BMJ Open Ophthalmology

Increased choroidal stromal area in patients with active Graves' ophthalmopathy based on binarisation method of optical coherence tomographic images

Min Zhou,[1] Dide Wu,[2] Leqi Cai,[1] Congyao Wang,[1] Yihua Su,[1] Ye Li,[1] Wanyi Ke,[1] Tingting Chen,[1] Shubin Hong,[2] Haipeng Xiao,[2] Pengxia Wan [1]

To cite: Zhou M, Wu D, Cai L, *et al*. Increased choroidal stromal area in patients with active Graves' ophthalmopathy based on binarisation method of optical coherence tomographic images. *BMJ Open Ophthalmology* 2024;**9**:e001443. doi:10.1136/bmjophth-2023-001443

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/bmjophth-2023-001443).

MZ, DW and LC contributed equally.

Received 7 August 2023
Accepted 29 September 2024

[1]Department of Ophthalmology, Sun Yat-sen University First Affiliated Hospital, Guangzhou, Guangdong, China
[2]Department of Endocrinology, Sun Yat-sen University First Affiliated Hospital, Guangzhou, Guangdong, China

Correspondence to
Dr Pengxia Wan; wanpengx@mail.sysu.edu.cn and

Professor Haipeng Xiao; xiaohp@mail.sysu.edu.cn

ABSTRACT

Objective To investigate the change in choroidal components of patients with Graves' ophthalmopathy (GO) with different degrees of disease activity and severity by using the image binarisation method of optical coherence tomography (OCT).

Methods This cross-sectional study included 151 eyes of 90 patients with GO. Patients were grouped according to the clinical activity score (CAS) and disease severity. Total choroidal area (TCA), luminal area, stromal area (SA) and choroidal vascularity index (CVI) were acquired by image binarisation of the OCT. Ocular parameters between groups were compared using generalised estimating equations, accounting for intereye correlation and adjusting for relevant factors.

Results As for the included eyes, 104 eyes were inactive GO and 47 eyes were active GO. Local choroidal thicknesses were thicker in active GO than in inactive GO. TCA and SA were significantly larger in active GO than in inactive GO group (3.44±0.91 mm^2 vs 3.14±0.88 mm^2, p=0.046; 1.16 (1.03–1.50) mm^2 vs 1.10 (0.96–1.27) mm^2, p=0.002, respectively). CAS was positively correlated with TCA (r=0.171, p=0.036) and SA (r=0.172, p=0.035), and negatively associated with CVI (r=−0.174, p=0.032). In multiple regression models, age, diopter and intraocular pressure (IOP) exhibited significant correlations with the SA (β=−0.006, p=0.010; β=0.076, p<0.001; β=0.015, p=0.010, respectively).

Conclusions Thickened choroid was observed in active GO compared with inactive GO. The proportional increase of SA was augmented as the disease activity progressed. Age, diopter and IOP were independent factors that affected choroidal area and components in patients with GO. Multicentre prospective cohort studies with a large sample size are still needed.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Thickened choroid was observed in patients with Graves' ophthalmopathy (GO) compared with healthy controls.

WHAT THIS STUDY ADDS

⇒ Active GO had a thicker choroid than inactive GO even after adjusting for relevant factors.

⇒ The proportional increase of choroidal stromal area was augmented as the disease activity progressed.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Choroidal components show promising potential as biomarkers in patients with active GO.

INTRODUCTION

Approximately 50% of patients with Graves' disease (GD) will suffer from Graves' ophthalmopathy (GO) before, during or after the development of hyperthyroidism. Reportedly, the majority of patients (60%) are mild and only experience mild discomfort related to eyelid retraction, whereas unfortunately, 3%–7% will develop corneal breakdown or dysthyroid optic neuropathy (DON) as serious complications of GO.[1] DON can cause permanent loss of vision due to irreversible optic nerve damage if timely intervention such as high-dose intravenous steroids or surgical decompression is not sought.[2] Treatment decisions for GO depend on the disease activity, severity and duration according to the European Group on Graves' orbitopathy (EUGOGO) clinical practice guidelines.[3] However, both methods of measuring activity and severity are subjective, thus requiring training and may vary between observers. Hence, an accurate activity and severity evaluation of GO is crucial in order to guide treatment decisions and avoid unnecessary risks of side effects.

The choroid is known as the most vascularised layer of the eye and has vital functions like nourishing the retina.[4] Over the last decades, choroid has been considered to be affected by systemic autoimmune diseases for its high vascular component, which might

be susceptible to inflammation.[5] Several studies have measured the choroid thickness in autoimmune diseases, including GO, using optical coherence tomography (OCT) with a self-contained software system and have proposed that it could be the potential inflammatory biomarker.[6–8] However, previous thickness measurements may not sufficiently reflect the detailed structure of the choroid because it was only a linear measurement at a certain point that could not reveal which component was more affected. Sonoda *et al* reported a new method to quantify the choroidal luminal area (LA) and stromal area (SA) using ImageJ software through the binarisation method of OCT images that was reproducible and acceptable.[9 10] In recent years, this approach has been used in a number of studies of various ocular diseases involving the retina and choroid, and has shown the ability to serve as a new additional assessment tool.[11–13]

Therefore, our study was designed to further investigate the choroidal components change in patients with GO with various degrees of disease activity and severity by using this binarisation method to find an objective way to classify GO's activity and severity.

