Relationship between Optical Coherence Tomography Angiography Vessel Density and Severity of Visual Field Loss in Glaucoma

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Purpose: To evaluate the association between vessel density measurements using optical coherence tomography angiography (OCT-A) and severity of visual field loss in primary open-angle glaucoma.

Design: Observational, cross-sectional study.

Participants: A total of 153 eyes from 31 healthy participants, 48 glaucoma suspects, and 74 glaucoma patients enrolled in the Diagnostic Innovations in Glaucoma Study.

Methods: All eyes underwent imaging using OCT-A (Angiovue; Optovue, Fremont, CA), spectral-domain OCT (Avanti; Optovue), and standard automated perimetry (SAP). Retinal vasculature information was summarized as vessel density, the percentage of area occupied by flowing blood vessels in the selected region. Two measurements from the retinal nerve fiber layer (RNFL) were used: circumpapillary vessel density (cpVD) (750-μm-wide elliptical annulus around the optic disc) and whole-image vessel density (wiVD) (entire 4.5×4.5-mm scan field).

Main Outcome Measures: Associations between the severity of visual field loss, reported as SAP mean deviation (MD), and OCT-A vessel density.

Results: Compared with glaucoma eyes, normal eyes demonstrated a denser microvascular network within the RNFL. Vessel density was higher in normal eyes followed by glaucoma suspects, mild glaucoma, and moderate to severe glaucoma eyes for wiVD (55.5%, 51.3%, 48.3%, and 41.7%, respectively) and for cpVD (62.8%, 61.0%, 57.5%, 49.6%, respectively) (P < 0.001 for both). The association between SAP MD with cpVD and wiVD was stronger (R² = 0.54 and R² = 0.51, respectively) than the association between SAP MD with RNFL (R² = 0.36) and rim area (R² = 0.19) (P < 0.05 for all). Multivariate regression analysis showed that each 1% decrease in wiVD was associated with 0.66 decibel (dB) loss in MD and each 1% decrease in cpVD was associated with 0.64 dB loss in MD. In addition, the association between vessel density and severity of visual field damage was found to be significant even after controlling for the effect of structural loss.

Conclusions: Decreased vessel density was significantly associated with the severity of visual field damage independent of the structural loss. Optical coherence tomography angiography is a promising technology in glaucoma management, potentially enhancing the understanding of the role of vasculature in the pathophysiology of the disease.


Glucoma...
measurements and compares this with standard spectral-domain OCT (SD OCT) structural measurements.

Methods

This was an observational cross-sectional study including 153 eyes from 31 healthy participants, 48 glaucoma suspects, and 74 patients with primary open-angle glaucoma enrolled in the Diagnostic Innovations in Glaucoma Study who underwent OCT-A (Angiovue; Optovue Inc., Fremont, CA)13–20 and SD OCT ONH imaging (Avanti; Optovue Inc.).

The Diagnostic Innovations in Glaucoma Study eligibility criteria and methodological details have been reported in previous publications.21 In brief, all participants completed a comprehensive ophthalmologic examination, including best-corrected visual acuity, slit-lamp biomicroscopy, intraocular pressure (IOP) measurement with Goldmann applanation tonometry, gonioscopy, dilated fundus examination, stereoscopic optic disc photography, ultrason ophthalmometry, and standard automated perimetry (SAP) in both eyes. Only participants older than 18 years of age with open angles on gonioscopy, and spherical refraction within ±10 diopters were included.

Written informed consent was obtained from all participants. The institutional review board at the University of California San Diego approved all protocols, and the methods described were in agreement with the tenets of the Declaration of Helsinki and the Health Insurance Portability and Accountability Act.

Healthy subjects were required to have an IOP of 21 mmHg or less with no history of elevated IOP, normal-appearing optic discs, and if needed the margin was adjusted manually and confirmed by an SSI lower than 37 and scans with segmentation failures or artifacts were excluded from the analysis. Graders also reviewed the location of the optic disc margin for accuracy, and if needed the margin was adjusted manually and confirmed by 2 graders.

All participants underwent visual field testing using the 24-2 pattern Swedish interactive threshold algorithm on the Humphrey Field Analyzer (Carl Zeiss Meditec, Dublin, CA) within 6 months of imaging. Only reliable tests (≤33% fixation losses and false-negatives, and ≤15% false-positives) were included. The quality of visual field tests was also reviewed by the Visual Field Assessment Center23 staff to identify and exclude visual fields with evidence of inattention or inappropriate fixation, artifacts such as eyelid and lens rim artifacts, fatigue effects, and abnormal results caused by diseases other than glaucoma.

