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Structure–Function Relationships and Glaucoma Detection with Magnification Correction of OCT Angiography

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Purpose: To investigate the effects of adjusting the ocular magnification during OCT-based angiography imaging on structure–function relationships and glaucoma detection.

Design: Cross-sectional study.

Participants: A total of 96 healthy control participants and 90 patients with open-angle glaucoma were included.

Methods: One eye of each patient in the control group and the patient group was evaluated. The layers comprising the macula vascular density (VD) and circumpapillary VD were derived from swept-source OCT angiography imaging. The mean sensitivity (MS) of the standard automated perimetry was measured using the Humphrey 24-2 test. Structure–function relationships were evaluated with simple and partial correlation coefficients. A receiver operating characteristic analysis was performed to evaluate the diagnostic accuracy for glaucoma using the area under the receiver operating characteristic curve (AUC). Ocular magnification was adjusted using Littmann's formula modified by Bennett.

Main Outcome Measures: The association between the axial length and VD, structure–function relationships, and glaucoma detection with and without magnification correction.

Results: The superficial layer of the macular region was not significantly correlated to the axial length without magnification correction ($r = 0.0011$; $P = 0.99$); however, it was negatively correlated to the axial length with magnification correction ($r = -0.22$; $P = 0.028$). Regarding the nerve head layer in the circumpapillary region, a negative correlation to the axial length without magnification correction was observed ($r = -0.22$; $P = 0.031$); however, this significant correlation disappeared with magnification correction. The superficial layer of the macula and the nerve head layer of the circumpapillary region were significantly correlated to Humphrey 24-2 MS values without magnification correction ($r = 0.22$ and $r = 0.32$, respectively); however, these correlations did not improve after magnification correction ($r = 0.20$ and $r = 0.33$, respectively). Glaucoma diagnostic accuracy in the superficial layer (AUC, 0.63) and nerve head layer (AUC, 0.70) without magnification correction did not improve after magnification correction (AUC, 0.62 and 0.69, respectively).

Conclusions: Adjustment of the ocular magnification is important for accurate VD measurements; however, it may not significantly impact structure–function relationships and glaucoma detection. *Ophthalmology Science 2022;2:100120 © 2022 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Ocular magnification is corrected when the actual size of the retina, especially the circumpapillary retinal nerve fiber layer (cpRNFL) thickness and macular ganglion cell layer (GCL) thickness, is analyzed with OCT. The optical system of the fundus camera was designed based on the Gullstrand schematic eye. Because the apparent size is magnified in hyperopic eyes (short axial length) and minified in myopic eyes (long axial length), the area undergoing analysis must be enlarged and reduced, respectively, even if their measurements are obtained at the same angle of view, to analyze the same area.

Littmann's formula modified by Bennett has been frequently used for magnification correction.[1–3] Briefly, the actual size is calculated using the formula $t = p \times q \times s$, where t is the actual fundus dimension, p is the magnification factor of the imaging system, q is the magnification factor for the individual eye, and s is the value obtained from the imaging device. The ocular magnification factor q of the eye can be determined by the formula $q = 0.01306 \times (\text{axial length} - 1.82)$.[1] Furthermore, p is a constant in the telecentric system. It has been reported that magnification correction affects the cpRNFL thickness and that the cpRNFL thickness is negatively correlated to the axial length without magnification correction; however, it has shown no correlation or positive correlation to the axial length with magnification correction.[4–8] Similar findings were also observed using macular GCL imaging.[5,9]

The recent development of OCT angiography (OCTA) has enabled the assessment of vascular perfusion and its quantification with indices such as microvascular density.[10] Additionally, OCTA detects structural changes with early

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glaucoma.[11–13] However, the area measured using OCTA differs among devices. Therefore, ocular magnification correction should be applied to analyze the vascular density (VD) accurately. Lal et al[14] reported that transverse magnification with induced refractive error in contact lenses was reduced with magnification correction. Transverse magnification in the retina can be influenced by ocular refraction, corneal curvature, and axial length,[1–3,15] whereas the axial length can be the parameter with the lowest error to predict the actual size of optic nerve head.[16] Therefore, further investigations of the effect of ocular magnification correction on the axial length observed using OCTA are needed. Additionally, it is clinically important to clarify the effects of performing and not performing magnification correction on the structure–function relationships and the detectability of glaucoma. Therefore, this study aimed to investigate the effects of adjusting the ocular magnification of OCTA images on structure–function relationships and glaucoma detection.

