Comparison of Sectoral Structure-Function Relationships in Glaucoma: Vessel Density Versus Thickness in the Peripapillary Retinal Nerve Fiber Layer

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KS and TH contributed equally to the work presented here and should therefore be regarded as equivalent authors.

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PURPOSE. To compare the sectoral structure-function relationships in glaucoma between two structural parameters, vessel density (VD) and thickness, in the peripapillary retinal nerve fiber layer (RNFL).

METHODS. Optical coherence tomography (OCT) angiography (AngioVue/RTVue-XR) for VD and OCT (RS-3000) for peripapillary RNFL thickness were performed in 129 eyes from 129 subjects (94 glaucoma, 17 glaucoma suspects, and 18 normal eyes). Humphrey visual field (VF) tests were performed using a SITA standard 24-2 program. Sectoral structure-function relationships based on the Garway-Heath map were compared between VD and thickness using a semipartial correlation coefficient (sr) and partial residual plots determined by multiple linear regression models controlling for the effects of sex, age, axial length, disc area, beta- and gamma- parapapillary atrophy (PPA) zones, and signal strength index (SSI).

RESULTS. For all VF sectors, the sr2 of VD was largest when the VF sector corresponded to the VF sector (sr2: 0.17–0.39). In contrast, in only three sectors was the sr2 of thickness largest when the thickness sector corresponded to the VF sector (sr2: 0.02–0.34): the inferior temporal, temporal, and superior temporal sectors. The partial residual plots against VD showed more linear relationships with VF sensitivity than those against thickness in the qualitative evaluation of fitting by locally weighted scatterplot smoothing. Eyes with larger SSI had higher VD, whereas eyes with a larger extension of the PPA zone into the VF measurement area had lower VD.

CONCLUSIONS. Peripapillary RNFL VD and thickness have different characteristic sectoral structure-function relationships in glaucoma.

Keywords: optical coherence tomography, angiography, glaucoma, structure-function relationships, peripapillary retinal nerve fiber layer

Optical coherence tomography (OCT) angiography (OCTA), an OCT-based vascular flow detection system with high-resolution three-dimensional imaging, has succeeded in noninvasive visualization and quantification of human retinal vessels in vivo, including the radial peripapillary capillaries (RPCs), which supply the inner portion of the peripapillary retinal nerve fiber layer (RNFL). In healthy subjects, RPC imaging was much better than fluorescein angiography which, to date, has been the sole method for in vivo imaging of retinal vessels. Almost a half century ago, a histologic study revealed the selective atrophy of RPCs in postmortem eyes with glaucoma. As expected, OCTA studies demonstrated reduced blood flow and vessel density (VD) in peripapillary vessels, including RPCs in eyes with glaucoma. Furthermore, the peripapillary vascular changes were well correlated with the severity of glaucoma, and the diagnostic utility of peripapillary VD for RNFL thickness had been the sole method for in vivo imaging of retinal vessels. This work presented here and should therefore be regarded as equivalent authors.

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In glaucoma, the structural changes attributed to retinal ganglion cell damage are supposed to be spatially correlated with functional alterations (i.e., loss of visual field [VF] sensitivity) based on the characteristic projection of the retinal nerve fibers. Given this, a reasonable spatial relationship between structure and function is necessary for the structural parameters derived from imaging devices to be used for glaucoma diagnosis. In this regard, peripapillary VD may be a promising diagnostic parameter given that vascular impairment was reported to exactly colocalize with RNFL defects. Several studies investigated the sectoral correlation between peripapillary VS and VF loss, and found a strong correlation in the inferior temporal (IT) and superior temporal (ST) sectors, which was comparable to the correlation between RNFL thickness and VF loss. However, a comprehensive comparison of sectoral structure-function relationships between peripapillary VD and RNFL thickness in glaucoma has yet to be reported.

Several factors have been identified to influence peripapillary RNFL thickness. Older age, longer axial length, smaller disc area, lower signal strength index (SSI), and female sex were reported to be associated with thinner RNFL thickness. In contrast, the determinants of peripapillary VD have yet to be well explored. For example, RPC density measured by OCTA was not shown to be significantly affected by age, sex, or optic disc size in 52 healthy subjects with spherical equivalent within ±2.00 diopters (D).
Gao et al.\textsuperscript{24} reported that signal strength variation could lead to artifacts in identification of capillary dropout or regions with low RNFL measurements.

Recently, the region devoid of Bruch’s membrane in beta-parapapillary atrophy (PPA) was differentiated from the region having Bruch’s membrane (beta-PPA) based on histology and OCT images; this zone was termed “gamma-PPA.”\textsuperscript{25,26} Dai et al.\textsuperscript{26} reported that gamma-PPA is associated with myopia rather than glaucoma, whereas the zone of beta-PPA having Bruch’s membrane is associated with glaucoma. The influence of beta- or gamma-PPA on peripapillary RNFL thickness has not been reported.

The aim of the present study was to compare the sectoral structure-function relationships in glaucoma between two structural parameters, RNFL thickness and VD in the peripapillary RNFL, controlling for potential confounders such as axial length, age, disc area, beta- and gamma-PPA zones, and SSI.