Optical Coherence Tomography Angiography

The OCT-A imaging system provides a noninvasive method for visualizing the ONH and retinal vasculature. The image acquisition technique is optimized for the Split-Spectrum Amplitude-Decorrelation Angiography algorithm described in detail by Jia et al.13 The Split-Spectrum Amplitude-Decorrelation Angiography method captures the dynamic motion of moving scatterers, such as red blood cells in a flowing blood vessel, and computes a high-resolution 3-dimensional visualization of perfused vasculature.

The OCT-A characterizes vascular information at each retinal layer as an en face angiogram, a vessel density map (Fig 1), and quantitatively as vessel density (percentage), calculated as the percentage area occupied by flowing blood vessels in the selected region.

For this study, we used vessel density measurements within the peripapillary RNFL in scans with a 4.5×4.5-mm field of view centered on the ONH. Vessel density within the RNFL was measured from the internal limiting membrane to RNFL posterior boundary using standard AngioVue software (version 2015.1.0.90). Measurements were calculated in 2 areas. Whole-image vessel density (wVD) was obtained over the entire 4.5×4.5-mm scan field, and circumpapillary vessel density (cpVD) was measured in a 750 μm-wide elliptical annulus extending outward from the optic disc boundary, where the inner elliptical contour is obtained by fitting an ellipse to the disc margin on the OCT en face retinal angiogram, and the ring width between inner and outer elliptical contour is defined as the circumpapillary region (Fig 1).

The Imaging Data Evaluation and Analysis Reading Center established a standard protocol for OCT-A image quality review. Trained graders reviewed all images to identify poor-quality scans, defined as blurred images, scans with a signal strength index less than 48, residual motion artifacts visible as irregular vascular pattern or disc boundary on the enface angiogram, local weak signal caused by floaters, and RNFL segmentation errors. Graders also reviewed the location of the optic disc margin for accuracy, and if needed the margin was adjusted manually and confirmed by 2 graders.

Spectral-Domain Optical Coherence Tomography

Avanti SD OCT uses an 840-nm central wavelength, a 22-μm focal spot diameter, and a 70-kHz axial line scan rate that yields an axial resolution of 5 μm in tissue. The ONH map image acquisition protocol was used to obtain RNFL thickness measurements in a 10-pixel-wide band along a 3.45-mm-diameter circle centered on the ONH and rim area measurements.

All participants had both SD OCT and OCT-A imaging performed on the same day. Participants with poor-quality ONH scans defined by an SSI lower than 37 and scans with segmentation failure or artifacts were excluded from the analysis. A total of 351 eyes of 213 subjects had OCT-A and SD OCT imaging within 6 months of visual field testing and were potentially eligible for inclusion in the analysis. Fifty-four eyes were excluded because of poor-quality OCT-A scans, 16 eyes were excluded because of poor-quality SD OCT images, and 35 eyes were excluded because of unreliable visual field tests. A total of 246 eyes of 153 subjects had good-quality OCT-A, SD OCT, and
Visual field tests. One eye of each of these 153 subjects was randomly selected to be included in the analysis.

**Statistical Analysis**

Descriptive statistics were calculated as the mean and standard deviation, and categoric variables were compared using the chi-square test. Analysis of variance (ANOVA) was performed to compare mean values among the healthy, glaucoma suspect, mild glaucoma, and severe to moderate glaucoma eyes.

Relationships between visual field parameters and OCT-A vessel density and SD OCT RNFL and rim area were evaluated using simple linear ($y = a + bx$) and second-order polynomial (or quadratic) models ($y = ax^2 + bx + c$). Results were reported as $R^2$ (coefficient of determination) with differences between the $R^2$ values calculated using bootstrapping procedures to estimate the 95% confidence intervals of the difference in coefficients of determination. Akaike’s information criterion (AIC) was used to compare the models for goodness of fit. The smaller the AIC, the better the model. Univariable linear regression models were built using visual field MD as the dependent variable and OCT-A parameters, wiVD and cpVD, and SD OCT RNFL thickness and rim area measurements and other demographic and ocular characteristic variables as the independent variables. Multivariable models also were used to evaluate the relationship between the visual field MD with vessel density and SD OCT RNFL and rim area while adjusting for potential confounding parameters, such as age, IOP, central corneal thickness (CCT), and axial length.

All statistical analyses were performed with Stata version 14 (StataCorp LP, College Station, TX) and JMP version 11.2.0 (SAS Institute Inc., Cary, NC). The alpha level (type I error) was set at 0.05 for all comparisons.

**Results**

The study population consisted of 31 healthy subjects (mean age, 69.0±7.7 years; SAP MD, 0.3±1.3 dB), 48 glaucoma suspects (mean age, 71.4±9.4 years; SAP MD –0.6±1.5 dB), 46 patients with mild glaucoma (mean age, 72.9±10.7 years; SAP MD –3.0±1.8 dB), and 28 patients with moderate to severe glaucoma (mean age, 75.7±10.7 years; SAP MD, –13.6±6.6 dB) (Table 1). Healthy subjects tended to be younger than glaucoma suspects and glaucoma patients, but this difference was not statistically significant ($P = 0.063$, ANOVA).
Qualitative Assessment

Healthy eyes generally appeared to have denser capillary networks in the RNFL layer compared with eyes with early glaucomatous optic nerve damage, and a trend of a sparser microvascular network could be detected with advancing stages of the disease (Fig 1).

