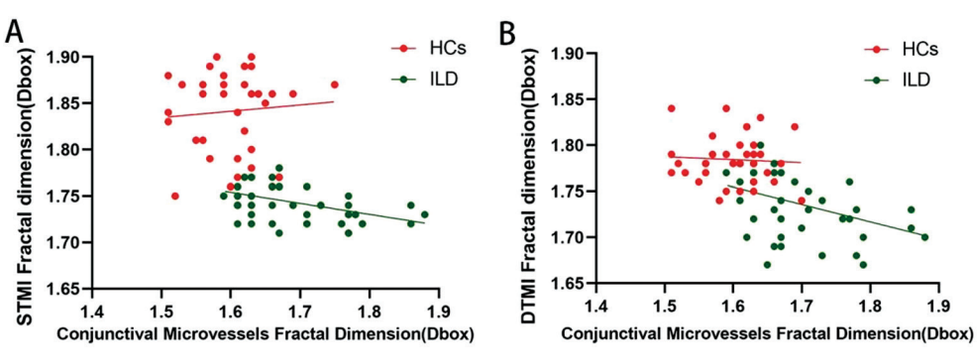

Figure 5 Conjunctival microvascular density and retinal microvascular density in HCs and ILD groups A: Superficial retinal layer; B: Deep retinal layer; ILD: Interstitial lung disease; HC: Healthy control.

endothelial damage, infiltration of monocytes into the vascular wall, and the development of occlusive lesions, which in turn can cause catastrophic multi-organ failure[17]. Patients diagnosed with DM, a suspected autoimmune disorder, may experience various ocular symptoms including asymptomatic retinopathy, double vision, or blurred vision[23]. According to a report, 14% (6 out of 43) individuals with PM and DM were found to have retinopathy[24]. Additionally, ocular vascular disease can arise from RA, and the underlying pathology of RA-induced retinopathy can be attributed to retinal vasculitis[25-26]. According to color Doppler imaging of the eye, individuals with RA exhibit significantly higher values for the resistivity index and peak contraction velocity of the central retinal artery[27]. ILD is commonly observed in patients with SSc[28], RA[29], and PM/DM[30]. ILD, characterized by varying degrees of interstitial lung fibrosis and inflammation[31], represents a severe manifestation of lung involvement that progresses to CTD. The therapy and prognosis of visual function in patients with CTD-ILD are significantly influenced by the visualization and assessment of fundus microvessels, which also serve as a means to identify and measure the advancement of the disease during subsequent examinations. Coherence in OCTA is a newly discovered OCT modality in which dynamic OCT focuses on motionless surrounding tissue while detecting changes in backscattering velocity brought on by movement, such as moving blood cells in blood arteries. Notably, interference-related changes can be observed in OCT images that are continuously acquired, as a result of the interference occurring in the backscattered light[32]. OCTA is utilized for assessing chronic obstructive pulmonary disease , reflecting the severity of inflammation and hypoxia in patients with chronic obstructive pulmonary disease, and correlating with alterations in retinal blood vessels[33]. It also plays a crucial role in evaluating retinal changes associated with ocular retinal density in CTD-ILD. The identified fundus changes can indicate the initial stage of the disease and serve as a means to monitor its progression.

The exact etiology of CTD-ILD remains unidentified. One prevailing hypothesis suggests that disease-specific auto-targeting antibodies may be responsible for lung damage by attacking the pulmonary tissue[34]. Conversely, certain subtypes of CTD may arise from lung-based mechanisms, wherein local inflammation induced by lung injury facilitates the production of autoantibodies against the lungs' own autoantigens. The subsequent binding of autoantibodies and antigens associated with lung pathology can lead to further inflammation and fibrosis[19,35]. The most prevalent pattern of non-specific interstitial pneumonia, a more inflammatory form of ILD, was observed in SSc (68%–77%)[25,27], PM/DM (65%–82%)[28-29], Sjogren's syndrome (28%–61%)[28,36], and undifferentiated CTD (83%)[37]. However, in RA patients with ILD, there is a higher susceptibility to develop acute interstitial pneumonia, a more fibrotic subtype of ILD, as indicated by high resolution CT or histology[38-41]. In a succinct manner, the overproduction of inflammatory chemicals by cells, the presence of autoantibodies, and the formation of immune complexes contribute to the deposition of inflammation in retinal blood vessels, subsequently leading to intimal proliferation. This growth of the intima disrupts hemodynamics, hinders laminar blood flow, and elevates the likelihood of thrombosis in patients, ultimately culminating in ischemia and hypoxia[36].

The central retinal artery is responsible for supplying the inner layer of the retina, while the choroidal vessels provide nourishment to the outer four layers. Additionally, the macula and optic disc receive their blood supply from the choroidal capillaries. Acute vascular occlusive retinopathy is characterized by vascular stenosis, occlusion, and extensive capillary nonperfusion[42]. Scott *et al*[43] have observed the presence of immunoglobulin and complement deposits, perivascular single-cell infiltration, and a rare cellulose-like necrosis in the retinal vascular wall, which can lead to small vessel infarction and ganglion cell atrophy. Immunoglobulin and complement deposition were also observed in the histopathological analysis. A star-shaped arrangement of capillaries forms a connected nerve plexus between the arterioles and venules in the SRL. Because SRL covers the retina's superficial surface as well as the capillaries of the retinal pigment epithelium, a decline in SRL density may be a sign of obstructive vascular disease, which has a clinically undetectable antecedent. Either a general decrease in blood flow velocity within the retinal arteries or a general reduction in blood vessel obstruction can explain the overall decline in SRL thickness. This finding supports our assertion that the density of various retinal regions, including SL, SR, IL, S, I, and C2-C4, decreased significantly (P<0.05), indicating a potential precursor to vascular occlusive retinopathy. However, in comparison to the HCs group, CTD-ILD patients exhibited a substantial decrease in SRL thickness in the macula, specifically in the SMIR and STMI regions, which may be attributed to microvascular blockage.

