Correlation Between Retinal Vessel Diameters and Uveitis Activity

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Purpose. To assess retinal vein and artery diameters during active and inactive intraocular inflammation in eyes with uveitis.

Methods. Color fundus photographs and clinical data of eyes with uveitis collected during two visits (active disease [i.e., T0] and inactive stage [i.e., T1]) were reviewed. The images were semi-automatically analyzed to obtain the central retina vein equivalent (CRVE) and central retina artery equivalent (CRAE). Changes of CRVE and CRAE from T0 to T1 were calculated, and their possible correlation with clinical data, including age, gender, ethnicity, uveitis etiology, and visual acuity, were investigated.

Results. Eighty-nine eyes were enrolled in the study. Both CRVE and CRAE reduced from T0 to T1 ($P < 0.0001$ and $P = 0.01$, respectively), with active inflammation being able to influence the CRVE and CRAE ($P < 0.0001$ and $P = 0.0004$, respectively) after accounting for all other variables. The degree of venular (ΔV) and arteriolar (ΔA) dilation was influenced only by time ($P = 0.03$ and $P = 0.04$, respectively). Best-corrected visual acuity was influenced by time and ethnicity ($P = 0.003$ and $P = 0.0006$).

Conclusions. CRVE and CRAE are increased in eyes with active intraocular inflammation regardless of the type of uveitis, and they decrease when the inflammation wears off.

Keywords: uveitis, intraocular inflammation, retina, vessels, veins, diameter, vasculature, infectious uveitis, non-infectious uveitis

The quantification of inflammation and its changes over time are critical for the management of patients with uveitis.[1–3] Clinical uveitis grading is currently based on semiquantitative measures of clinical parameters including anterior chamber flare and cells and vitreous haze, all of which are subject to critical interobserver and intraobserver variation.[4,5] In the last decade very precise yet expensive devices able to provide an objective quantification of anterior segment inflammation have been validated.[6,7] Experts in the field are also exploring the application of widely available imaging devices like optical coherence tomography to grade inflammation in the anterior and posterior chamber, with variable results.[8–10] As of today, no reliable imaging biomarker of posterior segment inflammation is available.

Color fundus photography (CFP) is routinely used in clinical practice to monitor changes in the retina over time, and it has been chosen for medical screening and telemedicine thanks to its widespread availability and ease of use.[11,12] In addition, retinal vessel diameters have been successfully assessed using semi-automatic analysis of CFPs and consistently associated with the risk for cardiovascular diseases.[13,14]

Our group has recently used CFP to assess retinal vessel diameters in patients with acute COVID-19 and found a clear association between disease severity and vessel diameters.[15] A follow-up study on the same cohort has subsequently shown a decrease in vessel diameters six months after disease resolution, suggesting a possible correlation between systemic inflammation and retinal vessel calipers.[16]

Vessel walls are susceptible to change in diameter in response to both intravascular and extravascular cytokine-mediated stimuli. As such, if the size of retinal vessels varies of a measurable amount in response to systemic inflammation, it is reasonable to hypothesize that local increase of inflammatory cytokines concentration would induce similar changes. The aim of the present study was to measure the retinal vessel diameters in eyes with uveitis during active and inactive intraocular inflammation to assess changes, if any.

Material and Methods

This was a retrospective longitudinal multicenter study conducted at two retina and uveitis referring centers including the Luigi Sacco Hospital, University of Milan, Italy, and

the Eye Institute, Cleveland Clinic Abu Dhabi, Abu Dhabi, United Arab Emirates.

Research and Publication Ethics

The research described adhered to the tenets of the Declaration of Helsinki. The study was conducted in compliance with the local Institutional Review Board from each center.

Study Population and Imaging Methods

Medical charts and imaging studies of patients older than 18 years of age from the study centers diagnosed with uveitis between January 2016 and December 2020 were reviewed. The inclusion criteria were as follows: (1) diagnosis of posterior uveitis only (according to the standardization of uveitis nomenclature (SUN) classification), either infectious or noninfectious; (2) availability of clinical data and fundus color photography centered onto the optic nerve head at two different times: when active inflammation was present (T0) and after its clinical resolution (T1); (3) treatment naïve uveitis or patient not receiving any treatment for at least 3 months at T0.