Quantitative Assessment

Vessel density measurements were lower in more severe disease (Fig 2). Specifically, the mean wiVD in moderate to severe glaucoma eyes was significantly lower (41.7% ± 5.5%) than in mild glaucomatous eyes (48.3% ± 4.2%), glaucoma suspects (51.3% ± 4.6%), and healthy eyes (55.5% ± 3.2%) ($P < 0.001$).

Table 1. Demographics and Ocular Characteristics of Study Population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Healthy (n = 31)</th>
<th>Glaucoma Suspect (n = 48)</th>
<th>Mild Glaucoma (n = 46)</th>
<th>Moderate and Severe Glaucoma (n = 28)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>69.0 (7.7)</td>
<td>71.4 (9.4)</td>
<td>72.9 (10.7)</td>
<td>75.7 (10.7)</td>
<td>0.063</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>11/20</td>
<td>18/30</td>
<td>20/26</td>
<td>17/11</td>
<td>0.181</td>
</tr>
<tr>
<td>Ethnicity (AD/ED)</td>
<td>12/19</td>
<td>14/34</td>
<td>14/32</td>
<td>7/21</td>
<td>0.707</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>130.4 (15.8)</td>
<td>127.5 (19.6)</td>
<td>127.2 (13.1)</td>
<td>127.0 (15.4)</td>
<td>0.816</td>
</tr>
<tr>
<td>Diastolic</td>
<td>84.3 (16.4)</td>
<td>78.3 (9.8)</td>
<td>78.6 (9.6)</td>
<td>77.8 (9.0)</td>
<td>0.076</td>
</tr>
<tr>
<td>Mean</td>
<td>99.2 (12.2)</td>
<td>94.7 (11.4)</td>
<td>94.8 (9.0)</td>
<td>94.2 (9.4)</td>
<td>0.195</td>
</tr>
<tr>
<td>MOPP (mmHg)</td>
<td>56.3 (7.7)</td>
<td>52.1 (8.2)</td>
<td>54.4 (6.6)</td>
<td>54.6 (7.2)</td>
<td>0.101</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>70.2 (13.1)</td>
<td>67.6 (11.4)</td>
<td>66.2 (12.1)</td>
<td>64.8 (8.2)</td>
<td>0.334</td>
</tr>
<tr>
<td>Self-reported history of diabetes (%)</td>
<td>16.1</td>
<td>10.4</td>
<td>20.0</td>
<td>14.0</td>
<td>0.657</td>
</tr>
<tr>
<td>Self-reported history of hypertension (%)</td>
<td>45.16</td>
<td>58.3</td>
<td>61.0</td>
<td>68.0</td>
<td>0.338</td>
</tr>
<tr>
<td>IOP (mmHg)</td>
<td>14.5 (2.9)</td>
<td>16.5 (5.1)</td>
<td>13.2 (4.1)</td>
<td>12.3 (5.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CCT (μm)</td>
<td>542.7 (43.4)</td>
<td>548.4 (38.1)</td>
<td>535.4 (37.0)</td>
<td>513.1 (36.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>Axial length (mm)</td>
<td>23.7 (1.2)</td>
<td>24.2 (1.0)</td>
<td>23.9 (1.1)</td>
<td>24.8 (1.7)</td>
<td>0.005</td>
</tr>
<tr>
<td>Disc area (mm²)</td>
<td>2.0 (0.4)</td>
<td>2.1 (0.4)</td>
<td>2.1 (0.5)</td>
<td>2.0 (0.5)</td>
<td>0.596</td>
</tr>
<tr>
<td>Rim area (mm²)</td>
<td>1.4 (0.3)</td>
<td>1.0 (0.3)</td>
<td>0.9 (0.4)</td>
<td>0.7 (0.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average RNFL thickness (μm)</td>
<td>96.6 (9.6)</td>
<td>86.8 (12.8)</td>
<td>78.6 (11.6)</td>
<td>65.2 (10.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SAP MD (dB)</td>
<td>0.3 (1.3)</td>
<td>–0.6 (1.5)</td>
<td>–3.0 (1.8)</td>
<td>–13.6 (6.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SAP mean sensitivity (dB)</td>
<td>29.7 (1.6)</td>
<td>28.5 (1.8)</td>
<td>26.1 (2.0)</td>
<td>15.5 (6.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SAP PSD (dB)</td>
<td>1.6 (0.4)</td>
<td>2.0 (0.7)</td>
<td>4.3 (2.2)</td>
<td>10.2 (2.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OCT-A wiVD (%)</td>
<td>55.5 (3.2)</td>
<td>51.3 (4.6)</td>
<td>48.3 (4.2)</td>
<td>41.7 (5.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OCT-A cpVD (%)</td>
<td>62.8 (3.9)</td>
<td>61.0 (4.7)</td>
<td>57.5 (4.4)</td>
<td>49.6 (6.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