**Subjects and Methods**

**Study Design**

This cross-sectional study conducted at Kanazawa University Hospital adhered to the tenets of the Declaration of Helsinki and approval of the institutional review board was obtained. Written informed consent was obtained from each subject.

Participants underwent the following ophthalmic examinations: autorefractometry, autokeratometry, measurement of best-corrected visual acuity (BCVA) using a Landolt chart, axial length measurement (OA-1000; Tomey, Tokyo, Japan), slit-lamp examination, intraocular pressure (IOP) measurements using a Goldmann applanation tonometer, gonioscopy, dilated fundus examination, stereoscopic fundus photography (Kowa nonmyd WX; Kowa Company Ltd., Tokyo, Japan), standard automated perimetry (SAP; Swedish Interactive Threshold Algorithm standard 24-2, Humphrey Field Analyzer II; Humphrey-Zeiss Instruments, Dublin, CA, USA), spectral-domain OCT examination for peripapillary RNFL thickness (RS-3000 Advance Retina Scan; Nidek Inc., Gamagori, Aichi, Japan) and OCTA (AngioVue/RTVue-XR Fourier-domain OCT; Optovue Inc., Fremont, CA, USA). OCT for RNFL thickness and OCTA were performed on the same visit and VF testing was performed within 3 months of these OCT imaging assessments.

The study consisted of three groups: normal (nonglaucoma), glaucoma suspect (GS), and primary open angle glaucoma (POAG). Healthy subjects had an IOP $\leq 21$ mm Hg, normal appearance of the optic disc in both eyes, and no VF abnormalities corresponding to the definition of glaucoma. Glaucoma specialists evaluated the stereoscopic photos of the fundus and the VF test results under conditions whereby other clinical data were masked. POAG was defined as the presence of glaucomatous optic disc changes (enlarged cupping, neuroretinal rim thinning, and RNFL defects) and reproducible VF defects corresponding to the optic disc changes in reliable SAP results (fixation losses $<20\%$, false-positive rate $<15\%$, and false-negative rate $<20\%$). A glaucomatous VF defect was defined as follows: (1) a cluster of three points with a probability $<5\%$ on a pattern deviation map in at least one hemifield and including at least one point with a probability $<1\%$; (2) glaucoma hemifield test results outside normal limits; and (3) pattern standard deviation (SD) $<5\%$. GS subjects had a glaucomatous appearance of the optic disc, but lacked reproducible glaucomatous VF defects. Ocular hypertension, defined as having an elevated IOP $>21$ mm Hg, no glaucomatous optic disc appearance, and no VF abnormalities, was also regarded as GS. If both eyes met the definition for one of the three groups, one eye was randomly chosen for analysis.

Exclusion criteria were as follows: previous intraocular surgeries including cataract surgery and retinal photocoagulation, BCVA $<0.2$, refractive errors outside of the OCT focal range ($\pm 15$ D to $\pm 10$ D), high astigmatism ($>3$ D), gonioscopic findings other than normal open angle, media opacity affecting fundus imaging, fundus abnormalities, such as diabetic retinopathy, uveitis, macular degeneration, retinitis pigmentosa, retinal vein occlusion, or retinal detachment. Low-quality OCT images (low SSI, image artifacts, or segmentation errors) and unreliable SAP results were also criteria for exclusion. SSI $<6$ for the RS-3000 and $<40$ for the RTVue-XR were regarded as low quality.

**OCT Imaging**

Although OCTA could be analyzed in six sectors subdivided based on the Garway-Heath map\textsuperscript{3} using built-in software (AngioVue, v2015.100.0.33), the same sector analysis was not available for RNFL thickness measurement using the same OCT device (Figs. 1A–D). Therefore, we used another OCT device, the RS-3000, which allowed the use of custom software to apply the same Garway-Heath sector analysis as for peripapillary RNFL thickness. OCT examinations with the two devices were performed after mydriasis by tropicamide.

The RNFL thickness was measured using the RS-3000 along a circle with a diameter of 3.45 mm derived from a raster scan over a $6 \times 6$-mm area centered on the optic disc at a scan density of 512 A-scans vertically $\times 128$ B-scans horizontally with a scanning speed of 53,000 A-scans per second. This OCT device has a $7$-\mu m depth resolution and 20-\mu m transverse resolution in fundus imaging. Each 1024 A-scan data point along the circle was grouped into one of the six sectors of the Garway-Heath map, and the average RNFL thickness value for each sector was exported using the custom software (Figs. 1B, 1C).