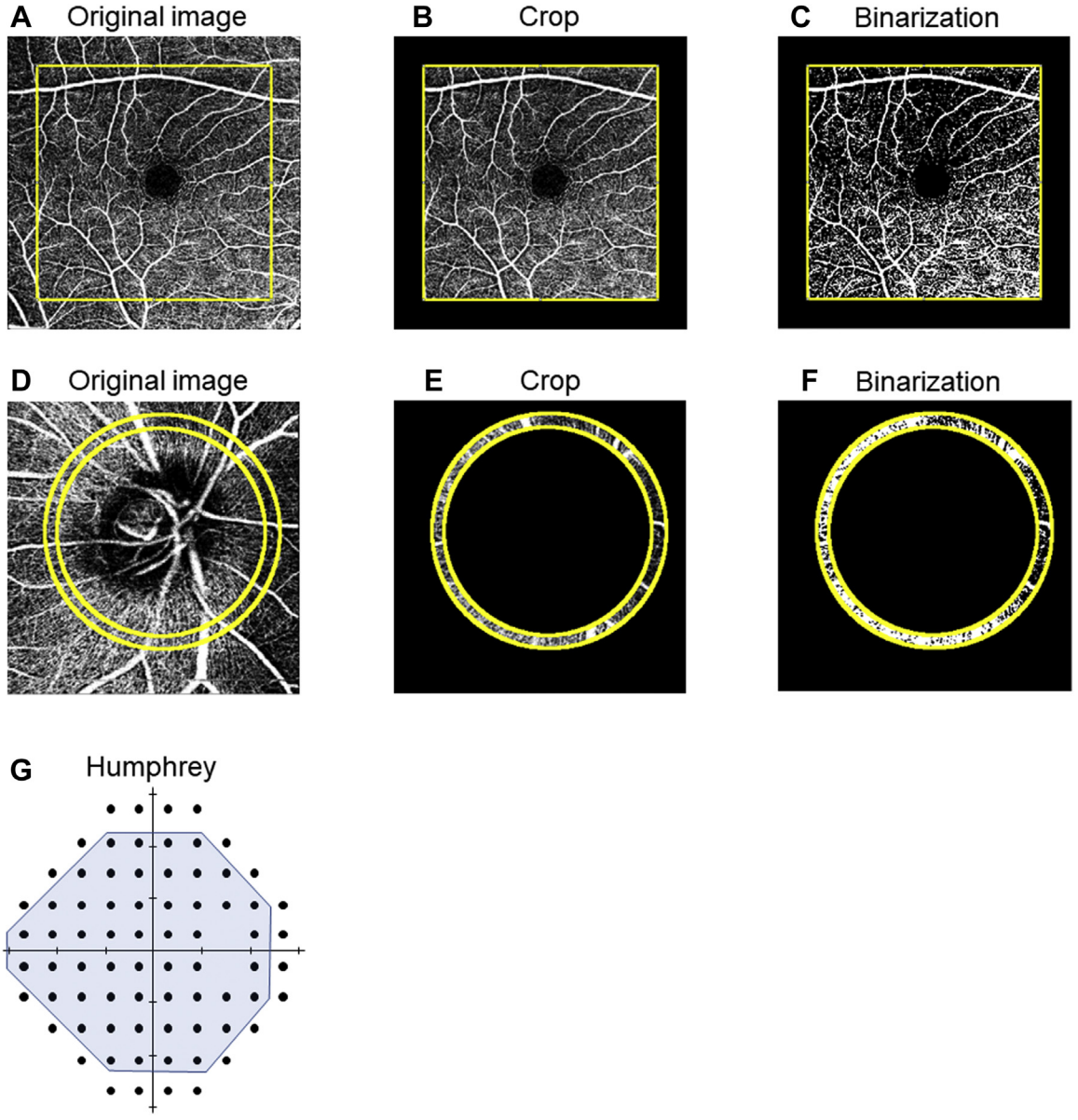

Figure 1. Analysis region and method of measuring (**A–C**) the macular vascular density (mVD) and (**D–F**) circumpapillary VD (cpVD; yellow). **A**, Macular scan showing an area measuring 4.8 × 4.8 mm that was analyzed to evaluate the mVD. **D**, Optic disc scan showing the annulus region between the 2 3.2-mm and 3.6-mm diameter concentric circles centered on the disc that was analyzed to evaluate the cpVD. **B**, **E**, First, the regions of interest in the mVD (**B**) and cpVD (**E**) were cropped from original image based on the magnification of each eye. **C**, **F**, Second, OCT angiography images were set to 8-bit color and binarized with the Otsu autothresholding algorithm method to white and black areas. Then, the microvascular density was defined as the percentage of white areas occupied by vessels in the defined area. **G**, To measure the visual field, the mean sensitivity of test scores corresponding to the 24-2 test scores were used.

Table 1. Participant Characteristics by Group

Characteristic	Control Group (n = 96)	Glaucoma Group (n = 90)	*P* Value	Effect Size
Sex (female/male)	46/50	54/36	0.11	0.24*
Lens (IOL/phakic)	20/76	12/78	0.22	0.18*
Age (yrs)	58.4 (40.1–78.9)	59.1 (42.6–75.6)	0.66	0.064†
Visual acuity (logMAR)	−0.20 (−0.30 to −0.079)	−0.19 (−0.30 to −0.079)	0.25	0.12†
Spherical equivalent (D)	−2.0 (−7.3 to 1.6)	−2.6 (−7.7 to 1.6)	0.26	0.23†
Axial length (mm)	24.6 (22.1–27.3)	24.8 (22.4–27.0)	0.19	0.15†
IOP (mmHg)	14.7 (8.0–20.6)	15.2 (9.0–24)	0.62	0.15†
HFA mean deviation (dB)	0.11 (−2.9 to 2.1)	−1.9 (−5.4 to 0.78)	<0.01	1.3†

D = diopter; HFA = Humphrey Field Analyzer; IOL = intraocular lens; IOP = intraocular pressure; logMAR = logarithm of the minimum angle of resolution.
Data are presented as the mean (95% confidence interval).
*Cohen's w values.
†Cohen's d value.

Methods

Study Design and Ethics

This cross-sectional study was approved by the Research Ethics Committee of the Kitasato University Hospital (identifier, B20-064) and registered in the UMIN Clinical Trials Registry (http://www.umin.ac.jp/) under trial number R000046076 UMIN000040372 (date of registration, May 12, 2020). This study was performed according to the tenets of the Declaration of Helsinki. All participants provided written informed consent. Data were collected between July 2020 and May 2021.

Participants

One eye from each of the 100 patients with early open-angle glaucoma and 1 eye from each of 100 healthy control participants were analyzed. All participants underwent comprehensive ophthalmic examinations before OCTA, including biomicroscopy, gonioscopy, funduscopy, visual field assessment, intraocular pressure (IOP) measurement, refraction, and axial length measurement. A best-corrected visual acuity test was also performed. Healthy control participants were recruited among the medical staff at Kitasato University Hospital or corresponded to the healthy eye of patients with unilateral retinal disease, such as age-related

Table 2. Measurement Values of the Macular and Circumpapillary Vascular Density with and without Magnification Correction in the 2 Groups

Variable	Control Group (n = 96)	Glaucoma Group (n = 90)	*P* Value	Effect Size*
Macular vascular density				
Image quality (AU)	65.2 (46.7–72.0)	63.0 (45.8–71.0)	<0.01	0.42
	—	—	—	—
Superficial (%)	31.8 (28.5–34.8)	31.0 (26.4–34.5)	<0.01	0.43
	31.7 (27.4–35.1)	30.9 (27.1–34.5)	<0.01	0.39
Deep (%)	43.1 (39.2–52.3)	42.6 (39.2–46.5)	0.19	0.19
	43.1 (39.0–52.7)	42.7 (39.2–47.1)	0.33	0.18
Outer retina (%)	20.4 (18.4–22.8)	20.2 (18.2–22.6)	0.20	0.19
	20.4 (18.3–23.0)	20.0 (18.2–22.5)	0.066	0.27
Choriocapillaris (%)	51.8 (48.7–53.9)	51.8 (49.8–53.5)	0.86	0.025
	51.7 (48.4–53.7)	51.8 (49.5–53.3)	0.98	0.0082
Circumpapillary vascular density				
Image quality (AU)	65.7 (46.5–73.0)	64.2 (50.4–71.8)	<0.01	0.28
Nerve head (%)	60.4 (50.5–69.8)	55.9 (43.7–66.9)	<0.01	0.79
	58.6 (48.6–67.2)	54.5 (41.4–64.4)	<0.01	0.74
Vitreous (%)	69.5 (61.8–76.2)	67.5 (57.5–76.3)	<0.01	0.46
	67.3 (59.3–73.7)	65.4 (56.9–73.8)	<0.01	0.45
RPC (%)	67.3 (58.3–74.7)	63.6 (52.4–72.3)	<0.01	0.75
	67.3 (59.3–73.7)	61.8 (51.7–70.1)	<0.01	0.70
Choroid disc (%)	77.5 (63.8–91.5)	79.0 (67.0–90.9)	0.18	0.20
	75.3 (63.7–87.8)	76.4 (65.5–88.6)	0.23	0.18

AU = arbitrary unit; RPC= radial peripapillary capillary; — = not available.
Measurement values without (top) and with (bottom) magnification correction are shown as mean (95% confidence interval).
*Effect sizes are expressed as Cohen's d value.