AD = African descent; BP = blood pressure; CCT = central corneal thickness; cpVD = circumpapillary vessel density; dB = decibels; ED = European descent; IOP = intraocular pressure; MD = mean deviation; MOPP = mean ocular perfusion pressure; OCT-A = optical coherence tomography angiography; PSD = pattern standard deviation; RNFL = retinal nerve fiber layer; SAP = standard automated perimetry; wiVD = whole image vessel density.
*Statistical significance tested by ANOVA.
Mean cpVD values were significantly lower in patients with moderate to severe glaucoma (49.6% \pm 6.9%) compared to mild glaucoma (57.5% \pm 4.4%), glaucoma suspects (61.0% \pm 4.7%), and healthy eyes (62.8% \pm 3.9%) (ANOVA; Tukey honestly significant difference $P < 0.05$ for all comparisons) (Table 1). Standard structural and functional measurements also showed statistically significant differences among groups ($P < 0.001$) (Table 1).

ANOVA: Tukey honestly significant difference $P < 0.05$ for all pairwise comparisons except between healthy and glaucoma suspect eyes ($P = 0.322$). Standard structural and functional measurements also showed statistically significant differences among groups ($P < 0.001$) (Table 1).

Table 2. Age-adjusted Pearson Correlation Coefficient Matrix on Vessel Density, Visual Field, and Structural Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>wiVD (%) (n = 153)</th>
<th>cpVD (%) (n = 153)</th>
<th>Average RNFL Thickness (μm) (n = 153)</th>
<th>SD OCT Rim Area (mm²) (n = 153)</th>
<th>SAP MD (dB) (n = 153)</th>
<th>SAP Pattern Standard Deviation (dB) (n = 153)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cpVD (%)</td>
<td>*0.91 (&lt;0.001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average RNFL thickness (μm)</td>
<td>*0.83 (&lt;0.001)</td>
<td>*0.73 (&lt;0.001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD OCT rim area (mm²)</td>
<td>*0.65 (&lt;0.001)</td>
<td>*0.44 (&lt;0.001)</td>
<td></td>
<td>*0.69 (&lt;0.001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual field MD (dB)</td>
<td>*0.71 (&lt;0.001)</td>
<td>*0.73 (&lt;0.001)</td>
<td></td>
<td>*0.59 (&lt;0.001)</td>
<td>*0.46 (&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>Visual field PSD (dB)</td>
<td>*-0.63 (&lt;0.001)</td>
<td>*-0.61 (&lt;0.001)</td>
<td></td>
<td>*-0.60 (&lt;0.001)</td>
<td>*-0.48 (&lt;0.001)</td>
<td>*-0.79 (&lt;0.001)</td>
</tr>
<tr>
<td>Visual field mean sensitivity (dB)</td>
<td>*0.73 (&lt;0.001)</td>
<td>*0.74 (&lt;0.001)</td>
<td></td>
<td>*0.62 (&lt;0.001)</td>
<td>*0.50 (&lt;0.001)</td>
<td>*1.02 (&lt;0.001)</td>
</tr>
<tr>
<td>Visual field SAP mean sensitivity (1/lambert)</td>
<td>*0.69 (&lt;0.001)</td>
<td>*0.65 (&lt;0.001)</td>
<td></td>
<td>*0.64 (&lt;0.001)</td>
<td>*0.55 (&lt;0.001)</td>
<td>*0.80 (&lt;0.001)</td>
</tr>
</tbody>
</table>

*Pearson’s $r$ ($P$ value to test $r=0$).

$\text{cpVD} = \text{circumpapillary vessel density}; \text{dB} = \text{decibels}; \text{MD} = \text{mean deviation}; \text{PSD} = \text{pattern standard deviation}; \text{RNFL} = \text{retinal nerve fiber layer}; \text{SAP} = \text{standard automated perimetry}; \text{SD OCT} = \text{spectral-domain optical coherence tomography}; \text{wiVD} = \text{whole image vessel density}.$