METHODS

Participants

This study enrolled 90 patients (49 men and 41 women, 151 eyes) with GO who visited the First Affiliated Hospital of Sun Yat-sen University between March 2019 and August 2022. The diagnosis of GO was established based on Bartley's diagnostic criteria.[14] DON diagnosis was based on the presence of CT scan signs of apical crowing or optic nerve stretching and at least two of the following: the deterioration of best-corrected visual acuity (BCVA), altered visual field (VF), loss of colour vision, relative afferent pupillary defect or optic disc swelling in the absence of any other explanation.[15 16] All participants received a detailed assessment of their medical history, including the history of GD and GO, current medication use and history of drug use.

The exclusion criteria were the following: (1) use of glucocorticoids and immunosuppressive agents within the past 3 months; (2) the history of ocular surgery and trauma; (3) refractive errors over +5.0 D or under −6.0 D of spherical equivalent; (4) concomitant ocular diseases such as infectious keratitis, glaucoma or retinal disease; (5) poor image due to exposure keratitis, dense cataract; (6) systemic diseases such as neurodegenerative diseases, diabetes mellitus, malignant hypertension or systemic lupus erythematosus, which might affect the retina. Patients were divided into groups according to the EUGOGO recommendations with clinical activity score (CAS) and disease severity.[17]

Ophthalmological examinations

An experienced ophthalmologist (CW) who was blinded to study protocol performed all the ophthalmological examinations, including measurement of BCVA, intraocular pressure (IOP), proptosis and biomicroscopy of the anterior and posterior segments. All patients underwent VF testing using a Humphrey Field Analyzer II (Carl Zeiss Meditec, Dublin, California, USA) with the 24-2 threshold SITA-FAST protocol. VFs with >20% fixation losses, or >15% false-positive and 15% false-negative errors were excluded. The general light sensitivity of VF was expressed in mean deviation (MD), and the local VF defect was expressed in pattern SD (PSD), as calculated by the perimetry software.

Spectral-domain optical coherence tomography image acquirement and analysis

The imaging of all subjects was performed using the spectral-domain OCT instrument (Spectralis OCT, Heidelberg Engineering, Heidelberg, Germany) with enhanced depth imaging technology. All measurements were carried out between 14:30 hours and 17:30 hours to minimise the effects of the circadian rhythm. Single line scans of 30° (9 mm) were taken horizontally focused on the centre of the fovea with high-speed protocol scans. The best central scan passing through the fovea was selected to measure the thickness of the retina and choroid. The choroidal thickness measurement was performed manually from the inferior margin of retinal pigment epithelium (RPE) to the inner portion of the sclerochoroidal junction at the central fovea and points 1500 µm, 3000 µm nasal and temporal from the central fovea with a self-contained software system. The retinal thickness was measured manually from the internal limiting membrane to the outer boundary of the RPE at the central fovea and points 1500 µm, 3000 µm nasal and temporal from the central fovea. The retinal nerve fibre layer (RNFL) thickness was measured using 1024 A-scan points from a 3.45 mm circle centred on the optic disc. The RNFL thickness map of the optic disc was segmented into four quadrants as follows: temporal, nasal, inferior and superior. Two ophthalmologists (YS and WK) reviewed all images in consensus. The image exclusion criteria are as follows: (1) a signal strength index <25 dB, (2) non-centred scans (RNFL measurement), (3) motion artefacts and (4) unclear choroidal boundary.

The best central scan passing through the fovea was then imported into ImageJ (V.1.49, National Institutes of Health, Bethesda, Maryland, USA) for further processing. The specific measurement method was used based on previous reports.[9 10] The examined area was determined to be 7500 µm wide in the subfoveal choroid. The nasal boundary was the edge of the optic nerve head and the temporal boundary was determined as the 7500 µm temporal from the edge of the optic nerve head. The Oval Selection Tool on the ImageJ toolbar was used to select three choroidal vessels with lumens >100 µm, then the reflectivity of these areas was calculated. A threshold was set at the average brightness of the LA to exclude background noise in the OCT image. The image was transformed to an 8-bit image, and subsequently, a Niblack Auto Local Threshold tool was applied. After processing, the binarised image was transformed into

RGB images again, and the LA was measured using the Threshold Tool. After setting the data of the distance of each pixel, the total choroidal area (TCA), LA and SA were automatically calculated. The choroidal vascularity index (CVI) was calculated by the following formula: CVI%=LA/TCA×100%. Measurements were performed by the same trained individual (LC).

Statistical analyses

All statistical evaluations were conducted using SPSS V.22.0 statistical software package (SPSS, Armonk, New York, USA). Descriptive data were expressed as mean (SD) or median (IQR) for continuous variables and frequency (percentage) for categorical variables.

Comparisons among three groups were performed using the one-way analysis of variance for normally distributed continuous data and the Kruskal-Wallis test for non-normally distributed continuous data. When comparing two groups, a Student's t-test or Mann-Whitney U test was performed. Data from both eyes among the groups were analysed using generalised estimating equations (GEE) accounting for the intereye correlation and adjusting the age, sex and diopter. The least significant difference (LSD) post hoc test was used to determine significant pairwise comparisons. Correlations between disease severity and activity and ocular parameters were analysed by using the Spearman's correlation. Univariate linear regression and multiple stepwise linear regression analyses were performed to identify the influence factors of choroidal components. Age, sex and all variables significant with p<0.05 in the simple regression analysis were included in the final multiple regression model. We considered a p value <0.05 to be statistically significant.