In particular the inflammation was considered active in case any of the following signs were present: anterior segment cells/flare $\geq$ 0.5, vitreous haze $\geq$ 0.5, presence of clinically active retinal or choroidal lesions, presence of active vasculitis, or macular edema assessed through fluorescein angiography or optical coherence tomography, respectively. The inflammation was considered clinically resolved when none of the signs listed above were identifiable.

The demographic and clinical data included in the analysis were patients age, gender and ethnicity, uveitis etiology (infectious/noninfectious) and underlying diagnosis, and logarithm of the minimum angle of resolution visual acuity (VA). The color fundus photographs were collected with the Carl Zeiss Clarus 500 (Carl Zeiss Meditec, Jena, Germany). With this device the focus is by default automatically adjusted before image acquisition. However, in case of failure, the operator adjusted it manually to obtain the best sharpness of the images at the optic nerve head area to ensure a good visualization of the peripapillary vessels for subsequent analysis.

Exclusion criteria were (1) poor color fundus photography quality (because of either corneal, lens, and IOL (Intraocular Lens) opacities or poor patient cooperation); (2) vitreous haze $\geq$ 2 (national eye institute (NEI)-developed SUN scale) because this could affect the reliability of vessels' boundary identification and diameters measurement; (3) vitreal abnormalities other than vitritis (i.e., vitreous hemorrhage, asteroid hyalosis, and inherited vitreoretinal disorders); (4) systemic or ocular vasculitis because