**OCT Angiography**

OCTA over the disc area ($4.5 \times 4.5$ mm$^2$) was performed using the AngioVue OCT system, which obtains volumetric scans of 304 $\times$ 304 A-scans (two repeats/B-scan) at a scanning speed of 70,000 A-scans per second (Figs. 1E, 1F). Vascular flow information with a low signal-to-noise ratio of flow detection was derived using the split-spectrum amplitude-decorrelation angiography algorithm.\textsuperscript{4} Motion correction technology using orthogonal scan patterns was used to reduce motion artifacts in all three dimensions caused by heartbeat, respiration, and saccades.\textsuperscript{27} Angiography information was displayed en face as the maximum of the decorrelation values within the corresponding layer derived from the automated segmentation of the volume scans. We evaluated peripapillary VD in the RNFL layer (from the inner limiting membrane to the posterior boundary of the RNFL) including the RPC. VD (%) was defined as the proportion of the area with flow signal (i.e., a decorrelation value above the default threshold) to that of the measurement area. The built-in software (AngioVue, v2015.100.0.33) automatically set an annular area around the optic disc (750 \mu m width with an inner elliptical border automatically fitted to the optic disc margin), and provides the values for sector-wise VD (i.e., six sectors based on the Garway-Heath map) within the annular area (Figs. 1D, 1E).

Given that magnification correction was not possible for the AngioVue, OCT images from the RS-3000 were also uncorrected for magnification to align the sectors between RNFL VD and thickness. The matched alignment of sectors from both OCT devices was verified by superimposing the VD images from the AngioVue onto the scanning laser ophthalmoscopy (SLO) images from the RS-3000 (Fig. 1F).
Measurements of Disc Area and PPA Area

The disc area and PPA area were determined using the same RS-3000 raster scan over the optic disc. The built-in OCT software automatically determines the disc margin as the Bruch’s membrane opening (BMO). The region between the BMO and the clinical disc margin, defined by the Elschnig’s scleral ring observed in the fundus photos, corresponds with gamma-PPA, whereas the region between the BMO and the end of the retinal pigment epithelium (RPE) corresponds with beta-PPA. To measure the disc area and beta- and gamma-PPA zones, we first inspected, and corrected as needed, the OCT-determined disc area (i.e., the BMO area) by viewing the BMO positions in the B-scan images (Fig. 2A). We then determined the clinical disc area by modifying the circle of the BMO to match the clinical disc margin on the SLO image with reference to the fundus photo presented in another display (Fig. 2B). Last, we modified the circle again to match the outer border of beta-PPA, viewing the SLO image and the B-scan images (Fig. 2C). The values of the clinical disc area and the area inside the outer border of beta-PPA were automatically shown as altered values of the OCT-determined disc area. The beta-PPA area was derived by subtracting the BMO area from the area inside the outer border of beta-PPA. The gamma-PPA area was derived by subtracting the clinical disc area from the BMO area. A modified Littmann’s formula (Bennett’s formula) was used to correct the ocular magnification effect associated with OCT scans on the area measurements. By entering the axial length data into the formula, a magnification-corrected scan length was provided on the RS-3000 screen, with which the magnification effect was corrected for the area measurements.

One well-trained examiner (SU) performed the measurements for all eyes under conditions whereby other clinical data were masked. To assess intraobserver reproducibility, the measurements were repeated for 15 randomly chosen eyes. In addition, another examiner (TH) twice performed the measurements in these eyes to assess interobserver reproducibility.

Measurements of the Degree of PPA Extension into the Measurement Areas for VD and Thickness of Peripapillary RNFL

A VD image (Fig. 3) containing an annular measurement area was superimposed onto the SLO image from the RS-3000 (Fig. 3B). The positions of the clinical disc margin (CDM), BMO, and
Figure 2. Measurements of disc area and beta- and gamma-PPA zones. (A) The built-in OCT software automatically determines the disc margin as the BMO (inner red circle in the SLO image). (B) The disc area was derived by modifying the circle of the BMO to match the CDM on the SLO image with reference to the fundus photo presented in another display (inner red circle). (C) The outer border of beta-PPA was determined by viewing the SLO image and the B-scan image. The beta-PPA zone corresponds to the area between the inner red circles in (A) and (C). The gamma-PPA zone corresponds to the area between the inner red circles in (A) and (B). The lower images in (A), (B), and (C) are B-scan images along the horizontal white lines in the corresponding SLO images.

Figure 3. Measurements of the degree of PPA extension into the measurement areas of VD and thickness of peripapillary RNFL. (A) Fundus photograph of a glaucomatous eye with PPA. (B) An AngioVue image of vessels superimposed with the annular measurement area (light blue) on the SLO image from the RS-3000. The positions of the CDM (red solid circle), BMO (red dotted circle), and the outer border of beta-PPA zone (yellow circle) were also superimposed. Within each sector of the annular measurement area, the area between the CDM and BMO (i.e., gamma-PPA zone) and the area between the CDM and the outer border of the beta-PPA zone (i.e., beta- plus gamma-PPA) were semiquantified as 0%, 10%, 25%, 50%, 75%, or 100%. The degree of beta-PPA extension was determined as the difference between the values of beta- plus gamma-PPA and gamma-PPA. (C) The position of the circle for thickness measurements using the RS-3000 is shown in blue. The extent of BMO or the outer border of the beta-PPA zone that overlapped with each sector of the measurement circle was semiquantified as 0%, 10%, 25%, 50%, 75%, or 100%.
the outer border of beta-PPA, which were determined in the SLO image from the RS-3000 as described above, were also superimposed. Within each sector of the annular measurement area, the area between the CDM and the outer border of the beta-PPA zone (i.e., beta- plus gamma-PPA) were semiquantified as 0%, 10%, 25%, 50%, 75%, or 100%. The degree of beta-PPA extension was determined as the difference between the values of beta- plus gamma-PPA and gamma-PPA. The semiquantification was performed twice by a single rater (KS) in a masked fashion; a third evaluation was performed if the first and second evaluations were different, and was adopted as the final decision. The position of the measurement circle for RNFL thickness was superimposed onto the SLO images with markings for the CDM, BMO, and the outer border of the beta-PPA zone (Fig. 3C). The extent of BMO or the outer border of the beta-PPA zone that overlapped each sector of the measurement circle was semiquantified as 0%, 10%, 25%, 50%, 75%, or 100%. The semiquantification was performed similar to the VF measurements.