Table 3. Correlation Coefficients of the Vascular Density, Visual Field Sensitivity, Axial Length, Age, and Image Quality of the Control and Glaucoma Groups

	Humphrey Field Analyzer 24-2 Mean Sensitivity		Axial length		Age		Image Quality	
	r Value	*P Value*	*r Value*	*P Value*	*r Value*	*P Value*	*r Value*	*P Value*
Humphrey Field Analyzer	—	—	0.12	0.25	**−0.56**	**<0.01**	—	—
24-2 mean sensitivity (dB)	—	—	−0.012	0.91	**−0.35**	**<0.01**	—	—
Axial length (mm)	0.12	0.25	—	—	**−0.23**	**0.027**	—	—
	−0.012	0.91	—	—	**−0.26**	**0.015**	—	—
Age (yrs)	**−0.56**	**<0.01**	**−0.23**	**0.027**	—	—	—	—
	0.35	**<0.01**	**−0.26**	**0.015**	—	—	—	—
Macular vascular density								
Image quality (AU)	**0.30**	**<0.01**	**−0.28**	**<0.01**	−0.18	0.072	—	—
	0.11	0.31	−0.11	0.32	**−0.22**	**0.033**	—	—
Superficial (%)	**0.21**	**0.044**	0.011	0.99	**−0.21**	**0.033**	**0.21**	**0.037**
	0.061	0.57	0.049	0.64	0.092	0.39	0.13	0.22
Deep (%)	0.10	0.32	0.11	0.28	−0.081	0.43	**−0.44**	**<0.01**
	−0.088	0.41	**0.23**	**0.026**	−0.059	0.58	**−0.33**	**<0.01**
Outer retina (%)	−0.13	0.20	0.084	0.42	0.16	0.12	**−0.37**	**<0.01**
	0.076	0.48	0.092	0.39	**0.23**	**0.026**	**−0.47**	**<0.01**
Choriocapillaris (%)	**0.34**	**<0.01**	−0.16	0.11	**−0.25**	**0.013**	**0.62**	**<0.01**
	0.044	0.68	−0.052	0.62	**−0.27**	**<0.01**	**0.37**	**<0.01**
Circumpapillary vascular density								
Image quality (AU)	**0.23**	**0.027**	**−0.23**	**0.025**	−0.12	0.25	—	—
	0.046	0.067	−0.084	0.43	−0.11	0.28	—	—
Nerve head (%)	**0.21**	**0.036**	**−0.22**	**0.031**	**−0.27**	**<0.01**	**0.30**	**<0.01**
	0.13	0.23	−0.19	0.070	−0.071	0.51	0.098	0.36
Vitreous (%)	**0.21**	**0.044**	−0.070	0.50	**−0.31**	**<0.01**	−0.13	0.22
	0.020	0.85	**−0.26**	**0.014**	0.057	0.60	−0.17	0.10
RPC (%)	0.15	0.13	**−0.26**	**0.012**	**−0.28**	**<0.01**	−0.0002	0.99
	0.095	0.37	**−0.24**	**0.024**	0.095	0.87	−0.090	0.40
Choroid disc (%)	**−0.22**	**0.031**	−0.0087	0.93	0.11	0.30	**−0.24**	**0.017**
	0.068	0.52	−0.039	0.71	−0.17	0.12	0.079	0.46

AU = arbitrary unit; RPC = radial peripapillary capillary; − = not available. Boldface indicates statistical significance.

macular degeneration, retinal vein occlusion, epiretinal membrane, or retinal detachment.

The inclusion criteria were as follows: 40 to 79 years of age, IOP of 21 mmHg or less for control participants and 30 mmHg or less for patients with glaucoma, spherical equivalent of between −8 and +5 diopters and astigmatism of 2 diopters or less, axial length of 22 to 28 mm, and best-corrected visual acuity score of 0 logarithm of the minimum angle of resolution or less. For patients with open-angle glaucoma, the inclusion criteria were gonioscopically wide-open angles and presence of typical glaucomatous changes in the optic nerve head, including rim thinning, rim notch, or retinal nerve fiber layer (RNFL) defects, and mean deviation of −6 dB or more with a Humphrey Field Analyzer (HFA; Carl Zeiss Meditec) 24-2 or 30-2 test point program. For healthy control participants, the inclusion criteria were no abnormal findings except for clinically insignificant senile cataract on biomicroscopy and funduscopy and normal HFA 24-2 or 30-2 results according to the Anderson-Patella criteria.[17]

The exclusion criteria were possible secondary ocular hypertension and other systemic or ocular disorders that could affect the study results. Eyes with a history of refractive surgery and multifocal intraocular lens implantation were also excluded. If both eyes of a patient met these criteria, then 1 eye was randomly chosen for the study.

OCT Angiography Imaging

OCT angiography imaging was performed using the swept-source OCT (DRI OCT Triton; Topcon) without pupil dilation. Imaging was performed with an eye-tracking system using the optic disc horizontal scan mode (320 × 320-pixel scan resolution in a 4.5 × 4.5-mm scan area) and macular horizontal raster scan (6 × 6-mm scan area). The macular VD (mVD) was measured using the macular scanned areas of 4.8 × 4.8 mm (Fig 1A). The circumpapillary VD (cpVD) was measured using the annulus region between 2 concentric circles (diameters of 3.2 mm and 3.6 mm) centered on the optic disc (Fig 1B). To ensure the reliability of OCTA imaging, an image quality index of more than 40 was set.

Two-dimensional OCTA images of the macular region were derived using the following predefined axial slabs in the device software: superficial, from 2.6 μm below the internal limiting membrane (ILM) to 15.6 μm below the inner plexiform layer (IPL) plus the inner nuclear layer (INL); deep, from 15.6 μm below the IPL plus INL to 70.2 μm below the IPL plus INL; outer retina, from 70.2 μm below the IPL plus INL to the Bruch membrane (BM); and choriocapillaris, from the BM to 10.4 μm below the BM. For the optic disc scan, the following predefined axial slabs were derived: nerve head, from the top position on the B-scan to 130 μm below the ILM; vitreous, from the top position on the

Table 4. Correlation Coefficients of the Axial Length of the Macular Vascular Density and Circumpapillary Vascular Density with and without Ocular Magnification Correction in the Control Group

	Axial Length				
	Without Magnification Correction		*With Magnification Correction*		**P Value (Without vs. With Magnification Correction)**
	r Value	*P Value*	*r Value*	*P Value*	
Macular vascular density					
Superficial (%)	0.0011	0.99	**−0.22**	**0.028**	0.13
	0.015	0.83	**−0.24**	**<0.01**	0.080
Deep (%)	0.11	0.28	0.13	0.21	0.89
	0.078	0.29	0.096	0.19	0.90
Outer retina (%)	0.084	0.42	−0.018	0.86	0.49
	0.085	0.24	−0.012	0.87	0.51
Choriocapillaris (%)	−0.16	0.11	**−0.25**	**0.013**	0.52
	−0.16	**0.024**	**−0.25**	**<0.01**	0.52
Circumpapillary vascular density					
Nerve head (%)	**−0.22**	**0.031**	0.050	0.63	0.060
	−0.24	**<0.01**	0.029	0.69	0.060
Vitreous (%)	−0.070	0.50	0.026	0.80	0.51
	−0.12	0.10	−0.015	0.84	0.47
RPC (%)	**−0.26**	**0.012**	−0.12	0.24	0.32
	−0.30	**<0.01**	**−0.16**	**0.027**	0.31
Choroid disc (%)	−0.0087	0.93	0.051	0.62	0.68
	−0.0099	0.89	0.051	0.48	0.68

RPC = radial peripapillary capillary. Boldface indicates statistical significance.
Pearson's correlation coefficients (top) and partial correlation coefficients adjusted for age and image quality (bottom) are shown.