Patient and public involvement statement

Patients and the public were not involved in the design and conduct of this study.

RESULTS

Demographic data

Of the 90 patients included in this study, 58 patients (27 males, 31 females) had inactive GO with a mean age of 45.60±12.76 years and 32 patients (22 males, 10 females) had active GO with a mean age of 45.63±11.19 years. As for the included eyes, 104 eyes had inactive GO and 47 eyes had active GO. No significant difference was found between the two groups for age, sex and duration of GD and GO (table 1).

When the patients were grouped in accordance with the disease severity recommended by EUGOGO, 32 patients (mean age: 43.38±13.22 years; 11 males, 21 females) with mild GO, 50 patients (mean age: 46.28±11.20 years; 31 males, 19 females) with moderate-to-severe GO and 8 patients (mean age: 50.38±13.28 years; 7 males, 1 female) with DON, of these, 59 eyes had mild GO, 81 had moderate-to-severe GO and 11 had DON. There was a statistical difference in sex among the three groups (p=0.007), but no statistical differences were found in other demographic data (online supplemental table 1).

Ophthalmological examinations

After adjustment for age, sex and intereye correlation, BCVA, MD, PSD, IOP, diopter and proptosis were no significant differences between the active GO group and inactive GO group (all p>0.05) (online supplemental table 2).

Among the groups according to the disease severity, there was a significant difference in BCVA among the three groups (p=0.001). Pairwise comparisons revealed that the BCVA of the DON group was the worst, compared with the mild GO group (DON vs mild GO=0.10 (0.05–0.30) vs 0.00 (0.00–0.00), p=0.002) and moderate-to-severe group (DON vs moderate-to-severe GO=0.10 (0.05–0.30) vs 0.00 (0.00–0.10), p=0.001). There was a significant difference in diopter among the three groups (p=0.004). Proptosis was found to be significantly different among the three groups (p=0.001). In pairwise comparisons, the moderate-to-severe GO group had greater proptosis than the mild GO group (20.3±3.0 mm vs 17.7±2.3 mm, p<0.001), whereas no significant difference was found in patients with moderate-to-severe GO versus DON. There were no significant differences in IOP and VF parameters, including MD and PSD, among the three groups (all p>0.05) (online supplemental table 3).

Optical coherence tomography image data

All analyses were adjusted for age, sex, diopter and intereye correlation. Choroidal thicknesses measured 1500 µm nasal and 3000 µm nasal away from the foveal centre were thicker in active GO than in inactive GO (319±101 µm vs 285±97 µm, p=0.036; 232±87 µm vs 199±79 µm, p=0.036, respectively). In the comparison

Table 1 Comparison of demographic and clinical characteristics between inactive GO group and active GO group

	Inactive GO	Active GO	P value
	N=58 patients	N=32 patients	
Age (years)	45.60±12.76	45.63±11.19	0.994
Male sex (%)	46.60%	68.8%	0.050
GD duration (months)	12.00 (5.75–24.00)	12.00 (5.25–24.00)	0.767
GO duration (months)	6.00 (3.00–18.25)	6.00 (3.00–12.00)	0.397

GD, Graves' disease; GO, Graves' ophthalmopathy.

Table 2 Comparison of choroidal thickness at various locations and choroidal components between inactive GO group and active GO group

	Inactive GO	Active GO	P value*
	N=104 eyes	N=47 eyes	
Choroidal subfoveal (μm)	331±102	362±106	0.105
Nasal choroidal 1500 μm (μm)	285±97	319±101	**0.036**
Nasal choroidal 3000 μm (μm)	199±79	232±87	**0.036**
Temporal choroidal 1500 μm (μm)	320 (262–358)	320 (276–393)	0.177
Temporal choroidal 3000 μm (μm)	280±72	289±69	0.687
TCA (mm^2)	3.14±0.88	3.44±0.91	**0.046**
LA (mm^2)	2.02±0.58	2.18±0.58	0.229
SA (mm^2)	1.10 (0.96–1.27)	1.16 (1.03–1.50)	**0.002**
CVI (%)	64.3 (62.9–65.6)	62.9 (61.7–64.4)	**0.021**

Bold font indicates statistical significance(P<0.05).
*Generalised estimating equations, adjusted for age, sex, diopter and intereye correlation.
CVI, choroidal vascularity index; GO, Graves' ophthalmopathy; LA, luminal area; SA, stromal area; TCA, total choroidal area.

of the choroidal parameters obtained from the image binarisation of OCT, TCA and SA were significantly larger in active GO group than in inactive GO group (3.44±0.91 mm^2 vs 3.14±0.88 mm^2, p=0.046; 1.16 (1.03–1.50) mm^2 vs 1.10 (0.96–1.27) mm^2, p=0.002, respectively), but no significant difference was observed in LA between these two groups (p=0.229) (table 2 and figure 1). CVI was significantly higher in inactive GO than in active GO (64.3 (62.9–65.6)% vs 62.9 (61.7–64.4)%, p=0.021). There were no significant differences in retinal and RNFL thickness at different locations or quadrants between the active and inactive GO groups (all p>0.05) (online supplemental table 4).