Statistical Analysis

Comparisons between the POAG, GS, and healthy groups were performed using Fisher’s exact test for categorical data and by a Kruskal-Wallis test with post hoc Bonferroni correction for numerical data. Comparisons between different sectors were performed using mixed-effects models with subject-specific random effects accounting for the possible interaction between sectors. The intraobserver and interobserver reproducibility of the measurements of disc area and beta- and gamma-PPA zones were evaluated by intra-class correlation coefficients (ICCs). Correlations between two variables were examined using a Spearman’s rank correlation. For the sectorwise analysis of VF, the antilogged values of the visual sensitivity at 52 non-blind spot locations were grouped into six sectors, and the average values by sector were logged to convert back to a dB scale.30 The labeling of each VF sector in this study was based on the location of structural parameters rather than the actual VF location (Fig. 1A). For example, IT in the VF sector indicated ST in the VF location.

Multiple linear regression analysis adjusting for confounders was used to examine the relationships between two of the three variables: mean deviation (MD), average RNFL VD, and average RNFL thickness. To examine the influence of RNFL VD and RNFL thickness on the VF sensitivity by sector, a semipartial correlation coefficient (sr) was determined using multiple linear regression models adjusting for potential confounders.30 A significant sr indicates that a significant amount of variance in the objective variable is attributed to an explanatory variable independent of the other covariates. A partial residual plot was used to visualize the relationship between a given explanatory variable and the objective variable controlling for the effect of other covariates in the multiple linear regression model. In a residual plot, the residual plus the contribution of the respective independent variable to the multiple linear regression is plotted against the values for that independent variable, and the least squares line through the plotted points is shown. A locally weighted scatterplot smoothing (LOWESS) curve was added to the partial residual plot in comparison with the least squares line to assess the nonlinearity. LOWESS estimates a curve of best fit without assuming a specific mathematical model.31 A straighter LOWESS fitting indicates a more linear relationship.

Statistical analyses were performed with SPSS software (IBM SPSS Statistics 20, IBM Corp., New York, NY, USA) and STATA software (STATA/MP version 14.0; StatCorp, College Station, TX, USA). For all analyses, a $P < 0.05$ was considered statistically significant.

RESULTS

A total of 129 eyes from 129 subjects (94 eyes from 94 patients in the POAG group, 17 eyes from 17 patients in the GS group, and 18 eyes from 18 subjects in the healthy group) were...
Table 2. Correlation Between Variables Using Spearman's Rank Correlation Coefficients