B-scan to 49.4 μm below the ILM; radial peripapillary capillary (RPC), from the top position on the B-scan to 70.2 μm below the ILM; and choroid disc, from 130.0 μm below the ILM to 390.0 μm below the BM.

Analysis of OCT Angiography Images

All OCTA images were exported as TIFF files and analyzed using ImageJ free image analysis software version 1.53e (National Institutes of Health, Bethesda, MD).[18] First, the region of interest of both the mVD and cpVD were cropped from the original image based on the magnification of each eye. Second, OCTA images were set to 8-bit color and binarized with the Otsu autothresholding algorithm method to white and black areas.[19] Then, the microvascular density was defined as the percentage of white areas occupied by vessels in the defined area (Fig 1). Ocular magnification was corrected using Littmann's formula modified by Bennett as follows[1–3]:

$$\text{Actual size} = 3.3820 \times 0.01306 \times (\text{axial length} - 1.82) \times \text{scan area}.$$

The magnification factor of 3.3820 of the imaging system was based on the Zeiss fundus camera. However, this value was applied during the current study because the conversion factor of the Topcon fundus camera was close to a constant and because it can be considered to function close to the telecentric design of the Zeiss fundus camera.[20] Poor-quality images with poor clarity, motion artifacts, an irregular vessel pattern or disc boundary, weak signal caused by vitreous opacity, or segmentation errors were excluded from OCT and OCTA analysis.

Visual Field Measurement

Standard visual field measurements were obtained using the HFA 24-2 or 30-2 test point program (Swedish Interactive Threshold Algorithm Standard and Goldmann size III stimulus). Only reliable visual fields, defined as those with fixation losses of $< 20\%$, false-positive responses of $< 15\%$, and false-negative responses of $< 33\%$, were used for analysis.[21] The mean sensitivity (MS) values corresponding to the HFA 24-2 test points were used for statistical analyses because patients who underwent HFA 24-2 or 30-2 testing were also included in the current study (Fig 1C).

Statistical Analysis

Continuous data were calculated as the mean and 95% confidence intervals. Differences within factors were compared using paired *t* tests. Group differences were compared using the unpaired *t* test. Categorical data were compared using the chi-square test. The correlation coefficient (*r*) was calculated using Pearson's product-moment correlation coefficient. Detectability of glaucoma was evaluated according to the area under the receiver operating characteristic curve (AUC) calculated using receiver operating characteristic analysis.

An a priori sample size was calculated based on a simple linear regression analysis of the largest sample size in this study using G*Power3 version 3.1.9.2 (Franz Faul, University of Kiel, Kiel, Germany). With an effect size f^2, an α error, and power $(1 - \beta)$ set to 0.15 (middle size), 0.05, and 0.95, respectively, at least 89 participants were required for both the control and patient groups. Considering a dropout rate of approximately 10%, a total of 100 eyes of 100 healthy control participants and 100 patients with open-angle glaucoma were estimated as the required a priori sample size. All statistical analyses were performed using the statistical programming language R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was defined as $P < 0.05$.

Results

A total of 4 eyes of 4 healthy control participants and 10 eyes of 10 glaucoma patients were excluded. Finally, 96

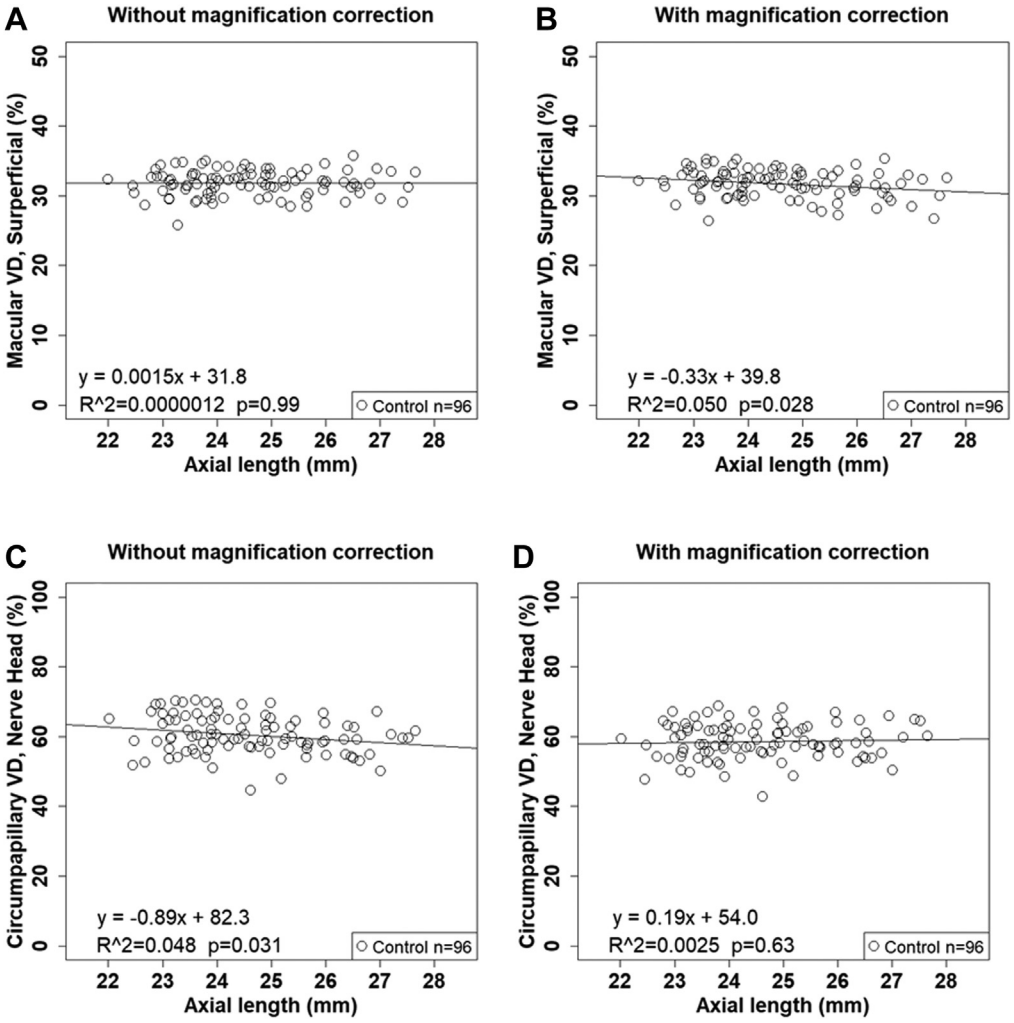

Figure 2. **A**, **B**, Scatterplots showing the association between the axial length and the superficial layer of the macular vascular density (VD) without (**A**) and with (**B**) ocular magnification correction. **C**, **D**, Scatterplots similarly showing the association between the axial length and nerve head layer of the circumpapillary VD without (**C**) and with (**D**) ocular magnification correction.

eyes of 96 healthy control participants and 90 eyes of 90 patients with glaucoma were included in the analysis. The clinicodemographic characteristics of the control and glaucoma groups are shown in Table 1. No significant differences were found in the characteristics of the participants in the control and glaucoma groups. The measurement values of each layer comprising the VD of the macula and the circumpapillary region are shown in Table 2. The signal strength (macular and disc), superficial layer (macular), nerve head layer (disc), vitreous layer (disc), and RPC layer (disc) were significantly lower in the glaucoma group than in the control group, both with and without magnification correction (all $P < 0.05$).