There were no significant differences in choroidal parameters among the groups classified by disease severity based on the EUGOGO criteria, except for the SA (p=0.040). Post hoc pairwise analyses revealed that SA was significantly larger in DON group compared with the mild GO group (1.43±0.37 mm^2 vs 1.09±0.33 mm^2, p=0.021) (online supplemental table 5). Foveal retinal thickness in eyes with DON was the thinnest, compared with those in the mild and moderate-to-severe GO group (mild GO vs moderate-to-severe GO vs DON=219±15 μm vs 220±18 μm vs 210±18 μm, p=0.002), whereas no significant difference was shown between mild and moderate-to-severe GO group (p=0.859). However, no statistically significant difference was observed in retinal thickness among the three groups at other locations. There were no significant differences in RNFL thickness at any quadrants among the three groups (all p>0.05) (table 3).

Correlations between OCT parameters and disease activity and severity

CAS was significantly positively correlated with choroidal thickness at 1500 μm and 3000 μm nasal locations (r=0.174, p=0.033; r=0.188, p=0.021, respectively), whereas not statistically associated with other locations. TCA and SA were significantly positively correlated with CAS (r=0.171, p=0.036; r=0.172, p=0.035, respectively), while CVI was found to be significantly negatively correlated with CAS (r=−0.174, p=0.032) (figure 2 and online supplemental table 6). Disease severity had positive associations with choroidal components, including TCA (r=0.187, p=0.021), LA (r=0.176, p=0.030) and SA (r=0.202, p=0.013), but not with other parameters. Correlations between the OCT parameters and disease severity are presented in online supplemental table 7.

Factors associated with the choroid

TCA was found to be significantly associated with the sex (β=0.489, p=0.001), BCVA (β=−0.691, p=0.046), diopter (β=0.202, p<0.001) and IOP (β=0.032, p=0.033), but not age (β=−0.004, p=0.561) and proptosis (β=0.037, p=0.132) in the univariate linear regression. Based on the results of univariate analyses and clinical knowledge, age, sex, BCVA, diopter and IOP were included in multiple linear regression. Age, sex, diopter and IOP were independent factors affecting the TCA (β=−0.019, p=0.002; β=0.355, p=0.014; β=0.201, p<0.001; β=0.033, p=0.034, respectively) (online supplemental table 8).

The univariate linear regression showed that SA was significantly associated with sex (β=0.157, p=0.003), diopter (β=0.069, p<0.001) and IOP (β=0.012, p=0.026). Age and all significant predictors were subsequently entered into multiple regression models. Age, diopter and IOP exhibited significant correlations with the SA (β=−0.006, p=0.010; β=0.076, p<0.001; β=0.015, p=0.010, respectively) after adjusting for relevant factors (online supplemental table 9).

DISCUSSION

This binarisation method demonstrated that active GO exhibited a thicker choroid than inactive GO even after