<table>
<thead>
<tr>
<th>Variables</th>
<th>RNFLT, µm</th>
<th>MD, dB</th>
<th>Sex, male</th>
<th>Age, y</th>
<th>SE, D</th>
<th>AL, mm</th>
<th>Disc area, * mm²</th>
<th>Beta-PPA area, * mm²</th>
<th>Gamma-PPA area, * mm²</th>
<th>Beta-PPA in VD measurement area, %</th>
<th>Gamma-PPA in VD measurement area, %</th>
<th>Beta plus gamma-PPA in VD measurement area, %</th>
<th>SSI, VD</th>
<th>SSI, RNFLT</th>
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<tr>
<td>VD in RNFL, %</td>
<td>rs 0.74</td>
<td>0.68</td>
<td>−0.21</td>
<td>−0.10</td>
<td>0.24</td>
<td>−0.21</td>
<td>−0.02</td>
<td>−0.28</td>
<td>−0.41</td>
<td>−0.44</td>
<td>−0.36</td>
<td>−0.47</td>
<td>0.58</td>
<td>0.14</td>
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<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.02</td>
<td>0.27</td>
<td>0.005</td>
<td>0.02</td>
<td>0.85</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.11</td>
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<tr>
<td>RNFLT, µm</td>
<td>rs 0.72</td>
<td>−0.12</td>
<td>0.004</td>
<td>0.16</td>
<td>−0.15</td>
<td>−0.01</td>
<td>−0.18</td>
<td>−0.16</td>
<td>−0.23</td>
<td>−0.17</td>
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<td>0.37</td>
<td>0.16</td>
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<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>0.19</td>
<td>0.96</td>
<td>0.08</td>
<td>0.08</td>
<td>0.90</td>
<td>0.04</td>
<td>0.07</td>
<td>0.009</td>
<td>0.06</td>
<td>0.08</td>
<td>&lt;0.001</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>MD, dB</td>
<td>rs 0.85</td>
<td>0.68</td>
<td>0.005</td>
<td>0.07</td>
<td>0.46</td>
<td>&lt;0.001</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
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<td>0.03</td>
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<tr>
<td>Disc area, * mm²</td>
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<td>−0.07</td>
<td>0.11</td>
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<td>0.07</td>
<td>−0.005</td>
<td>0.23</td>
<td>−0.14</td>
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<tr>
<td>P</td>
<td>0.43</td>
<td>0.46</td>
<td>0.23</td>
<td>0.32</td>
<td>0.43</td>
<td>0.96</td>
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<td>0.47</td>
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<tr>
<td>Beta-PPA area, *</td>
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<td>−0.31</td>
<td>0.19</td>
<td>−0.09</td>
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<td>0.32</td>
<td>0.34</td>
<td>0.61</td>
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<tr>
<td>mm²</td>
<td>P 0.04</td>
<td>&lt;0.001</td>
<td>0.03</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.02</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Gamma-PPA area, *</td>
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<td>−0.20</td>
<td>0.19</td>
<td>−0.21</td>
<td>−0.73</td>
<td>0.72</td>
<td>0.57</td>
<td>0.72</td>
<td>0.71</td>
<td>−0.35</td>
<td>−0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mm²</td>
<td>P 0.07</td>
<td>0.03</td>
<td>0.03</td>
<td>0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-PPA in VD</td>
<td>rs −0.25</td>
<td>−0.32</td>
<td>0.16</td>
<td>0.03</td>
<td>−0.40</td>
<td>0.35</td>
<td>0.56</td>
<td>0.91</td>
<td>−0.26</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>measurement area, %</td>
<td>P 0.009</td>
<td>&lt;0.001</td>
<td>0.07</td>
<td>0.71</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>0.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamma-PPA in VD</td>
<td>rs −0.17</td>
<td>−0.20</td>
<td>0.19</td>
<td>−0.28</td>
<td>−0.61</td>
<td>0.59</td>
<td>0.80</td>
<td>−0.29</td>
<td>−0.007</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>measurement area, %</td>
<td>P −0.06</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.94</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta plus gamma-PPA in VD</td>
<td>rs −0.23</td>
<td>−0.32</td>
<td>0.18</td>
<td>−0.10</td>
<td>−0.55</td>
<td>0.51</td>
<td>−0.32</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VD measurement area,%</td>
<td>P 0.008</td>
<td>&lt;0.001</td>
<td>0.04</td>
<td>0.27</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.998</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VD and RNFLT are the average values of total peripapillary area. AL, axial length; MD, mean deviation of static automated perimetry; RNFLT, retinal nerve fiber layer thickness. * Magnification-corrected values.
<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>MD, dB vs. VD$^*$</th>
<th>MD, dB vs. Thickness$^*$</th>
<th>VD,$^+$ % vs. Thickness$^*$</th>
<th>VD,$^+$ % vs. MD</th>
<th>Thickness,$^+$ µm vs. MD</th>
<th>Thickness,$^+$ µm vs. VD$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter in comparison</td>
<td>B, P value, (95% CI)</td>
<td>B, P value, (95% CI)</td>
<td>B, P value, (95% CI)</td>
<td>B, P value, (95% CI)</td>
<td>B, P value, (95% CI)</td>
<td>B, P value, (95% CI)</td>
</tr>
<tr>
<td>Parameter in comparison</td>
<td>0.78, &lt;0.001, (0.64 to 0.92)</td>
<td>0.64, &lt;0.001, (0.53 to 0.76)</td>
<td>0.26, &lt;0.001, (0.21 to 0.31)</td>
<td>1.65, &lt;0.001, (1.52 to 1.98)</td>
<td>1.83, &lt;0.001, (1.48 to 2.18)</td>
<td></td>
</tr>
<tr>
<td>Sex, male</td>
<td>2.12, 0.03, (0.25 to 4.01)</td>
<td>-2.14, 0.02, (-3.84 to -0.43)</td>
<td>-1.30, 0.15, (-3.06 to -0.46)</td>
<td>-3.20, 0.20, (-8.11 to -1.71)</td>
<td>1.76, 0.46, (1.48 to 2.18)</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>0.003, 0.93, (0.11 to 0.05)</td>
<td>-0.02, 0.16, (-0.12 to 0.02)</td>
<td>-0.05, 0.16, (-0.15 to 0.23)</td>
<td>0.04, 0.67, (0.02 to 0.46)</td>
<td>1.00, 0.26, (0.07 to 0.07)</td>
<td></td>
</tr>
<tr>
<td>Axial length, mm</td>
<td>-0.66, 0.10, (-1.43 to 0.12)</td>
<td>-0.60, 0.10, (-0.11 to 0.11)</td>
<td>0.18, 0.21, (0.02 to 0.16)</td>
<td>0.18, 0.21, (0.04 to 0.16)</td>
<td>0.10, 0.67, (0.02 to 0.16)</td>
<td></td>
</tr>
<tr>
<td>Disc area,$^{†}$ mm$^2$</td>
<td>-0.72, 0.41, (-2.46 to 1.31)</td>
<td>0.02, 0.98, (2.47 to 1.31)</td>
<td>0.22, 0.79, (1.57 to 1.60)</td>
<td>0.65, 0.78, (1.84 to 1.40)</td>
<td>0.92, 0.67, (5.20 to 5.37)</td>
<td></td>
</tr>
<tr>
<td>Beta-PPA area,$^{†}$ mm$^2$</td>
<td>-0.94, 0.29, (-2.70 to 0.81)</td>
<td>0.76, 0.35, (-2.47 to 1.31)</td>
<td>0.18, 0.21, (1.57 to 1.60)</td>
<td>-1.29, 0.50, (1.57 to 1.60)</td>
<td>-2.33, 0.29, (5.20 to 5.37)</td>
<td></td>
</tr>
<tr>
<td>Gamma-PPA area,$^{†}$ mm$^2$</td>
<td>1.67, 0.18, (-0.80 to 4.15)</td>
<td>0.77, 0.35, (-2.47 to 1.31)</td>
<td>0.18, 0.21, (1.57 to 1.60)</td>
<td>-1.29, 0.50, (1.57 to 1.60)</td>
<td>-2.33, 0.29, (5.20 to 5.37)</td>
<td></td>
</tr>
<tr>
<td>Beta plus gamma-PPA in VD measurement area, %</td>
<td>0.08, 0.14, (0.03 to 0.20)</td>
<td>-2.47 to 1.31, (-2.47 to 1.31)</td>
<td>-1.29, 0.50, (1.57 to 1.60)</td>
<td>-2.33, 0.29, (5.20 to 5.37)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSI, VD$^*$</td>
<td>-0.16, 0.02, (0.29 to 0.81)</td>
<td>-0.16, 0.001, (0.29 to 0.81)</td>
<td>-0.18, 0.001, (0.29 to 0.81)</td>
<td>-0.24, 0.09, (0.29 to 0.81)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSI, thickness$^*$</td>
<td>not included</td>
<td>0.33, &lt;0.001, (0.29 to 0.81)</td>
<td>0.28, &lt;0.001, (0.29 to 0.81)</td>
<td>-0.17, 0.31, (0.29 to 0.81)</td>
<td>-0.51 to 0.16, (0.29 to 0.81)</td>
<td></td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.57</td>
<td>0.50</td>
<td>0.72</td>
<td>0.51</td>
<td>0.57</td>
<td></td>
</tr>
</tbody>
</table>