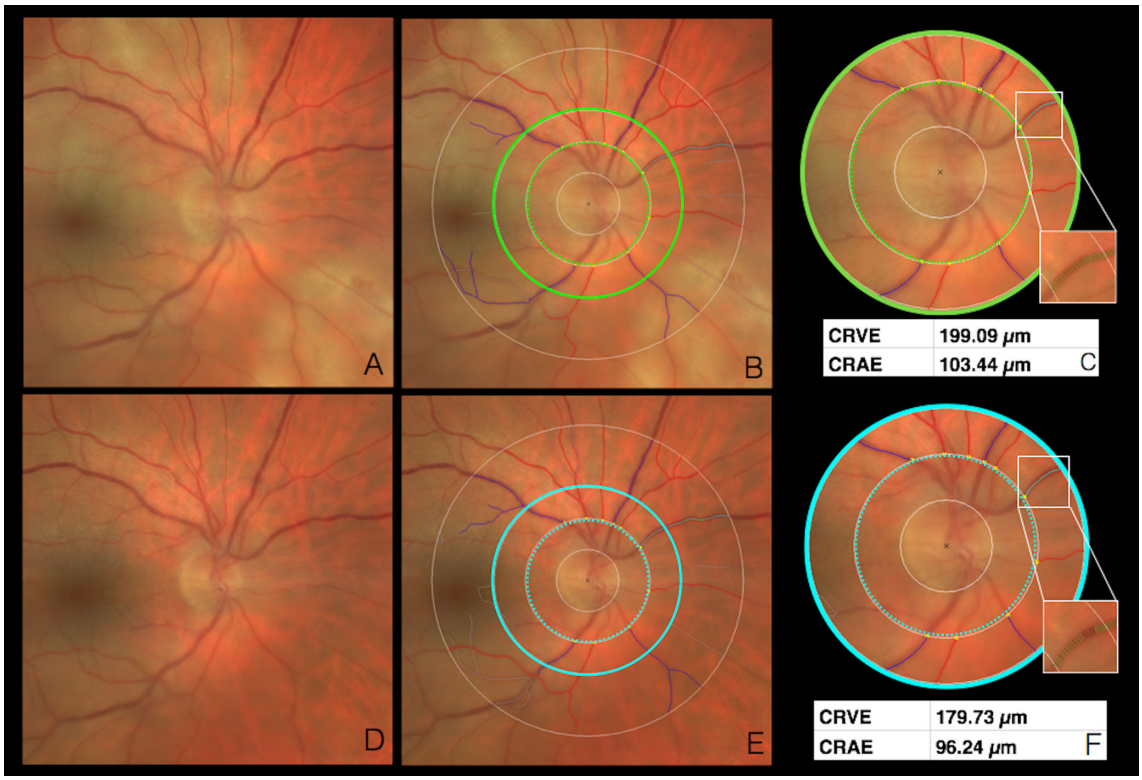

Figure 1. Example of vessel diameter analysis on the same patient with active (**A–C**) and inactive (**D–F**) inflammation. CFP images without (**A** and **D**) and with (**B** and **E**) the superimposed vessel analysis are displayed, as well as the CRVE and CRAE during active (**C**) and inactive (**F**) uveitis. When ocular inflammation is present, the veins and arteries are dilated and engorged, with higher CRVE and CRAE. In the quiet eye, the vessels shrink, and both CRVE and CRAE are reduced. Please note only the clearly visible vessels have been selected and highlighted (veins in *blue* and arteries in *red*) at T0 and that the same vessels have been selected for paired comparison at T0 and T1. The only portions of the vessels being analyzed are those comprised between the *dotted circle* and the *continuous circle* in *green* at T0 and *light blue* at T1. The magnifications in **C** and **F** show the multiple measurements the software runs on each vessel segment.

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they could directly affect retinal vessel diameters, thus representing a bias to the analysis.

Imaging Analysis

Color fundus images were assessed for their quality by two readers (FZ and CR), and, if vascular architecture was not clearly visible because of defocused or not centered image, the eye was not included in the analysis. Each gradable image was then analyzed to estimate the central retinal artery equivalent (CRAE), the central retinal vein equivalent (CRVE), and the arteriovenous ratio as the ratio of the two using a computer-assisted program (Singapore I Vessel Assessment [SIVA], version 4.0, National University of Singapore, Singapore) validated and extensively adopted for retinal vessel analysis.[13,17,18] The SIVA software automatically identifies the optic nerve head center and the major retinal vessels included in a 0.5 to 1 disc diameter radius from the optic nerve head margin,[19] and it divides them into arteries and veins and measures their diameter. The operator is allowed to correct segmentation errors and remove/include vessels segments showing uncorrectable artifacts. In particular, if some vessels were either not visible due to focal vitritis or directly crossing an area of retinitis affecting their shape/visibility, they were excluded from the analysis. After the choice of the vessel segments to include in the analysis, the software automatically provides a measure in micrometers of the CRAE and CRVE calculated from the included vessel segments (Fig. 1). At T1 only the vessels originally selected for the analysis at T0 were re-measured to allow for a correct paired comparison.

Statistical Analysis

Descriptive statistics for continuous variables included the mean and standard deviation (SD) where appropriate. Qualitative variables were summarized as percentages.

Normality distribution of continuous variables was tested using the Shapiro-Wilk test. Differences in CRAE and CRVE between T0 and T1 were assessed with paired *t*-testing.

The influence on CRAE and CRVE of age, gender, and uveitis etiology (infectious or non-infectious) and disease activity (active/inactive) was tested with a mixed linear model accounting for the patient and eyes within patients as random effects. The amount of change from T0 and T1 in CRAE (ΔA) and CRVE (ΔV) was calculated by subtracting T0 values to T1 values.

The influence of age, gender and uveitis etiology (infectious or non-infectious) on ΔA and ΔV was tested with a mixed linear model with nesting of eyes within patients as random effect. The Bonferroni correction was applied to account for repeated measures. The statistical analyses were run on the open access R software (R Studio Version 1.1.383, R Project, www.r-project.org). *P* values < 0.05 were considered statistically significant.

Results

A total of 120 eyes from 87 patients were initially considered for inclusion. Of these, 89 eyes of 70 patients were deemed eligible and included in the analysis, whereas the remaining 31 eyes from 17 patients were excluded because of inadequate image quality. Of the 70 enrolled patients, 26 (37%) were females, the mean age was 37 years (median = 34;

Table. Etiology of the Posterior Uveitis Included in the Study

		Diagnosis							
		Noninfectious Uveitis					Infectious Uveitis		
Uveitis Etiology	Total	Behcet	Sarco	VKH	MFC	Idiopathic	Syphilis	TB	Toxo
Eyes, n	89	2	4	11	13	23	3	29	4

Sarco, sarcoidosis; VKH, Vogt-Koyanagi-Harada disease; MFC, multifocal choroiditis; TB, tuberculosis; Toxo, toxoplasmosis.