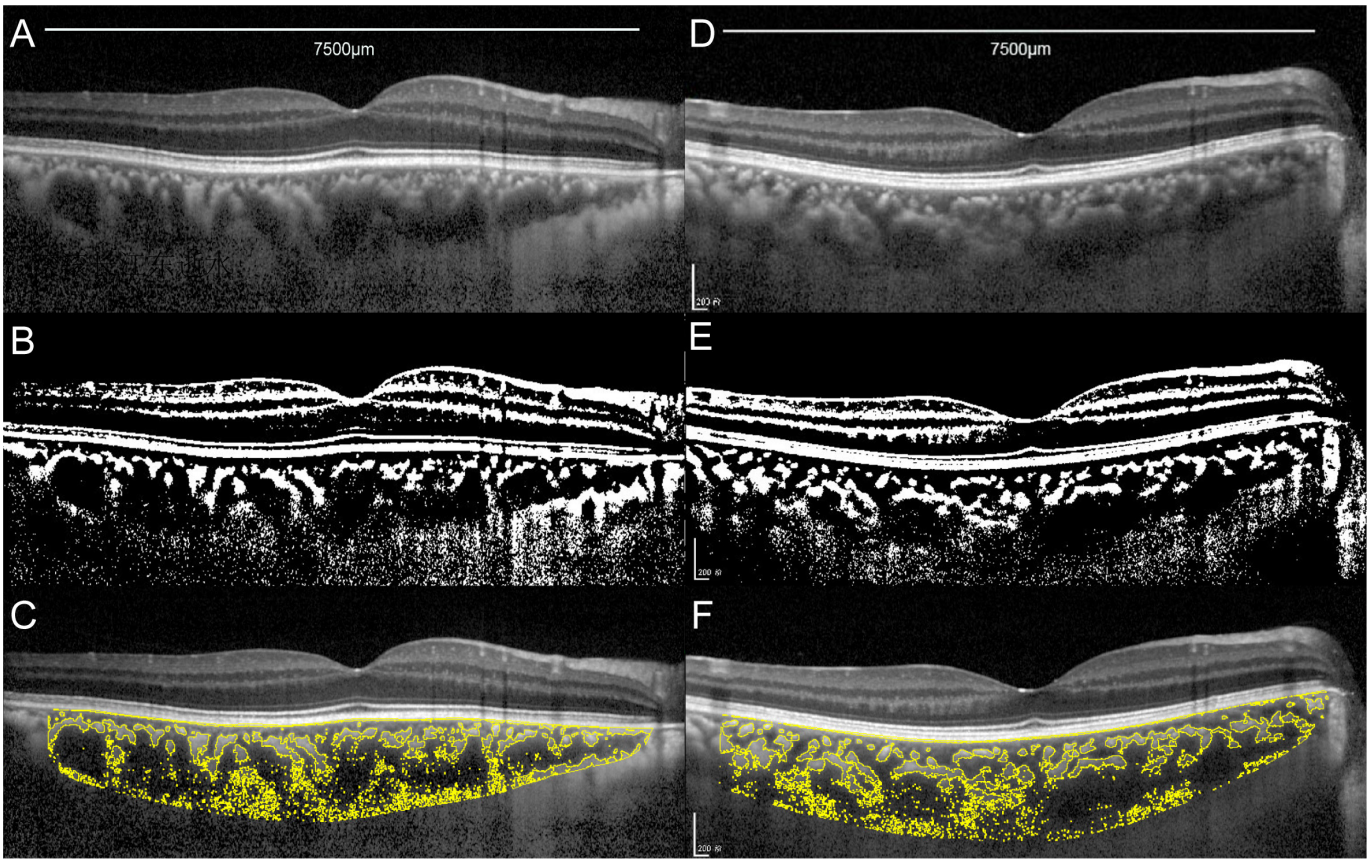

Figure 1 Representative choroidal images obtained by the binarisation method in patients with inactive Graves' ophthalmopathy (GO) and active GO. (1) A patient with inactive GO with clinical activity scores (CAS) of 2 points. (A) Image acquired by optical coherence tomography (OCT) with the enhanced-depth image (EDI) mode. (B) Image binary representation. (C) Target choroidal region overlapped in the original image (black areas circled by yellow lines indicate luminal area). (2) A patient with active GO with CAS of 4 points. (D) Image acquired by EDI-OCT. (E) Image binary representation. (F) Target choroidal region overlapped in the original image (black areas circled by yellow lines indicate luminal area). Active GO eye (F) exhibited a larger stromal area compared with inactive GO eye (C) after adjusting for age, sex, diopter and intereye correlation.

adjusting for relevant factors known to be associated with the choroid. SA, but not LA, excessively increased in active GO. Additionally, TCA and SA were positively correlated with CAS scores, indicating gradual thickening and change in choroid as disease activity progressed. Age, diopter and IOP were independent factors that affected choroidal area and components in patients with GO.

A previous study has demonstrated that superior rectus muscle enlargement alone may compress the superior ophthalmic vein causing reduced venous outflow from susceptible orbits, thereby expanding the apparent orbital fat volume and producing proptosis.[18] Thickening choroid in GO is thought to be the result of choroidal vascular congestion and stasis secondary to raised orbital venous pressure. Loiudice *et al* found that LA was higher in patients with GO compared with healthy controls with this binarisation method.[19] Yeter *et al* found that TCA, LA and SA were higher in patients with GO compared with healthy controls, they further carried out subgroup analysis by different activity, but no significant difference was found in TCA, LA and SA between active GO and inactive GO.[20] In contrast, our study showed that TCA and SA were increased in active GO compared with inactive GO. We speculate that this discrepancy may be caused by the following reasons: first, the limited number of studied populations; second, we adjusted for multiple confounders, such as age, sex and diopter, which are extensively accepted as influence factors for choroid. Additionally, TCA and SA showed positive correlations with CAS. These results indicated that SA excessively increased during the active phase. The choroid is composed of multiple cell types, including choroidal endothelial cells, melanocytes, fibroblasts, vascular smooth muscle cells and immune cells.[21] Insulin-like growth factor-1 (IGF-1) has been verified to stimulate choroidal endothelial cell proliferation in vitro.[22] A recent study demonstrated that choroidal thickness might be affected by elevated serum IGF-1 levels in patients with untreated acromegaly.[23] We speculate that activated choroid plexus fibroblasts, triggered by antithyrotropin receptor antibody or IGF-1 receptor antibody, secreted high levels of glycosaminoglycan, which increased SA in patients with active GO. Additionally, a study showed that the infiltration of T cells and monocytes/macrophages was more severe in early active GO than inactive GO.[24] Likewise, inflammatory infiltration may exist in the choroidal stroma, causing

Table 3 Comparison of retinal thickness and RNFL thickness at various locations among the three groups classified by disease severity based on the EUGOGO criteria