Table 3 shows the correlation coefficient among the VD, Humphrey 24-2 MS, axial length, age, and image quality of the control and glaucoma groups. Age and image quality tended to be weakly or moderately correlated to the VD or Humphrey 24-2 MS. Therefore, the partial correlation coefficient was also calculated during the analysis of the correlation of the VD to the axial length and Humphrey 24-2 MS.

The correlation coefficient of the axial length to the mVD and cpVD with and without ocular magnification correction in the control group is shown in Table 4. Regarding the mVD, the axial length was not significantly correlated to each layer without ocular magnification correction; however, it showed a significantly weak negative correlation to the superficial layer ($r = -0.22$; $P = 0.028$) and choriocapillaris layer ($r = -0.25$; $P = 0.013$) with magnification correction. A similar trend was observed after adjusting for age and image quality ($r = -0.24$ and $r = -0.25$, respectively).

Regarding the cpVD, we found a significant weak negative correlation between the axial length and both the nerve head layer ($r = -0.22$; $P = 0.031$) and RPC layer ($r = -0.26$; $P = 0.012$) without magnification correction; however, these significant correlations disappeared with magnification correction. No correlation was found in other

Table 5. Correlation between the Humphrey 24-2 Mean Sensitivity and Vessel Density with and without Ocular Magnification Correction for All Participants

	Humphrey 24-2 Mean Sensitivity				
	Without Magnification Correction		*With Magnification Correction*		**P Value (Without vs. With Magnification Correction)**
	r Value	*P Value*	*r Value*	*P Value*	
Macular vascular density					
Superficial (%)	**0.22**	**<0.01**	**0.20**	**<0.01**	0.92
	0.22	**<0.01**	**0.20**	**<0.01**	0.84
Deep (%)	0.062	0.40	0.063	0.39	0.99
	0.037	0.48	0.038	0.47	0.99
Outer retina (%)	0.034	0.65	0.043	0.56	0.93
	0.034	0.51	0.049	0.35	0.89
Choriocapillaris (%)	**0.15**	**0.036**	0.12	0.092	0.77
	0.15	**<0.01**	**0.13**	**0.014**	0.85
Circumpapillary vascular density					
Nerve head (%)	**0.32**	**<0.01**	**0.33**	**<0.01**	0.91
	0.32	**<0.01**	**0.32**	**<0.01**	1.0
Vitreous (%)	**0.19**	**<0.01**	**0.20**	**<0.01**	0.92
	0.18	**<0.01**	**0.18**	**<0.01**	1.0
RPC (%)	**0.28**	**<0.01**	**0.26**	**<0.01**	0.84
	0.27	**<0.01**	**0.25**	**<0.01**	0.84
Choroid disc (%)	−0.11	0.13	-0.12	0.11	0.92
	−0.12	**0.022**	-0.13	0.15	0.92

RPC = radial peripapillary capillary. Boldface indicates statistical significance.
Pearson's correlation coefficients (top) and partial correlation coefficients adjusted for age and image quality (bottom) are shown.

layers. A similar trend was observed after adjusting for age and image quality. Figure 2 shows the association of axial length with the superficial and nerve head layers with and without ocular magnification correction.

Table 5 shows the association of the Humphrey 24-2 MS with the mVD and cpVD with and without ocular magnification correction for all participants. Regarding the mVD, a significant positive correlation was observed between the Humphrey 24-2 MS and superficial layer without ($r = 0.22$; $P < 0.01$) and with ($r = 0.20$; $P < 0.01$) magnification correction. A similar trend was observed in the superficial layer ($r = 0.22$ and $r = 0.20$, respectively; each $P < 0.01$) after adjusting for age and image quality. The correlation in other layers was weak to nonexistent, and the correlation coefficients were very small ($0.034 \leq r \leq 0.15$). A similar trend was observed in other layers after adjusting for age and image quality ($0.034 \leq r \leq 0.13$).

Regarding the cpVD, the Humphrey 24-2 MS was significantly correlated to both the nerve head layer ($r = 0.32$; $P < 0.01$) and RPC layer ($r = 0.28$; $P < 0.01$) without magnification correction. A similar trend was observed in both the nerve head layer ($r = 0.32$; $P < 0.01$) and RPC layer ($r = 0.25$; $P < 0.01$) with magnification correction. A weak to no correlation was found with other layers, and the correlation coefficients were small ($-0.12 \leq r \leq 0.20$). A similar trend was observed after adjusting for age and image quality ($-0.13 \leq r \leq 0.18$). Figure 3 shows the association of the Humphrey 24-2 MS with both the superficial and nerve head layers with and without ocular magnification correction. The structure–function relationships between HFA 24-2 MS and both mVD and cpVD did not significantly improve with magnification correction.

The detectability of glaucoma using the mVD and cpVD with and without ocular magnification correction is shown in Table 6. Regarding the mVD, a small or moderate AUC was observed in the superficial layer (AUC, 0.63; $P < 0.01$) without magnification correction, but not in other layers without magnification correction. Regarding the cpVD, a moderate AUC was observed in the nerve head layer (AUC, 0.70; $P < 0.01$) and RPC layer (AUC, 0.70; $P < 0.01$) without magnification correction. Other layers without magnification correction showed small to moderate AUCs (AUC, 0.63 in the vitreous layer; AUC, 0.56 in the choroid disc layer). The AUCs of the mVD and cpVD did not significantly improve with magnification correction.

The superficial layer in the mVD and nerve head layer in the cpVD were significantly correlated without magnification correction ($r = 0.25$; $P < 0.01$) and with magnification correction ($r = 0.24$; $P < 0.01$). Similar trends were observed in the superficial layer in the mVD ($r = 0.25$; $P < 0.01$) and in the nerve head layer in the cpVD ($r = 0.25$; $P < 0.01$) after adjusting for age and image quality. Figure 4 shows the association between the superficial layer in the mVD and the nerve head layer in the cpVD with and without ocular magnification correction.