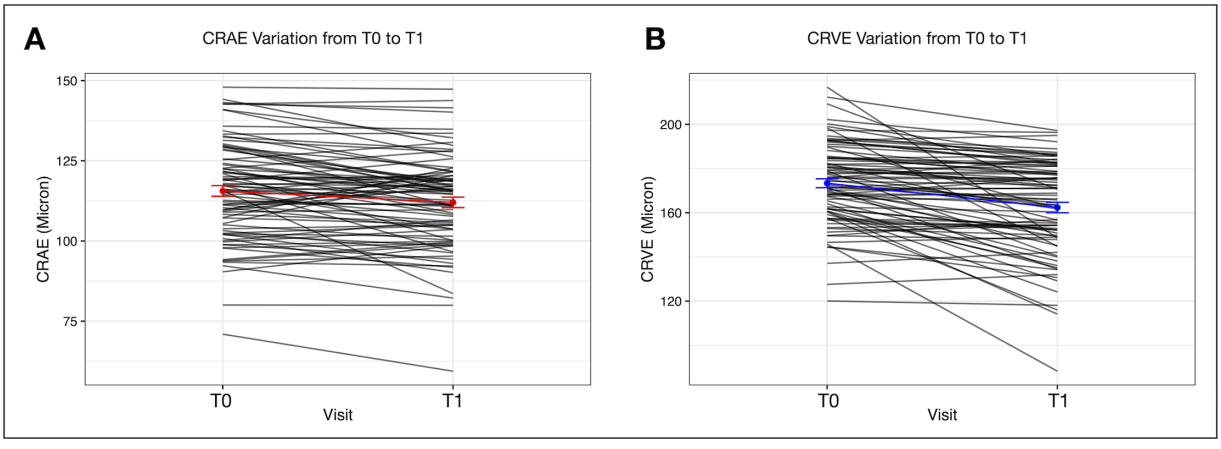

Figure 2. Graphical representation of artery (**A**) and vein (**B**) diameters and their variation from active (T0) to inactive (T1) inflammation. Both CRAE and CRVE decreased from T0 to T1. The paired analysis demonstrated a significant reduction for both measurements despite the quite large standard deviation because of the fact that 82% of patients showed a decrease in size of both arteries and veins when the inflamed eyes (T0) turned quiet (T1) and that the amount of this reduction was on average far greater than the increase seen in the remaining 16 eyes.

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SD = 12.39) and the eyes affected by infectious uveitis were 36 (40%). We had 19 (27.2%) Caucasian patients, 26 (37.1%) Asian Indians, and 25 Arabic (35.7%). Detailed clinical features of the enrolled eyes are reported in the Table.

The mean [SD] CRAE was 115.4 [15.5] at T0, when there was active intraocular inflammation and significantly reduced to 112.0 [14.9] at T1 when the eyes were quiet ($P = 0.01$). Similarly, the mean [SD] CRVE was 173.3 [18.3] at T0 and significantly reduced to 162.5 [22.0] at T1 ($P < 0.0001$; Fig. 2).

According to the linear mixed model, both CRAE and CRVE were significantly influenced ($P < 0.0001$ and $P = 0.0004$, respectively) only by the uveitis activity (active/inactive), whereas the uveitis cause (infectious/noninfectious), age, gender, uveitis etiology, and ethnicity had no significant effect.

The mean[SD] ΔA and ΔV were −3.4 [8.5] and −10.7 [15.7] respectively and 82% of eyes showed a decrease in both arterial and venous diameters from T0 to T1. The age of the subject significantly and negatively influenced the amount of change in both arterial and venous diameters from T0 to T1 ($P = 0.02$ and $P = 0.01$, respectively). All the other clinical variables mentioned above did not show any significant effect on the ΔA and ΔV.

Mean[SD] VA at T0 was 0.49 [0.32] and significantly improved to 0.44 [0.28} at T1. The linear mixed model that included uveitis activity (active/inactive), uveitis diagnosis, age, gender, ethnicity, CRAE and CRVE showed that only the uveitis activity (active/inactive) had a significant influence on the visual function ($P = 0.0006$).

Discussion

In this study we found that the diameter of both retinal arteries and veins was increased in inflamed eyes and that this dilation reduced when the eyes were quiet. These results suggest a possible correlation between retinal vessel diameters and the levels of intraocular inflammation.

The retinal vessel diameters is influenced by several factors, some of which can lead to permanent structural changes like the arterial narrowing seen in systemic hypertension, and some others can induce temporary changes, like the transient arterial dilation visible in response to hypercapnia or the increased vein diameters after a Valsalva maneuver. Recently we demonstrated that systemic inflammation may also have a relevant effect on the size of retinal vessels. The results of the current study strengthen the hypothesis that retinal vessel diameters vary in response to inflammation and suggest that this could also be true if the inflammation is confined within the eye.