	Mild GO	Moderate-to-severe GO	DON	P value*	Post hoc analysis p value†		
	N=59 eyes	N=81 eyes	N=11 eyes		Moderate-to-severe GO versus mild GO	Moderate-to-severe GO versus DON	Mild GO versus DON
Foveal (µm)	219±15	220±18	210±18	**0.002**	0.859	**<0.001**	**0.001**
Nasal retinal 1500 µm (µm)	355±19	354±23	350±17	0.272	0.324	0.237	0.126
Nasal retinal 3000 µm (µm)	300 (289–315)	300 (293–313)	305 (286–324)	0.632	0.476	0.486	0.706
Temporal retinal 1500 µm (µm)	325±17	326±19	320±15	0.439	0.799	0.2	0.281
Temporal retinal 3000 µm (µm)	255±16	258±17	257±16	0.736	0.909	0.434	0.475
Superior (µm)	134 (126–145)	139 (123–151)	130 (119–152)	0.22	0.153	0.147	0.419
Inferior (µm)	139 (132–148)	142 (132–154)	147 (139–177)	0.127	0.067	0.659	0.246
Nasal (µm)	69 (62–76)	67 (61–80)	73 (58–93)	0.872	0.677	0.7	0.82
Temporal (µm)	80 (72–87)	79 (73–90)	84 (72–90)	0.988	0.936	0.901	0.875

Bold font indicates statistical significance(P<0.05).
*Generalised estimating equations, adjusted for age, sex, diopter and intereye correlation.
†The post hoc test was performed using the least significant difference test.
DON, dysthyroid optic neuropathy; EUGOGO, European Group on Graves' orbitopathy; GO, Graves' ophthalmopathy; RNFL, retinal nerve fibre layer.

choroidal thickening and oedema. These factors likely outweigh the effect caused by choroidal vascular congestion and stasis and subsequently result in thickening TCA and SA in active GO.

Changing severity implies that increased vascular congestion, enlargement of orbital connective tissues and orbital inflammation are more severe. Interestingly, we found that choroidal components changed in patients with DON, while there was no change in choroidal thickness compared with mild GO. This indicated that choroidal components change may occur earlier than choroidal thickness under severe compression. We also found retinal thickness was thinnest in patients with DON compared with the other two groups. Degeneration of the retinal ganglion cell complex layer (GCCL) thickness is likely attributable to the thinning of the retina around the macula. Previous studies have demonstrated that GCCL thinning occurred in patients with DON.[25 26] Mechanical compression of the retina by enlarged orbital soft tissue has contributed to the thinning of GCCL. Blum Meirovitch *et al* suggested that reduced blood flow as a consequence of vein congestion or arterial stenosis may be another possible reason.[27]

As reported by former studies, choroidal thickness has been found to decline with age and increase with refractive error. Age and diopter are widely considered influential factors for the choroid.[28 29] In particular, we found that TCA and SA increased with IOP elevation. Increasing tissue volume within the limited orbital space leads to an increase in intraorbital pressure. IOP may reflect intraorbital pressure to a large extent. A study has found that IOP decreased and superior ophthalmic vein blood flow velocity increased in patients with GO after orbital decompression, which hinted at the association between elevated IOP and increased episcleral venous pressure.[30] Therefore, based on our results, IOP may be a strong predictor of changes in the choroidal component that reflect disease activity. It also suggested that patients with GO with elevated IOP may need treatment as early as possible.

Strengths of the current study include its prospective study design and rigorous exclusion criterion. GEE analysis was used to account for the inclusion of two eyes from the same patient and to adjust for age, sex and diopter, thereby making the results more reliable and convincing. However, several limitations should be taken into account when interpreting the results of the current study. Our data obtained by image binarisation may differ from previous reports due to a diverse range of measurements and different scale bars. To maintain comparability, we selected the measurement range and scale bar used by Sonoda *et al*, who first published this binarisation method.[10] Furthermore, the small sample sizes in the subgroup analysis may have reduced the power to detect more significant differences. The results of subgroup analyses in the present study should be interpreted with caution because of the small and unbalanced sample sizes. Multicentre prospective cohort studies with a large sample size are necessary in the future.

In conclusion, active GO had a thicker choroid than inactive GO even after adjusting for relevant factors. The proportional increase of SA was augmented as the disease