Discussion

The current study found that all layers comprising the mVD are not correlated to the axial length without magnification

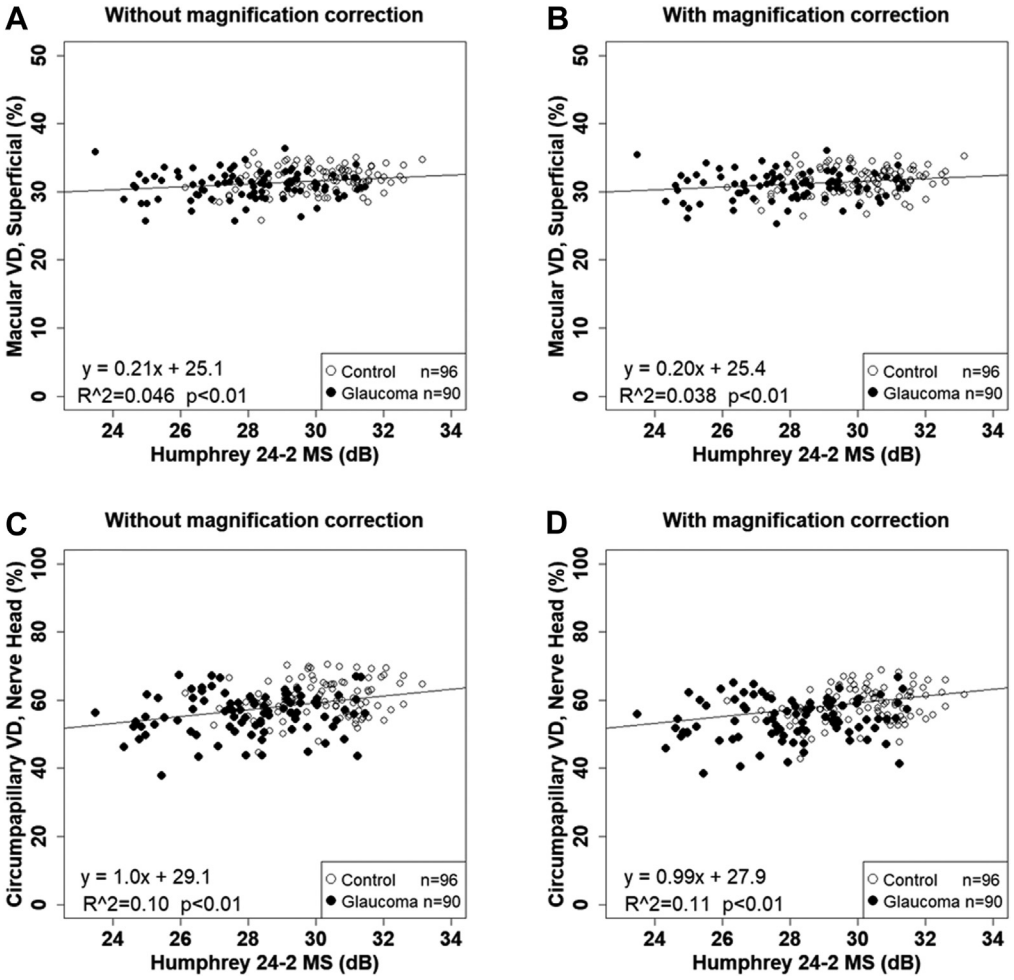

Figure 3. Scatterplots showing the association between the Humphrey 24-2 mean sensitivity (MS) and superficial layer of the macular vascular density (VD) without (**A**) and with (**B**) ocular magnification correction. **C**, **D**, Scatterplots similarly showing the association between the Humphrey 24-2 MS and nerve head layer of the circumpapillary VD without (**C**) and with (**D**) ocular magnification correction. Data of the control group (open circle) and glaucoma group (black dot) are shown.

correction. However, the superficial and choriocapillaris layers show a negative correlation to the axial length with magnification correction. Regarding the cpVD, the nerve head layer was negatively correlated to the axial length without magnification correction, but not with magnification correction. Structure−function relationships and glaucoma detectability were almost comparable even after correction for ocular magnification.

The thickness of the macular GCLs, which comprise the RNFL to the IPL, is negatively correlated to the axial length without ocular magnification correction.[22,23] However, the correlations of the axial length to the macular GCLs differ in each layer. Although it is positively correlated to the RNFL, it is negatively correlated to both the GCL and IPL and the GCL, IPL, and RNFL.[22] The positive correlation of the macular RNFL to the axial length may be attributable the temporal shift effect, whereby retinal arteries shift toward the fovea in the eye with a longer axial length. Yamashita et al[24] reported that the peak RNFL thickness also shifted according to the shift of the retinal arteries. This phenomenon could have affected the correlation between the axial length and mVD in the current study.

The microvascular density is calculated as the percentage of the occupied white area in the black background after the images are binarized. Large blood vessels were also calculated as white areas during this analysis. For eyes with a long axial length, large blood vessels could be included in the area undergoing analysis because of the temporal shift in the mVD image without magnification correction. Furthermore, the mVD could be increased because it is calculated as the white area. However, large blood vessels could not be included after magnification correction because the area undergoing analysis is minimized based on magnification of the eye with a longer axial length. Therefore, a negative correlation might be observed for the mVD after ocular magnification correction.

Table 6. Glaucoma Detectability in the Vascular Density with and without Ocular Magnification Correction

	Area under the Receiver Operating Characteristic Curve (95% Confidential Interval)	*P* Value	Cutoff	Sensitivity/ Specificity (%)	Sensitivity at 80% Specificity (%)	Sensitivity at 90% Specificity (%)
Macular vascular density						
Superficial (%)	0.63 (0.55–0.71)	<0.01	≤31.2	53.3/70.8	33.3	20.0
	0.62 (0.54–0.70)	<0.01	≤31.3	61.1/64.6	27.8	20.0
Deep (%)	0.53 (0.45–0.61)	0.49	≤42.0	38.9/71.8	25.6	11.1
	0.51 (0.43. 0.60)	0.51	≤42.4	48.9/62.5	24.4	8.9
Outer retina (%)	0.55 (0.46–0.63)	0.26	≤19.2	25.6/88.5	27.8	12.2
	0.57 (0.49. 0.65)	0.096	≤19.9	44.4/69.8	25.6	16.7
Choriocapillaris (%)	0.50 (0.41–0.58)	0.91	≤49.7	2.2/90.6	22.2	3.3
	0.51 (0.43–0.60)	0.75	≤52.3	67.8/40.6	20.0	5.6
Circumpapillary vascular density						
Nerve head (%)	0.70 (0.63–0.77)	<0.01	≤57.6	61.1/70.8	46.7	33.3
	0.69 (0.62–0.76)	<0.01	≤55.1	53.3/78.1	46.7	36.7
Vitreous (%)	0.63 (0.55–0.70)	<0.01	≤69.2	65.6/57.3	28.9	16.7
	0.63 (0.55–0.70)	<0.01	≤67.7	74.4/53.1	27.8	23.3
RPC (%)	0.70 (0.63–0.76)	<0.01	≤66.6	74.4/59.4	40.0	32.2
	0.69 (0.61–0.75)	<0.01	≤66.2	87.8/43.8	37.8	27.8
Choroid disc (%)	0.56 (0.48–0.63)	0.17	>73.5	78.9/35.4	25.6	12.2
	0.55 (0.48–0.63)	0.22	>72.5	74.4/39.6	24.4	11.1

RPC = radial peripapillary capillary.
Descriptive statistics without magnification correction (top) and with magnification correction (bottom) are shown.