The arrangement of blood vessels in the DRL exhibits distinct characteristics. The DRL consists of polygonal structures, wherein capillaries converge radially to create clusters of capillaries. Blood is directed towards the deep venules through the DRL, which serves as the terminal point of the retinal capillary unit, originating from the superficial capillary cluster[44]. Consequently, any reduction in retinal blood flow may primarily impact the perfusion of the DRL. Based on previous studies, the underlying causes for the gradual restriction of flow in the deep choroid plexus,

as opposed to the superficial plexus, can be attributed to hemodynamic dysfunction or impaired neurovascular coupling. This dysfunction is characterized by the inability of neurons, glial cells, and vascular cells to effectively interact. The deep choroid plexus plays a crucial role in maintaining photoreceptor cell activity by providing oxygen and perfusion to the outer nuclear layer[45-46], where the photoreceptor axons are located. Based on the findings of Usui *et al*[47], it has been observed that elongate cells are absent in both DRL and SRL, while horizontal cells establish neurovascular units with capillaries, exhibiting a strong interdependence. Glial cell injury has been identified as a significant factor affecting the survival and functioning of photoreceptors[48]. Consequently, the reduction in blood vessel density in SRL and DRL among CTD-ILD patients can be attributed to the decline in DTMI alone, indicating early changes in retinal blood flow leading to organic loss. The potential consequences of long-term harm to the photoreceptors resulting in impaired visual effects remain uncertain, as it is unclear whether this is attributed to hemodynamic circumstances or a more intricate malfunction of glial vascular coupling. Our research findings indicate a statistically significant decrease in the density of SL, IL, S, I, R, and C3-C5 regions in the HCs group compared to the choroid (P<0.05). This suggests that the choroid, which serves as the primary blood vessel support in the eye, may be affected by the recurrence of chronic inflammatory diseases, potentially leading to vision impairment.

Moreover, to evaluate the influence of the disease on retinal involvement, we conducted AUC analyses on various macular retinal partitioning techniques in patients with CTD-ILD. Our findings demonstrate that OCTA exhibits a strong diagnostic capability for retinal microangiopathopathy. This is supported by the high positive probability ratios observed in the superficial SR area (0.9461) and MIR densities (0.9583). OCTA can directly see retinal microvessels and provide response to systemic disease microcirculation[15,49]. The iris and various conjunctival membranes receive their nutritional supply from branches of the anterior ciliary artery[50]. Conjunctival changes, including conjunctival vascularization, conjunctival varicose veins, telangiectasia, vasocongestion, intravascular congestion, and loss of fine arteries, have been associated with CTD such as systemic nodules[19]. In this study, it was observed that individuals with CTD-ILD exhibited a significantly elevated conjunctival vascular density. Furthermore, a negative correlation (-0.1885; P<0.001) was estimated between conjunctival vessel density and deep retinal DTMI in patients with CTD-ILD. Consequently, it is postulated that the increased blood vessel density in the conjunctiva of patients may be attributed to chronic inflammation, resulting in reduced conjunctiva perceptual and reflexia activity, as well as compensatory vasodilation, leading to heightened blood flow density. The first indication of systemic CTD may manifest as ocular involvement. To aid in the differentiation between abnormal and healthy eyes affected by CTD, and to enhance disease prognosis, the identification of retinal microcirculation changes through OCTA prior to the progression of CTD to severe ILD may be feasible. Our study stands as one of the limited investigations employing OCTA to analyze the characteristic modifications in retinal microvasculature associated with CTD-ILD.

However, it is important to acknowledge the limitations of this study. The small sample size and susceptibility of our study subjects to various confounding factors, such as medication usage, necessitate the validation of our research findings in larger-scale studies. While our primary assumption was that the reduction in retinal microvascular density among patients was attributed to inflammatory substances associated with CTD disease, it is crucial to consider the potential influence of decreased oxygen partial pressure on retinal microvasculature in ILD patients, likely due to the presence of dyspnea. One limitation associated with OCTA imaging pertains to the potential misinterpretation of data from uncooperative subjects, primarily attributed to the presence of motion artifacts.

In this study, OCTA was employed to gain a deeper understanding of macular retinal microcirculation in patients with CTD-ILD. Comparative analysis revealed significantly reduced superficial and deep retinal vascular density in CTD-ILD patients compared to HCs group. Moreover，this study observed an increase in conjunctival vascular density, which exhibited an inverse correlation with both superficial and deep retinal TMI in patients with CTD-ILD. This alteration in microcirculation may be implicated in the development of chronic inflammation and immune impairment in patients with CTD-ILD, leading to the associated visual impairment. Furthermore, the SR region and deep MIR region exhibited a higher positive likelihood ratio, suggesting that these regions may hold greater diagnostic value for CTD-ILD patients with retinal microvascular disease. These findings suggest that the OCTA has the potential to aid in the diagnosis of CTD-ILD by enabling the early detection of retinopathy and conjunctival vascular density.

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