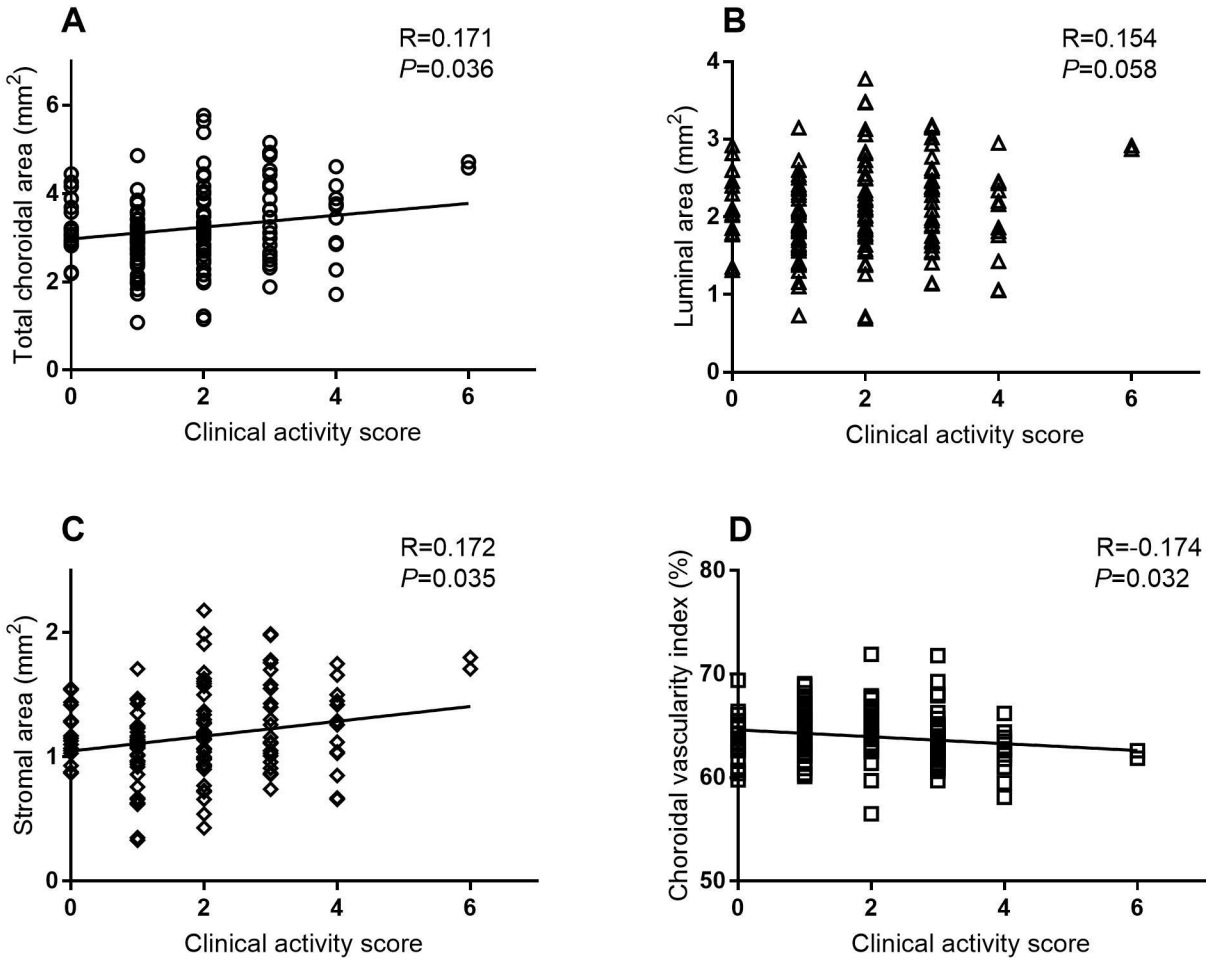

Figure 2 Correlation analyses between clinical activity score and the choroidal parameters obtained from the image binarisation of optical coherence tomography.

activity progressed. When OCT shows an increase in the proportion of SA, it may indicate that patients are in the active phase of the disease, necessitating close clinical follow-up and timely intervention. Choroidal components show promising potential as biomarkers in patients with active GO.

Acknowledgements The authors greatly appreciate the image analysis assistance by Zhouyue Li at State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China.

Contributors PW, HX, MZ and DW planned, designed and conceived the search. CW, YS and WK collected the data. LC and MZ performed statistical analysis. YL, TC and SH provided feedback on the report. MZ, DW and LC drafted the manuscript. PW, HX and MZ supervised the study. The manuscript was reviewed and approved by all authors. PW is responsible for the overall content as guarantor.

Funding This study was financially supported by the Guangdong Basic and Applied Basic Research Foundation (2019A1515110012), Natural Science Foundation of Guangdong Province (2021A1515010372) and Guangdong Medical Science and Technology Research Foundation (A2021017).

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study was approved by the institutional review board and the ethics committee of the First Affiliated Hospital of Sun Yat-sen University (2018-050) in China. This research adhered to the tenets of the Declaration of Helsinki. All subjects provided written informed consent and received physician approval to participate before enrolment in the study.

Provenance and peer review Part of a topic collection; not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