Although the layers comprising the cpVD and the mVD are calculated using the same procedure for magnification correction, we found a significant negative correlation to the nerve head layer of the cpVD without magnification correction, but not with magnification correction. This trend was similar to the relationship between the axial length and cpRNFL thickness with and without magnification correction.[5,7] Hirasawa et al[25] reported an association between the different circle diameters and cpRNFL thickness and a linear relationship between the circle diameters of 2.8 mm and 4.0 mm. If this result can also be applied to the cpVD, then magnification correction will be important in achieving accurate cpVD measurements.

The current study found weak to moderate structure–function relationships between the Humphrey 24-2 MS and mVD (superficial layer) and cpVD (nerve head layer and RPC layer) without ocular magnification correction. This relationship was comparable after magnification correction. Previous studies reported that the surface layer observed on the OCTA image is correlated to visual field sensitivity,[26,27] and our results showed a similar trend. The correlation coefficients of the mVD were lower than those of the cpVD. This may be because the macular damage was milder than that in the circumpapillary region because patients with a very early stage of glaucoma were included in the current study. Moreover, visual field

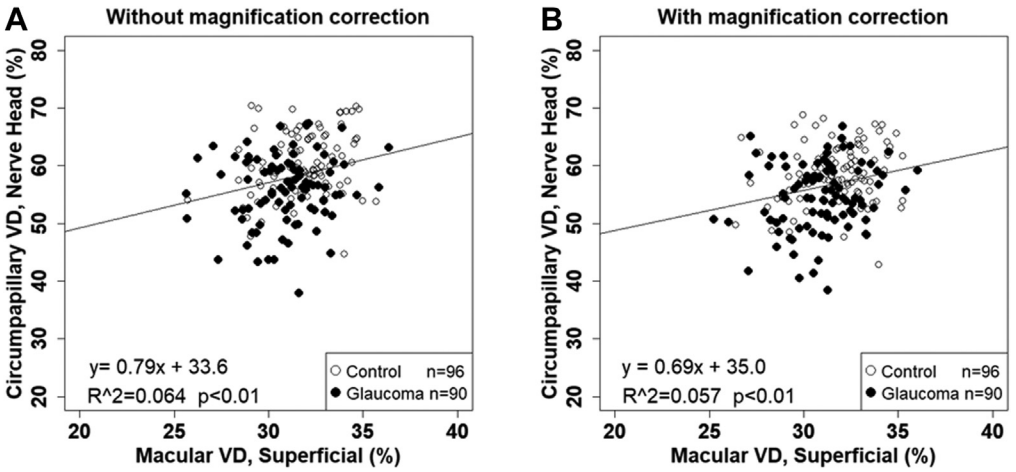

Figure 4. A, **B**, Scatterplots showing the association between the superficial layer of the macular vascular density (VD) and the nerve head layer of the circumpapillary VD without (**A**) and with (**B**) ocular magnification correction. Data of the control group (open circle) and glaucoma group (black dot) are shown.

Table 7. Correlation Coefficients of the Structure–Function Relationships Analyzed Using the 3 Models

	Humphrey 24-2 Mean Sensitivity		
	*Model A***	*Model B*†	*Model C*‡
Macular vascular density			
Superficial (%)	**0.22 (P < 0.01)**	**0.20 (P < 0.01)**	**0.22 (P < 0.01)**
Deep (%)	0.062 ($P = 0.40$)	0.063 ($P = 0.39$)	0.063 ($P = 0.39$)
Outer retina (%)	0.034 ($P = 0.65$)	0.043 ($P = 0.56$)	0.034 ($P = 0.64$)
Choriocapillaris (%)	**0.15 (P = 0.036)**	0.12 ($P = 0.092$)	**0.15 (P = 0.037)**
Circumpapillary vascular density			
Nerve head (%)	**0.32 (P < 0.01)**	**0.33 (P < 0.01)**	**0.33 (P < 0.01)**
Vitreous (%)	**0.19 (P < 0.01)**	**0.20 (P < 0.01)**	**0.20 (P < 0.01)**
RPC (%)	**0.28 (P < 0.01)**	**0.26 (P < 0.01)**	**0.28 (P < 0.01)**
Choroid disc (%)	−0.11 ($P = 0.11$)	−0.12 ($P = 0.11$)	−0.11 ($P = 0.13$)

RPC = radial peripapillary capillary. Boldface indicates statistical significance.
*Correlation coefficient without magnification correction.
†Correlation coefficient when the magnification was corrected only for vascular density.
‡Partial correlation coefficient adjusted for the axial length in model A.

sensitivity was measured using the 24-2 test point, but not the 10-2 test point. No improvement was found in the structure–function relationships even after magnification correction; this could be because only OCTA images were corrected for ocular magnification, and the 24-2 test point was not corrected for ocular magnification in the current study.

Current commercially available perimeters cannot correct each test point based on the ocular magnification. Therefore, a supplemental analysis was performed to identify which model is best for explaining the structure–function relationships. In model A, the magnification was not corrected. In model B, the magnification was corrected only on OCTA images. In model C, the magnification was statistically corrected using the axial length on the original OCTA image and HFA 24-2 MS (Table 7). As a result, the correlation coefficients were almost comparable among the 3 models. However, for the superficial layer, which was most correlated to visual field sensitivity, model A and model C explained the structure–function relationships slightly better than model B explained the mVD. For the cpVD, model B and model C explained the structure–function relationships slightly better than model A. These findings suggested that the magnification correction on only the OCTA images was not an important influencing factor in the structure–function relationships shown by OCTA images and visual fields measured using the 24-2 test point. Instead, it may be better to statistically correct both OCTA images and visual field sensitivity using the axial length.

The glaucoma detectability was comparable or worse in the mVD than in the cpVD, even with magnification correction; however, the difference was not significant. Nakanishi et al[9] reported that correction of ocular magnification does not improve the diagnostic accuracy for macular ganglion cell complex layers (the RNFL to the IPL) in glaucoma in non-highly myopic eyes and myopic eyes. A similar trend was observed during imaging of the mVD and cpVD during this study. No improvement was found in glaucoma detectability; it was the same or slightly worse even after magnification correction. However, the difference was not significant. This finding may be primarily attributable to correction of ocular magnification only on OCTA images (for reasons similar to those described for the aforementioned models). Although no significant between-group difference in participant characteristics was found, especially the axial length, in the current study, magnification correction may have an effect on glaucoma detectability if the background of the axial length differs between groups. Furthermore, magnification correction could have minimal significance in the analysis of glaucoma detectability using OCTA images if the axial lengths are comparable between groups.

One limitation of this study is that visual field sensitivity was not measured using the 10-2 test point. Further studies are needed to elucidate the structure–function relationships in the macular region.