B, regression coefficient; CI, confidence interval.
$^*$ The average values in the peripapillary RNFL were evaluated.
$^{†}$ Magnification-corrected values.
included in the study. The demographic data are shown in Table 1. The MD and pattern standard deviation of eyes in the POAG group were $-9.1 \pm 7.2$ dB and $8.4 \pm 4.6$ dB, respectively. Age, visual acuity, spherical equivalent, IOP, ocular perfusion pressure, and axial length were not significantly different between the groups. RNFL thickness were significantly different between groups, and none had a misalignment $>10^\circ$. The disc area of the GS group was significantly larger than in the other groups, whereas the beta- and gamma-PPA areas were not significantly different between groups, and the POAG group had significantly lower values than the other groups ($P < 0.001$).

With regard to the OCT and OCTA image analysis, the degree of sector misalignment between the two OCT devices was $2.3 \pm 2.1^\circ$. Most eyes (93.0%) showed a misalignment $\leq 5^\circ$, and none had a misalignment $>10^\circ$. The disc area of the GS group was significantly larger than in the other groups, whereas the beta- and gamma-PPA areas were not significantly different between groups. The mean intraobserver ICCs of the two examiners were 0.994, 0.982, and 0.967 for disc area and beta- and gamma-PPA areas, respectively. The interobserver ICCs were 0.995, 0.961, and 0.949 for the disc area and beta- and gamma-PPA zones, respectively. Concerning the degree of PPA extension into the measurement areas of VD and RNFL thickness, the number of eyes with beta- and gamma-PPA extending into the VD measurement area was 115 (89.1%) and 73 (56.6%), respectively. The mean frequency of disagreement between the first and second judgments in each VD sector was $2.3\% \pm 1.4\%$ and $4.9\% \pm 4.2\%$ for the gamma-PPA and beta-plus gamma-PPA zones, respectively. The degree of PPA extension was not significantly different between the groups (Table 1). Among all sectors, PPA extension was largest in the temporal sector ($P < 0.001$, Supplementary Table S1). In contrast, the number of eyes with beta- and gamma-PPA that overlapped with the thickness measurement circle was six (4.7%) and 0 (0%), respectively. There were no judgment disagreements in the evaluation of the measurement circles. Given the small number of eyes with this overlap, this factor was not included in the subsequent analyses.