ORCID iD

Pengxia Wan http://orcid.org/0000-0002-1729-8582

REFERENCES

1 Neigel JM, Rootman J, Belkin RI, *et al*. Dysthyroid optic neuropathy. The crowded orbital apex syndrome. *Ophthalmology* 1988;95:1515–21.
2 Blandford AD, Zhang D, Chundury RV, *et al*. Dysthyroid optic neuropathy: update on pathogenesis, diagnosis, and management. *Expert Rev Ophthalmol* 2017;12:111–21.
3 Bartalena L, Kahaly GJ, Baldeschi L, *et al*. The 2021 European Group on Graves' orbitopathy (EUGOGO) clinical practice guidelines for the medical management of Graves' orbitopathy. *Eur J Endocrinol* 2021;185:G43–67.
4 Nickla DL, Wallman J. The multifunctional choroid. *Prog Retin Eye Res* 2010;29:144–68.
5 Steiner M, Esteban-Ortega MDM, Muñoz-Fernández S. Choroidal and retinal thickness in systemic autoimmune and inflammatory diseases: A review. *Surv Ophthalmol* 2019;64:757–69.
6 Coskun E, Gurler B, Pehlivan Y, *et al*. Enhanced depth imaging optical coherence tomography findings in Behçet disease. *Ocul Immunol Inflamm* 2013;21:440–5.
7 Özkan B, Koçer ÇA, Altintaş Ö, *et al*. Choroidal changes observed with enhanced depth imaging optical coherence tomography in patients with mild Graves orbitopathy. *Eye (Lond)* 2016;30:917–24.
8 Gul A, Basural E, Ozturk HE. Comparison of choroidal thickness in patients with active and stable thyroid eye disease. *Arq Bras Oftalmol* 2019;82:124–8.
9 Sonoda S, Sakamoto T, Yamashita T, *et al*. Choroidal structure in normal eyes and after photodynamic therapy determined by binarization of optical coherence tomographic images. *Invest Ophthalmol Vis Sci* 2014;55:3893–9.
10 Sonoda S, Sakamoto T, Yamashita T, *et al*. Luminal and stromal areas of choroid determined by binarization method of optical coherence tomographic images. *Am J Ophthalmol* 2015;159:1123–31.
11 Pellegrini M, Giannaccare G, Bernabei F, *et al*. Choroidal Vascular Changes in Arteritic and Nonarteritic Anterior Ischemic Optic Neuropathy. *Am J Ophthalmol* 2019;205:43–9.
12 Kongwattananon W, Kumar A, Oyeniran E, *et al*. Changes in Choroidal Vascularity Index (CVI) in Intermediate Uveitis. *Trans Vis Sci Tech* 2021;10:33.
13 Demirel S, Özcan G, Yanık Ö, *et al*. A comparative study of the choroidal vascularity indexes in the fellow eyes of patients with pachychoroid neovasculopathy and central serous chorioretinopathy by binarization method. *Graefes Arch Clin Exp Ophthalmol* 2020;258:1649–54.
14 Bartley GB, Gorman CA. Diagnostic criteria for Graves' ophthalmopathy. *Am J Ophthalmol* 1995;119:792–5.
15 Currò N, Covelli D, Vannucchi G, *et al*. Therapeutic outcomes of high-dose intravenous steroids in the treatment of dysthyroid optic neuropathy. *Thyroid* 2014;24:897–905.
16 Zou M, Wu D, Zhu H, *et al*. Multiparametric quantitative MRI for the evaluation of dysthyroid optic neuropathy. *Eur Radiol* 2022;32:1931–8.
17 Bartalena L, Baldeschi L, Boboridis K, *et al*. The 2016 European Thyroid Association/European Group on Graves' Orbitopathy Guidelines for the Management of Graves' Orbitopathy. *Eur Thyroid J* 2016;5:9–26.
18 Hudson HL, Levin L, Feldon SE. Graves exophthalmos unrelated to extraocular muscle enlargement. Superior rectus muscle inflammation may induce venous obstruction. *Ophthalmology* 1991;98:1495–9.
19 Loiudice P, Pellegrini M, Marinò M, *et al*. Choroidal vascularity index in thyroid-associated ophthalmopathy: a cross-sectional study. *Eye Vis* 2021;8:18.
20 Yeter V, Koçak N, Subaşı M, *et al*. Choroidal vascularity index in thyroid-associated ophthalmopathy. *Can J Ophthalmol* 2023;58:27–33.
21 Brinks J, van Dijk EHC, Klaassen I, *et al*. Exploring the choroidal vascular labyrinth and its molecular and structural roles in health and disease. *Prog Retin Eye Res* 2022;87:100994.
22 Spraul CW, Baldysiak-Figiel A, Lang GK, *et al*. Octreotide inhibits growth factor-induced bovine choriocapillary endothelial cells in vitro. *Graefe's Arch Clin Exp Ophthalmol* 2002;240:227–31.
23 Zhang X, Ma J, Wang Y, *et al*. Elevated serum IGF-1 level enhances retinal and choroidal thickness in untreated acromegaly patients. *Endocrine* 2018;59:634–42.
24 Pappa A, Lawson JM, Calder V, *et al*. T cells and fibroblasts in affected extraocular muscles in early and late thyroid associated ophthalmopathy. *Br J Ophthalmol* 2000;84:517–22.
25 Zhang T, Xiao W, Ye H, *et al*. Peripapillary and Macular Vessel Density in Dysthyroid Optic Neuropathy: An Optical Coherence Tomography Angiography Study. *Invest Ophthalmol Vis Sci* 2019;60:1863–9.
26 Wu Y, Tu Y, Wu C, *et al*. Reduced macular inner retinal thickness and microvascular density in the early stage of patients with dysthyroid optic neuropathy. *Eye Vis* 2020;7:16.
27 Blum Meirovitch S, Leibovitch I, Kesler A. Retina and Nerve Fiber Layer Thickness in Eyes with Thyroid-Associated Ophthalmopathy. *Isr Med Assoc J* 2017;19:277–81.
28 Kim M, Kim SS, Koh HJ, *et al*. Choroidal thickness, age, and refractive error in healthy Korean subjects. *Optom Vis Sci* 2014;91:491–6.
29 Bafiq R, Mathew R, Pearce E, *et al*. Age, Sex, and Ethnic Variations in Inner and Outer Retinal and Choroidal Thickness on Spectral-Domain Optical Coherence Tomography. *Am J Ophthalmol* 2015;160:1034–43.
30 Onaran Z, Konuk O, Oktar SÖ, *et al*. Intraocular pressure lowering effect of orbital decompression is related to increased venous outflow in Graves orbitopathy. *Curr Eye Res* 2014;39:666–72.