In conclusion, the mVD, especially the superficial layer, is correlated to the axial length with magnification correction, but not to the axial length without magnification correction. Moreover, the cpVD, especially the nerve head layer, is negatively correlated to the axial length without magnification; however, this significant correlation disappeared with magnification correction. The structure–function relationships and glaucoma detectability did not improve even after correction for ocular magnification on OCTA images. Collectively, these results support that adjustment for ocular magnification is important for obtaining accurate VD measurements; however, it might not have significant effects on structure–function relationships and glaucoma detection.

Footnotes and Disclosures

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Analysis and interpretation: Hirasawa, Kanno, Shoji

Data collection: Hirasawa, Yamaguchi, Nagano

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Overall responsibility: Hirasawa, Yamaguchi, Nagano, Kanno, Kasahara, Shoji

Abbreviations and Acronyms:
AUC = area under the receiver operating characteristic curve; **BM** = Bruch membrane; **cpRNFL** = circumpapillary retinal nerve fiber layer; **cpVD** = circumpapillary vascular density; **GCL** = ganglion cell layer; **HFA** = Humphrey Field Analyzer; **ILM** = internal limiting membrane; **INL** = inner nuclear layer; **IOP** = intraocular pressure; **IPL** = inner plexiform layer; **MD** = mean deviation; **MS** = mean sensitivity; **mVD** = macular vascular density; **OCTA** = OCT angiography; **RNFL** = retinal nerve fiber layer; **RPC** = radial peripapillary capillary; **TD** = total deviation; **VD** = vascular density.

Keywords:
Early glaucoma, Glaucoma detection, OCT angiography, Ocular magnification.

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References

1. Bennett AG, Rudnicka AR, Edgar DF. Improvements on Littmann's method of determining the size of retinal features by fundus photography. *Graefes Arch Clin Exp Ophthalmol*. 1994;232(6):361−367.
2. Littmann H. [Determination of the real size of an object on the fundus of the living eye]. *Klin Monbl Augenheilkd*. 1982;180(4):286−289.
3. Littmann H. [Determining the true size of an object on the fundus of the living eye]. *Klin Monbl Augenheilkd*. 1988;192(1):66−67.
4. Hirasawa K, Shoji N, Yoshii Y, Haraguchi S. Determination of axial length requiring adjustment of measured circumpapillary retinal nerve fiber layer thickness for ocular magnification. *PLoS One*. 2014;9(9):e107553.
5. Francisconi CLM, Wagner MB, Ribeiro RVP, Freitas AM. Effects of axial length on retinal nerve fiber layer and macular ganglion cell-inner plexiform layer measured by spectral-domain OCT. *Arq Bras Oftalmol*. 2020;83(4):269−276.
6. Bae SH, Kang SH, Feng CS, et al. Influence of myopia on size of optic nerve head and retinal nerve fiber layer thickness measured by spectral domain optical coherence tomography. *Korean J Ophthalmol*. 2016;30(5):335−343.
7. Kausar A, Akhtar N, Afzal F, Ali K. Effect of refractive errors/axial length on peripapillary retinal nerve fibre layer thickness (RNFL) measured by Topcon SD-OCT. *J Pak Med Assoc*. 2018;68(7):1054−1059.
8. Kang SH, Hong SW, Im SK, et al. Effect of myopia on the thickness of the retinal nerve fiber layer measured by Cirrus HD optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2010;51(8):4075−4083.
9. Nakanishi H, Akagi T, Hangai M, et al. Effect of axial length on macular ganglion cell complex thickness and on early glaucoma diagnosis by spectral-domain optical coherence tomography. *J Glaucoma*. 2016;25(5):e481−e490.
10. Jia Y, Tan O, Tokayer J, et al. Split-spectrum amplitude-decorrelation angiography with optical coherence tomography. *Opt Express*. 2012;20(4):4710−4725.
11. Hou H, Moghimi S, Zangwill LM, et al. Macula vessel density and thickness in early primary open-angle glaucoma. *Am J Ophthalmol*. 2019;199:120−132.
12. Geyman LS, Garg RA, Suwan Y, et al. Peripapillary perfused capillary density in primary open-angle glaucoma across disease stage: an optical coherence tomography angiography study. *Br J Ophthalmol*. 2017;101(9):1261−1268.
13. Lee EJ, Lee KM, Lee SH, Kim TW. OCT angiography of the peripapillary retina in primary open-angle glaucoma. *Invest Ophthalmol Vis Sci*. 2016;57(14):6265−6270.
14. Lal B, Alonso-Caneiro D, Read SA, Carkeet A. Induced refractive error changes the optical coherence tomography angiography transverse magnification and vascular indices. *Am J Ophthalmol*. 2021;229:230−241.
15. Rudnicka AR, Burk RO, Edgar DF, Fitzke FW. Magnification characteristics of fundus imaging systems. *Ophthalmology*. 1998;105(12):2186−2192.
16. Garway-Heath DF, Rudnicka AR, Lowe T, et al. Measurement of optic disc size: equivalence of methods to correct for ocular magnification. *Br J Ophthalmol*. 1998;82(6):643−649.
17. Anderson D, Patella V. *Automated Static Perimetry*. St. Louis: Mosby; 1999:152−153.
18. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012;9(7):671−675.
19. Otsu N. A threshold selection method from gray level histograms. *IEEE Trans Syst Man Cybern*. 1979;9:62−66.

20. Knaapi L, Aarnisalo E, Vesti E, Leinonen MT. Clinical verification of the formula of Bennett et al. (1994) of determining the size of retinal features by fundus photography. *Acta Ophthalmol*. 2015;93(3):248−252.
21. Hodapp E, Parrish II R, Anderson D. *Clinical Decisions in Glaucoma*. St. Louis: Mosby; 1993.
22. Hirasawa K, Shoji N. Association between ganglion cell complex and axial length. *Jpn J Ophthalmol*. 2013;57(5): 429−434.
23. Ooto S, Hangai M, Tomidokoro A, et al. Effects of age, sex, and axial length on the three-dimensional profile of normal macular layer structures. *Invest Ophthalmol Vis Sci*. 2011;52(12):8769−8779.
24. Yamashita T, Nitta K, Sonoda S, et al. Relationship between location of retinal nerve fiber layer defect and curvature of retinal artery trajectory in eyes with normal tension glaucoma. *Invest Ophthalmol Vis Sci*. 2015;56(10), 6190-5.
25. Hirasawa H, Tomidokoro A, Araie M, et al. Peripapillary retinal nerve fiber layer thickness determined by spectral-domain optical coherence tomography in ophthalmologically normal eyes. *Arch Ophthalmol*. 2010;128(11): 1420−1426.
26. Lu P, Xiao H, Liang C, et al. Quantitative analysis of microvasculature in macular and peripapillary regions in early primary open-angle glaucoma. *Curr Eye Res*. 2020;45(5): 629−635.
27. Yarmohammadi A, Zangwill LM, Diniz-Filho A, et al. Relationship between optical coherence tomography angiography vessel density and severity of visual field loss in glaucoma. *Ophthalmology*. 2016;123(12):2498−2508.