Figure 3. Scatter plots illustrating the linear (grey line) and curvilinear (quadratic fit: dark lines) correlation between standard automated perimetry (SAP) mean deviation and optical coherence tomography angiography (OCT-A) whole-image vessel density, circumpapillary vessel density, spectral-domain optical coherence tomography (SD OCT) average retinal nerve fiber layer (RNFL) thickness, and rim area measurements. dB = decibels. *$R^2$: Adjusted $R^2$ from the linear regression model. $\ddagger R^2$: Adjusted $R^2$ from the quadratic regression model.
The results of age-adjusted univariate linear regressions are summarized in Table 2. Linear and curvilinear (quadratic) relationships between OCT-A vessel density and SD OCT structural measurements with visual field MD and visual field PSD are illustrated in Figures 3 and 4, respectively. The strength of the associations between vascular and structural measurements (expressed in linear scales) with different visual field indices (expressed in both decibels and linear scales) using both linear and quadratic regression models is summarized in Table 3. We used AIC to compare the linear and curvilinear regression models of OCT-A vessel density, and SD OCT RNFL thickness and rim area with visual field MD, PSD, mean sensitivity (dB), and mean sensitivity in 1/lambert (Table 3). The quadratic model was better than the linear model in assessing the relationship between visual field measurements and vessel density parameters, as well as between visual field measurements and structural measurements (Table 3).

Significant differences were found comparing the strength of the associations between MD and both OCT-A vascular parameters with the association between MD and RNFL and rim area measurements ($P ≤ 0.05$ for all pairwise comparisons). The association between MD and RNFL thickness also was significantly stronger than between MD and rim area ($P = 0.001$). The associations between wiVD and cpVD with MD were similar ($P = 0.500$). The strongest associations with visual field PSD were with wiVD, cpVD ($R^2 = 0.39$ and 0.36, respectively), and RNFL ($R^2 = 0.37$) followed by rim area ($R^2 = 0.23$). Significant differences were found between the associations of PSD with wiVD and rim area ($P = 0.026$) and between RNFL thickness and rim area ($P = 0.035$). The linear associations between visual field mean sensitivity were strongest with cpVD ($R^2 = 0.55$) followed by wiVD ($R^2 = 0.53$), RNFL thickness ($R^2 = 0.37$), and rim area ($R^2 = 0.19$). After converting mean sensitivity from logarithmic (dB) to linear units (1/lambert), a similar pattern was found: association with mean sensitivity (1/lambert) was highest for wiVD and cpVD ($R^2 = 0.44$ for both) followed by RNFL thickness ($R^2 = 0.34$) and rim area ($R^2 = 0.18$).

The strength of the associations between visual field MD with structural and OCT-A measures also was compared using a curvilinear quadratic model. The associations between OCT-A and visual field MD were significantly stronger than the associations between visual field MD and RNFL and rim area ($P < 0.05$ for all pairwise comparisons using bootstrapping procedure). Results from the univariate regression analysis for visual field MD as the dependent variable are summarized in Table 4. Multivariate linear regression analysis, while controlling for the potentially confounding effect of age, IOP, CCT, and axial length, showed that each 1% decrease in cpVD was associated with a...
0.64-dB loss in MD ($P < 0.001$) and that each 1% decrease in wiVD was associated with a 0.66-dB loss in MD ($P < 0.001$).

Multivariate regression analysis that controlled for the effect of potential confounders (age, IOP, CCT, and axial length) and adjusted for the effect of RNFL thickness (Table 5) showed that wiVD was independently associated with visual field MD. Similar results were found when cpVD was included in the model instead of wiVD. The association between RNFL and MD was no longer statistically significant when vessel density was included in the model. The multivariate regression analysis also was completed using rim area instead of RNFL thickness, and the results were similar. Each 1% decrease in wiVD was associated with a 0.71-dB loss in MD ($P < 0.001$), and the association between rim area and MD was no longer significant ($P = 0.285$) when wiVD was included in the model.

For completeness, associations between clinical and ophthalmic features and OCT-A vessel density also were evaluated. The OCT-A vessel density was significantly associated with RNFL and rim area measurements ($P < 0.001$) (Table 2). Because structural measurements, such as RNFL, ONH rim, and cup area, have been shown to be associated with disc size, we also evaluated the effect of disc area on OCT-A vascular measurements. There were no statistically significant correlations between disc area with wiVD and cpVD measurements in healthy eyes ($R^2 = 0.005$, $P = 0.696$, and $R^2 = 0.009$, $P = 0.614$, respectively). For this reason, disc area was not controlled for in the multivariable analyses. In addition, we did not find a significant association between MOPP and cpVD ($R^2 = 0.003$; $P = 0.49$) or wiVD ($R^2 = 0.000$; $P = 0.85$).

**Discussion**

The results of this study demonstrate a significant relationship between vessel density and severity of visual field damage. Qualitatively, the OCT-A vessel density map showed sparser peripapillary vascular networks in more severe glaucoma. Quantitatively, lower vessel density values were associated with more advanced stages of glaucomatous visual field damage. The principal finding of the study was a relatively strong association among cpVD, wiVD, and visual field loss expressed as MD ($R^2 = 0.54$ and 0.51, respectively; $P < 0.001$ for both), suggesting that reduced OCT-A vessel density is associated with more severe glaucoma. Our results also suggest the vascular-functional correlations were stronger than the standard structural (RNFL and rim area)—functional relationships whether comparing linear or nonlinear fitted models. Moreover, multivariate analyses indicated an independent relationship between reduced vessel density and visual field loss, even after adjusting for the severity of structural damage measured by rim area and RNFL thickness.