Variations in the size of vascular structures within the eye in response to uveitis activity have been extensively observed and reported at the level of the choroid, using choroidal thickness as an indirect measurement for choroidal vessels size or more sophisticated imaging analysis like choroidal vascularity index. Although these techniques have the advantage of multiple studies supporting their reliability, they still need good imaging of the choroid, relying on expensive optical coherence tomography devices including enhanced depth imaging function or swept source technology, often available only in tertiary referral centers. By contrast our approach is based on CFP analysis, thus offering the opportunity to objectively assess variations in intraocular inflammation using a cheap and extremely diffuse imaging technique.

In our population we had a large variability in vessel diameters at T0 and in the vessels that change from T0 to T1. This is likely related to the fact that retinal arterial and venous diameters is highly variable in healthy individuals, and it can be influenced by several anatomical, demographic, and clinical parameters. As such, establishing a normative dataset allowing detection of a pathological increase in diameter of arteries or veins because of inflammation is unrealistic. However, more than 80% of our subjects showed a decrease in CRVE and CRAE from T0 to T1 (Fig. 2) whereas healthy individuals show practically no changes in vessel diameters measured at follow-up visits performed six months apart.[16] This highlights the potential use of this technique to monitor inflammatory-driven variations over time.

On top of the CRAE and the CRVE absolute value, we also evaluated the extent of vessel change during and after inflammation, and we found that the veins changed by a larger extent compared to the arteries. This difference may be explained by the structural differences existing between arteries and vein walls making the latter less resistant to diameter modifications.[20,21] Interestingly our multivariate analysis exploring the relationship between demographic and clinical factors and the change in CRVE and CRAE identified the age of the patient as being inversely correlated with the amount of change. This could suggest age-related modifications of vessels structure making them more rigid and therefore less prone to inflammation-driven dilation.[13]

Variations in retinal vessel diameters was not significantly influenced by the nature of the uveitis (infectious/non-infection) or by the specific diagnosis. As such, this parameter cannot help clinicians in the differential diagnosis between various entities. On the other hand, its lack of specificity can be advantageous because it allows applicability to virtually any intraocular inflammatory condition. However, it must be underlined that the lack of significant correlation between type of uveitis and vessel diameters could just depend on the sample size and larger studies are needed to better explore this aspect.

Last, the study explored possible relations between patients' VA change and the demographic and clinical factors including CRVE and CRAE. VA improved when the uveitis was inactive (T1.) The linear mixed model recognized time of the visit (T0 vs. T1) and ethnicity as the two factors that influenced VA. Whereas the first is likely explained by the presence of active inflammation at T0 that can impair visual acuity, the latter is presumably explained by the variegated socioeconomic settings the patients come from.[22,23] CRVE and CRAE did not show a correlation with VA because of their collinearity with time.

The limits of the study must be acknowledged. To assess retinal vessel diameters, a clear image of the fundus is mandatory; thus this analysis cannot be applied in eyes with intense vitritis. This could be considered the greatest limitation of this technique, and in fact we had to exclude 31/120 eyes, most of which had a vitreous haze grade $\geq$2. However, eyes with intense inflammation are easier to clinically grade, and retinal vessel analysis could play a more relevant role at lower grades of inflammation when assessing changes in inflammation amount is more challenging. A detailed SUN grading[2] was not available for this cohort of eyes, so a correlation between the standardized clinical grading and the vessel diameter was not possible. The color fundus camera used had a micron/pixel ratio of 7; therefore the significance of the results from the arteries could

have been underestimated because of the smaller diameter and consequent smaller change in diameter compared to the veins. Finally, a sample with a more balanced spectrum of uveitic entities could have increased the power of the statistical models, possibly adding information on the effect of different uveitis on the vessel diameter.

To conclude, in this study we found that retinal artery and vein diameter was increased in inflamed eyes and that this dilation regressed when the uveitis was quiet. Retinal vessel diameters can be easily assessed on CFP and could help clinicians to objectively monitor intraocular inflammation when more sophisticated imaging technologies are not available, especially in challenging patients such as those with subclinical reactivations or showing lower degrees of inflammation.