Correlations between variables evaluated by Spearman’s rank correlation coefficients are shown in Table 2. MD, peripapillary RNFL VD, and RNFL thickness showed not only strong positive correlation with each other ($r = 0.68$ to $0.74, P < 0.001$), but also had significant correlation with other variables. Therefore, multiple linear regression analysis was used to examine the relationship between two variables adjusting for other confounders. Because refractive error and axial length were very strongly correlated each other ($r = -0.88, P < 0.001$), only axial length, the most significant
Table 4. Semipartial Correlation of VD and Thickness of the Peripapillary RNFL With VF Sensitivity (Sector-Wise Comparisons)

<table>
<thead>
<tr>
<th>Sectors</th>
<th>Nasal</th>
<th>IN</th>
<th>IT</th>
<th>Temporal</th>
<th>ST</th>
<th>SN</th>
</tr>
</thead>
<tbody>
<tr>
<td>VD, %</td>
<td>sr</td>
<td>sr²</td>
<td>P</td>
<td>sr</td>
<td>sr²</td>
<td>P</td>
</tr>
<tr>
<td>Nasal</td>
<td>0.418</td>
<td>0.17*&lt;0.001</td>
<td>0.452</td>
<td>0.20</td>
<td>&lt;0.001</td>
<td>0.387</td>
</tr>
<tr>
<td>IN</td>
<td>0.355</td>
<td>0.13</td>
<td>&lt;0.001</td>
<td>0.552</td>
<td>0.30*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IT</td>
<td>0.288</td>
<td>0.08</td>
<td>&lt;0.001</td>
<td>0.488</td>
<td>0.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temporal</td>
<td>0.219</td>
<td>0.05</td>
<td>&lt;0.001</td>
<td>0.347</td>
<td>0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ST</td>
<td>0.322</td>
<td>0.10</td>
<td>&lt;0.001</td>
<td>0.396</td>
<td>0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SN</td>
<td>0.289</td>
<td>0.08</td>
<td>&lt;0.001</td>
<td>0.301</td>
<td>0.09</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The sr was determined in multiple linear regression models controlling for the effects of sex, age, axial length, disc area, gamma- and beta-PPA zones, the beta-plus gamma-PPA zone extending into the VD measurement area in the same sector as VD, and SSI.

* Highest values of sr² among all sectors. The labeling of each VF sector was based on the location of structural parameters rather than the actual VF location.

determinant of ocular magnification in fundus imaging,29 was entered into the multiple regression models to avoid problems of multicollinearity. Among the PPA-related variables, all had significantly negative correlations with VD, whereas only the beta-PPA associated variables had significantly negative correlations with thickness.

As shown in Table 3, MD, peripapillary RNFL VD, and RNFL thickness were significant determinants of each other. The SSI of RNFL VD and beta-plus gamma-PPA extension into the VD measurement area were significant determinants of RNFL VD. Eyes with larger SSI had higher RNFL VD, whereas eyes with larger extensions of the beta-plus gamma-PPA zones into the VD measurement area had lower VD. When beta- and gamma-PPA were used separately as independent variables instead of beta-plus gamma-PPA, only beta-PPA extension was a statistically significant variable (Supplementary Table S2). We then examined the influence of structural parameters on RNFL VD in each sector. Besides RNFL thickness and SSI of VD, beta-plus gamma-PPA extension into the measurement area was a significantly negative determinant of VD in temporal and ST sectors (Supplementary Table S3). When beta-plus gamma-PPA was replaced by beta- and gamma-PPA in the multiple regression models, beta- and gamma-PPA extensions were significantly negative determinants in the nasal and temporal sectors and in the superior nasal (SN) sector, respectively (Supplementary Table S4). The IT sector had no significant association with PPA parameters, whereas males and older age appeared to have lower VD.

Figure 4 shows partial residual plots to illustrate the relationship between MD, peripapillary RNFL VD and RNFL thickness controlling for the effects of other variables listed in Table 4. MD, peripapillary RNFL VD, and RNFL thickness were positively correlated with each other, even after adjusting for potential confounders. According to the qualitative evaluation of LOWESS curves in comparison with the least squares lines, RNFL VD showed a more linear relationship with sectoral VF sensitivity than with RNFL thickness, especially in the IT (Figs. 5E, 5F), temporal (Figs. 5G, 5H), and ST (Figs. 5I, 5J) sectors where structure-function associations determined by sr² were strong.

**Discussion**

The sectoral structure-function relationship in glaucoma using peripapillary RNFL VD as a structural parameter was favorable in all six sectors. Within each sector, the correlation to VF sensitivity was strongest or a close second in the corresponding sector compared with other sectors, after controlling for the effects of confounders. In addition to the IT and ST sectors, where previous studies showed a strong correlation between VF and VD loss, the temporal and IN sectors also showed a comparable degree of correlation between them. When compared with RNFL thickness, the degree of correlation between VD and VF sensitivity was similar in the IT and ST sectors where RNFL thickness showed the strongest correlation with VF sensitivity. In contrast, the correlation with VF sensitivity was different in the nasal sector where VD had a relatively weak but significant correlation, whereas RNFL thickness showed no significant correlation. Thus, RNFL VD showed more uniform correlation with VF sensitivity around the optic disc than RNFL thickness, which may be advanta-
pecial interest in studying the structure-function relationships including the nasal sector.