Findings of a relatively strong correlation between vessel density measurements and visual field loss are in accordance with the study's associations. The OCT-A vessel density map showed sparser peripapillary vascular networks in more severe glaucoma. Quantitatively, lower vessel density values were associated with more advanced stages of glaucomatous visual field damage. The principal finding of the study was a relatively strong association among cpVD, wiVD, and visual field loss expressed as MD ($R^2 = 0.54$ and 0.51, respectively; $P < 0.001$ for both), suggesting that reduced OCT-A vessel density is associated with more severe glaucoma. Our results also suggest the vascular-functional correlations were stronger than the standard structural (RNFL and rim area)—functional relationships whether comparing linear or nonlinear fitted models. Moreover, multivariate analyses indicated an independent relationship between reduced vessel density and visual field loss, even after adjusting for the severity of structural damage measured by rim area and RNFL thickness.

**Table 4. Association Between Visual Field Mean Deviation and Vascular, Structural, Demographic, and Ocular Variables: Univariable Analysis**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient</th>
<th>$R^2$</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>wiVD (per 1% lower)</td>
<td>−0.666</td>
<td>0.511</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>cpVD (per 1% lower)</td>
<td>−0.641</td>
<td>0.533</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RNFL (per 1 μm thinner)</td>
<td>−0.228</td>
<td>0.360</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rim area (per 0.1 mm² lower)</td>
<td>−0.603</td>
<td>0.190</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (per 1 yr older)</td>
<td>0.121</td>
<td>0.024</td>
<td>0.011</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>0.945</td>
<td>0.207</td>
<td>0.322</td>
</tr>
<tr>
<td>Race (African descent)</td>
<td>0.984</td>
<td>0.026</td>
<td>0.337</td>
</tr>
<tr>
<td>IOP (per 1 mmHg higher)</td>
<td>−0.374</td>
<td>0.092</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CCT (per 10 μm thinner)</td>
<td>−0.351</td>
<td>0.059</td>
<td>0.003</td>
</tr>
<tr>
<td>Axial length (per 1 mm higher)</td>
<td>−0.867</td>
<td>0.035</td>
<td>0.022</td>
</tr>
<tr>
<td>Mean BP (per 10 mmHg higher)</td>
<td>−0.160</td>
<td>0.001</td>
<td>0.721</td>
</tr>
<tr>
<td>Hypertension (yes)</td>
<td>−0.217</td>
<td>0.000</td>
<td>0.821</td>
</tr>
<tr>
<td>Diabetes (yes)</td>
<td>−0.320</td>
<td>0.000</td>
<td>0.809</td>
</tr>
</tbody>
</table>

BP = blood pressure; CCT = central corneal thickness; cpVD = circum-papillary vessel density; IOP = intraocular pressure; RNFL = retinal nerve fiber layer; wiVD = whole-image vessel density.
with previous reports using OCT-A vessel density.\textsuperscript{16,18,20} However, it is worth mentioning that these reports measured vessel density in a thick retinal slab from internal limiting membrane to retinal pigment epithelium, whereas our results focused on vessel density in a more superficial layer, from the internal limiting membrane to the RNFL posterior boundary. Specifically, we found that glaucoma eyes had significantly sparser vessel density within the RNFL compared with both glaucoma suspects and healthy eyes, and that these vessel density measurements were lowest in the glaucoma eyes with the most severe disease.

To measure the vasculature within the RNFL, the boundaries were detected on the basis of conventional retinal layer segmentation of the SD OCT images. Glaucoma is characterized by remodeling of the ONH tissue. However, even in the presence of tissue remodeling, the RNFL boundaries can be reliably detected on the basis of the backscattered intensity image from the SD OCT. There is spatial colocalization laterally and in depth between the SD OCT intensity and the OCT-A image.

Findings of decreased OCT-A vascular parameters in patients with glaucoma also are in general agreement with the large body of evidence suggesting hemodynamic impairments in the ONH, retina, choroid, and retrobulbar circulations in glaucoma eyes.\textsuperscript{23–29} In addition, previous OCT-A reports compared the correlations between visual field loss and vessel density with the correlations between visual field loss and RNFL thickness.\textsuperscript{16,18} Consistent with our results, they reported stronger associations between visual field damage and vessel density. However, the current study went further and included 2 structural measures with vessel density in the multivariate model to demonstrate that vessel density was still strongly associated with visual field damage even after controlling for standard structural measures in the model. This finding is in agreement with Hwang et al,\textsuperscript{7} who reported an independent relationship between visual field MD and another measure of retinal vascular integrity, namely, total retinal blood flow measured by Doppler OCT.