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References

1. Denniston AK, Keane PA, Srivastava SK. Biomarkers and surrogate endpoints in uveitis: the impact of quantitative imaging. *Invest Ophthalmol Vis Sci*. 2017;58(6):BIO131–BIO140.
2. Jabs DA, Nussenblatt RB, Rosenbaum JT, et al. Standardization of uveitis nomenclature for reporting clinical data. Results of the first international workshop. *Am J Ophthalmol*. 2005;140:509–516.
3. Nussenblatt RB, Palestine AG, Chan CC, Roberge F. Standardization of vitreal inflammatory activity in intermediate and posterior uveitis. *Ophthalmology*. 1985;92:467–471.
4. Kempen JH, Ganesh SK, Sangwan VS, Rathinam SR. Interobserver agreement in grading activity and site of inflammation in eyes of patients with uveitis. *Am J Ophthalmol*. 2008;146:813–818.
5. Hornbeak DM, Payal A, Pistilli M, et al. Interobserver agreement in clinical grading of vitreous haze using alternative grading scales. *Ophthalmology*. 2014;121:1643–1648.
6. Herbort CP, Guex-Crosier Y, De Ancos E, Pittet N, Nussenblatt R. Use of laser flare photometry to assess and monitor inflammation in uveitis. *Ophthalmology*. 1997;104:64–72.
7. Tugal-Tutkun I, Herbort CP. Laser flare photometry: a noninvasive, objective, and quantitative method to measure intraocular inflammation. *Int Ophthalmol*. 2010;30:453–464.
8. Invernizzi A, Marchi S, Aldigeri R, et al. Objective quantification of anterior chamber inflammation: measuring cells and flare by anterior segment optical coherence tomography. *Ophthalmology*. 2017;124:1670–1677.
9. Sreekantam S, Macdonald T, Keane PA, Sim DA, Murray PI, Denniston AK. Quantitative analysis of vitreous inflammation using optical coherence tomography in patients receiving sub-Tenon's triamcinolone acetonide for uveitic cystoid macular oedema. *Br J Ophthalmol*. 2017;101:175–179.
10. Montesano G, Way CM, Ometto G, et al. Optimizing OCT acquisition parameters for assessments of vitreous haze for application in uveitis. *Sci Rep*. 2018;8:1–7.
11. Wong TY, Klein R, Sharrett AR, et al. The prevalence and risk factors of retinal microvascular abnormalities in older persons: The cardiovascular health study. *Ophthalmology*. 2003;110:658–666.
12. Xie Y, Nguyen QD, Hamzah H, et al. Artificial intelligence for teleophthalmology-based diabetic retinopathy screening in a national programme: an economic analysis modelling study. *Lancet Digit Heal*. 2020;2(5):e240–e249.
13. Wang JJ, Liew G, Klein R, et al. Retinal vessel diameter and cardiovascular mortality: Pooled data analysis from two older populations. *Eur Heart J*. 2007;28:1984–1992.
14. Tapp RJ, Owen CG, Barman SA, et al. Associations of retinal microvascular diameters and tortuosity with blood pressure and arterial stiffness United Kingdom biobank. *Hypertension*. 2019;74:1383–1390.
15. Invernizzi A, Torre A, Parrulli S, et al. Retinal findings in patients with COVID-19: Results from the SERPICO-19 study. *EClinicalMedicine*. 2020;27:100550.
16. Invernizzi A, Schiuma M, Parrulli S, et al. Retinal vessels modifications in acute and post – COVID – 19. *Sci Rep*. 2021;11(1):19373.
17. Kawasaki R, Wang JJ, Rochtchina E, Lee AJ, Wong TY, Mitchell P. Retinal vessel caliber is associated with the 10-year incidence of glaucoma: the Blue Mountains Eye Study. *Ophthalmology*. 2013;120:84–90.
18. Yip W, Tham YC, Hsu W, et al. Comparison of common retinal vessel caliber measurement software and a conversion algorithm. *Transl Vis Sci Technol*. 2016;5(5):11.
19. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BEK. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res*. 2003;27:143–149.
20. De Jong FJ, Ikram MK, Hofman A. Retinal vessel diameters and the role of inflammation in cerebrovascular disease. *Ann Neurol*. 2007;61:491–495.
21. Ikram MK, Ting Y, Cheung CY, Wong TY. Retinal vascular caliber measurements: clinical significance, current knowledge. 2013;119228:125–136.
22. Tsirouki T, Dastiridou A, Symeonidis C, et al. A focus on the epidemiology of uveitis. *Ocul Immunol Inflamm*. 2018;26:2–16.
23. Rao NA. Uveitis in developing countries. *Indian J Ophthalmol*. 2013;61:253–254.