The structure-function plots revealed different correlative behaviors across the glaucoma stages between the VD and thickness of the RNFL. The LOWESS fitting of global (Fig. 4B) and sectoral plots (Figs. 5B, 5D, 5F, 5H, 5J, 5L) for VF sensitivity in decibels against RNFL thickness were consistent with the curvilinear relationship of the log-linear model, as shown in many other studies. In contrast, the LOWESS fitting of global (Fig. 4A) and sectoral plots (Figs. 5A, 5C, 5E, 5G, 5I, 5K) of VF sensitivity in dB against VD was more linear than the plots against RNFL thickness, especially in the global plot and the IT, temporal, and ST sector plots. This indicates that RNFL VD may be more useful for assessing the structure-function relationship than RNFL thickness, even in advanced-stage glaucoma. However, the plots against VD showed a larger degree of scatter in the more advanced glaucoma stage (i.e., smaller VD values) than in the earlier stage. Given that OCTA quantifies VD as blood vessels with flow signals above the threshold rather than those actually present, the blood vessels with low flow signals may or may not be visible in OCTA imaging, depending on various factors other than true flow level.

The determinants of thickness and VD in the RNFL were different from each other in this study. Although the beta-PPA parameters had significant negative Spearman’s rank correlation coefficients with thickness, no variables other than MD or thickness were significant in the multiple linear regression models for the relationship between MD and thickness. In contrast, sex and SSI for VD were significant variables in the multiple regression models for the relationship between MD and VD. Therefore, these variables should be taken into account when interpreting RNFL VD data. PPA extension into the VD measurement area, rather than the magnification-corrected PPA and disc area, had a significant negative influence on peripapillary RNFL VD, indicating that RNFL VD measurement by OCTA over PPA may be affected by the presence of PPA. It is plausible that larger beta-PPA extension is associated with lower RNFL VD, as shown in the averages of all sectors and temporal and nasal sectors in this study, given that beta-PPA is associated with glaucoma. A recent study showed that POAG eyes with beta-PPA had lower juxtapapillary choroidal volume than those without. However, the peri-
papillary RNFL containing major vessels and capillary layers, including the RPC, receives its blood supply from the central retinal artery, but not from the choroid.3,5 In contrast, gamma-PPA, which is less relevant to glaucoma than beta-PPA, was a determinant of SSI in the SN sector. Gamma-PPA is indicative of morphologic changes around the optic disc due to axial elongation.26,34-35 In this study and previous ones, a larger beta-PPA zone was also significantly associated with longer axial length.35 Thus, the association of VD with beta-PPA rather than gamma-PPA in several sectors may not exclude the possibility of lower VD resulting from RNFL thinning due to stretching around the optic disc (a result of axial elongation). Although the exact mechanisms responsible for the association of PPA with lower RNFL VD are currently unknown, attention should be given to the area of PPA extending into the measurement area when evaluating peripapillary RNFL VD.

A previous study reported that SSI, an indicator of total reflectance intensity, showed a significant positive correlation with peripapillary VD in both normal eyes and eyes with POAG or ocular hypertension.23,24 Furthermore, the superficial VD in the macula and the optic disc was also significantly correlated with SSI using a fixed threshold.24 Thus, the quantification of VD may be more susceptible to SSI than thickness quantification.

This study has several limitations. The target vessels measured for RNFL VD not only included the RPCs but also the large retinal vessels. Therefore, the results of RNFL VD may not be the same as the VD of the RPCs. Several studies have evaluated RPC VD by removing the large vessels from the peripapillary OCTA images.8,23 The advantage of using only the VD of RPCs over RNFL VD as a structural parameter in glaucoma remains to be addressed. However, our goal was to compare VD with thickness in the peripapillary RNFL using the same six sectors in various stages of glaucoma. Thus, evaluation of VD in the RNFL, including the large retinal vessels, may be more appropriate than just capillaries alone given that the thickness of the RNFL also includes the large retinal vessels. In this study, we did not include pseudophakic subjects to exclude the possible confounding effects on VD measurements, which may limit the generalizability of our results to particular glaucoma populations. We adopted LOW-ESS curves to visually demonstrate the relationship in comparison with the least squares lines in the partial residual plots. Although we could find the different characteristics in LOW-ESS fitting for the structure-function relationship between peripapillary VD and thickness, the evaluation was qualitative, as was the case in previous studies.11

In conclusion, peripapillary RNFL VD and thickness had different characteristic sectoral structure-function relationships in glaucoma. In contrast to RNFL thickness, peripapillary RNFL VD may be influenced by SSI and the area of the PPA zone extending into the VD measurement area, which should be considered when interpreting VD data. Further studies delineating the usefulness of RNFL VD as a structural parameter for glaucoma are warranted.

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