There are several possible explanations for the independent association of vessel density and visual field MD: First, it may be due, at least in part, to the existence of dysfunctional (i.e., preapoptotic) retinal ganglion cells that may have reduced blood flow and therefore lower vessel density and poorer visual field sensitivity. Because these dysfunctional retinal ganglion cells have not yet atrophied, a reduction in RNFL thickness and rim area may not be detectable yet. Moreover, histologic studies also showed only moderate agreement between RNFL thinning and retinal ganglion cell loss,\textsuperscript{10,13} suggesting that RNFL thinning does not completely reflect the functional status of retinal ganglion cells. Therefore, the stronger correlation between vessel density and visual field damage might suggest that vessel density is a better reflection of retinal ganglion cell functioning than structural loss.

The relationship between detectable structural and functional damage and change in glaucoma is complex,\textsuperscript{32} and our results suggest that the relationship between OCT-A vessel density and visual field measures also is complex and influenced by many factors. Previous reports on standard structure-function relationships suggested that the strength of these relationships depends on the methods and scales of visual field expression, the type of structural measuring device, and the characteristics of the studied population.\textsuperscript{32} Therefore, several global visual field indices, including MD, PSD, and mean sensitivity, were assessed and compared using both linear and curvilinear associations between SAP functional measurements with vessel density, RNFL thickness, and rim area measurements.

Because the nature of the association between new vascular parameters and functional measures is not well established, we also investigated whether the relationship between vessel density and visual field damage was linear or curvilinear. Previous reports on OCT-A demonstrated a linear relationship between vessel density and visual field measurements.\textsuperscript{16,18} Our findings, based on AIC analysis, suggest that a quadratic model provides a somewhat better fit to the relationship between vessel density and visual function than a linear model. However, AIC values are only used for comparison between a set of candidate models and do not suggest the adequacy of the preferred model.\textsuperscript{24} Future studies are required to explore the exact nature of these relationships.

In our study, SAP MD, PSD, and mean sensitivity measured in a logarithmic scale reported in dB and mean sensitivity converted to a linear scale reported in 1/lambert were significantly associated with vessel density measurements ($P < 0.001$ for all). The associations among cpVD, wiVD, and MD ($R^2 = 0.54$ and 0.51, respectively) were higher than with PSD ($R^2 = 0.36$ and 0.39, respectively; $P < 0.001$). Reports comparing the strength of the association between OCT-based vascular measurements and different visual field summary measures are inconsistent. Hwang et al\textsuperscript{7} showed that total retinal blood flow measured by Doppler OCT was highly correlated with MD, but its relationship with PSD did not reach statistical significance. Another Doppler OCT–based study\textsuperscript{13} investigating hemispheric retinal blood flow measurements in eyes with glaucomatous visual field damage confined to a single hemifield reported significant differences in blood flow measurements between the affected and unaffacted retinal hemispheres in patients with glaucoma compared with healthy age-matched subjects, but failed to find an association between hemispheric retinal blood flow measurements and visual field mean retinal sensitivity measured as 1/lambert in the corresponding hemifield. In a recent OCT-A study, Liu et al\textsuperscript{18} reported that vessel density measured in a circumpapillary ring was more strongly correlated with visual field PSD compared with MD. Finally, more consistent with our findings, Wang et al\textsuperscript{23} demonstrated that optic disc OCT-A vessel density correlated with both SAP MD and PSD, but was more strongly correlated with MD.

The conflicting results in recent OCT-based Doppler and OCT-A studies investigating the relationship between vascular measurements and visual field abnormalities could be due, in large part, to different aspects of retinal vasculature that were measured. Moreover, the investigated study populations vary regarding the patients’ risk profiles, the severity and pattern of glaucoma damage, and the systemic factors that might have an effect on ocular hemodynamics.
Reduced vessel density can be the result of capillary dropout or very low or absent flow. If vessel density is lower in more advanced glaucoma eyes, it would indicate one or both scenarios may be occurring. Vessel density measurement within the RNFL is not a direct quantification of the blood flow. But if flow in certain vascular structures is reduced to a level below the detection limit of OCT-A in diseased eyes while clearly detectable in normal eyes, it is possible that lower vessel density may be a surrogate indicator of decreased blood flow in the diseased eyes. Comparison of validated measures of blood flow such as Doppler OCT with OCT-A vessel density in longitudinal studies may help answer this important question.

Although there is general consensus that ocular blood flow is reduced in glaucoma, studies investigating this issue have been hampered by the lack of a reproducible method to measure aspects of ocular circulation. Optical coherence tomography is a widely available tool for structural assessment in glaucoma management that is now used extensively in standard clinical care. Using OCT-A technology for ocular hemodynamic evaluations not only offers the advantage of providing a quantitative assessment of ocular circulation at a level of precision that has not been achieved with previous instruments that measured blood flow but also its feasibility from a clinical standpoint suggests that OCT-A may be a useful modality that may reflect hemodynamic considerations relevant to glaucoma management. Moreover, the device’s ability to visualize the vascular networks in easily interpretable images and density maps (Fig 1) may provide new clinically relevant information that can easily be incorporated into the routine management of patients with glaucoma.

The current OCT-A device detects vessels on the basis of amplitude decorrelation, which results from blood flow; however, it does not directly quantify flow within these vessels. In fact, decorrelation is linearly related to blood flow only over a limited range above a certain threshold of motion. In other words, vessels with very slow or absent flow below the detection threshold of the instrument will not be detected, and for the vessels detected, the current instrument does not differentiate a vessel with faster flow from one that has slower flow. For these reasons, the term “vessel density” is used as a quantitative summary measure of the vascular structures detected that reflects the proportion of area occupied by flowing vessels. Another known limitation of OCT-A technology is projection artifacts, which result from ghost images of anterior vessels projecting posteriorly. However, projection artifacts do not affect the measurements in the current study because microvasculature within the RNFL is the most anterior retinal vasculature and therefore is not affected by projection artifact. Although results of the present study and previous reports using OCT-A suggest lower vessel density in glaucoma and glaucoma suspect eyes may be relevant to the pathophysiology of the disease, the concept of vessel density is not well understood. In an earlier study with a similar technology, OCT microangiography, Zhi et al documented disappearance of signal from the peripapillary microvasculature in rats. However, there was persistence of some signal in large vessels in this region despite high IOP (100 mmHg). Comparison of in vivo assessment of OCT-A vessel density with histopathologic studies is needed to clarify what vessel density is measuring.

Study Limitations

This study has several other limitations. We used visual field indices to reflect the severity of glaucoma. However, other nonglaucomatous factors, such as refractive error and lenticular and media opacities, also may contribute to the visual field MD, PSD, and mean sensitivity. Moreover, our study population included few patients with advanced glaucoma. Recent reports suggest that standard structural measures such as RNFL thickness reach a floor effect and that visual field tests are highly variable in eyes with advanced glaucoma.11,30–42 It is important to evaluate whether OCT-A has a sufficient dynamic range to provide clinically relevant information across the full spectrum of glaucoma severity. In addition, we did not evaluate the possible confounding impact of various systemic conditions, BP and perfusion pressure, glaucoma eye drops, and systemic medications on vessel density and its relationship to standard structural and functional measures. It also should be noted that the cross-sectional design of this study limits the determination of the temporal relationship between OCT-A vessel density loss and glaucomatous structural and functional damage. Longitudinal studies are necessary to evaluate the topographic and temporal relationship between changes in OCT-A vessel density and glaucomatous changes in standard structural and functional measures in healthy participants, glaucoma suspects, and those with glaucoma.

In conclusion, OCT-A vessel density measurements are significantly associated with the severity of visual field damage. These associations are generally stronger than standard structural measures such as RNFL and rim area. Moreover, OCT-A vessel density measurements are still significantly associated with the severity of visual field loss even after adjusting for standard structural measurements. For these reasons, OCT-A is a promising technology that will allow clinical monitoring of vascular changes in glaucoma, and it could potentially enhance our understanding of the pathophysiology of the disease, specifically its underlying vascular mechanism.

References


Footnotes and Financial Disclosures

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Overall responsibility: Yarmohammadi, Zangwill, Diniz-Filho, Suh, Saunders, Medeiros, Weinreb

Abbreviations and Acronyms:
AIC = Akaike’s information criterion; ANOVA = analysis of variance; BP = blood pressure; CCT = central corneal thickness; cpVD = circumpapillary vessel density; dB = decibels; IOP = intraocular pressure; MAP = mean arterial pressure; MD = mean deviation; MOPP = mean ocular perfusion pressure; OCT = optical coherence tomography; OCT-A = optical coherence tomography angiography; ONH = optic nerve head; PSD = pattern standard deviation; RNFL = retinal nerve fiber layer; SAP = standard automated perimetry; SD OCT = spectral-domain optical coherence tomography; wiVD = whole-image vessel density.

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Pictures & Perspectives

Atypical Presentation of IgG4-Related Disease of the Eyelid

A 37-year-old HIV-positive woman presented with a 1-month history of progressive, painless, full-thickness destruction of the left upper, lower, and medial canthal eyelid with madarosis and symblepharon (Fig 1A). Incisional biopsy revealed a dense polytypic plasma cell infiltrate (Fig 1B), fibrosis, and phlebitis. Immunohistochemistry showed >100 IgG4 plasma cells per high-power field (Fig 1C) and a ratio of IgG4:IgG (Fig 1D) of >50% consistent with IgG4-related disease.

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