Projection-Resolved Optical Coherence Tomography Angiography of the Peripapillary Retina in Glaucoma

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• PURPOSE: To detect plexus-specific peripapillary retinal perfusion defects in glaucoma, using projection-resolved optical coherence tomography angiography (PR-OCTA).

• DESIGN: Prospective cross-sectional study.

• METHODS: One eye each of 45 perimetric glaucoma participants and 37 age-matched normal participants were scanned using 4.5-mm OCTA scans centered on the disc. The PR-OCTA algorithm removed flow projection artifacts in OCT angiograms. Five en face OCTA slabs were analyzed: nerve fiber layer plexus (NFLP), ganglion cell layer plexus (GCLP), superficial vascular complex (SVC [NFLP + GCLP]), deep vascular complex (DVC), and all plexi combined. Peripapillary retinal capillary density (CD) and vessel density (VD) were calculated using a reflectance-compensated algorithm.

• RESULTS: Focal capillary dropout could be visualized more clearly in the NFLP than in the other slabs. The NFLP, SVC, and all-plexus CD in the glaucoma group were significantly lower ($P < 0.001$) than in the normal group, but no significant differences in GCLP-CD and DVC-CD appeared between the 2 groups. Both NFLP-CD and SVC-CD had excellent diagnostic accuracy, as measured by the area under the receiver operating characteristic curve (AROC = 0.981 and 0.976), correlation with visual field mean deviation (Pearson r = 0.819 and 0.831), and repeatability (intraclass correlation coefficients = 0.947 and 0.942). Performances of NFLP-VD and SVC-VD were similar to the corresponding CD parameters.

• CONCLUSIONS: In this glaucoma group, reduction in perfusion was more pronounced in superficial layers of the peripapillary retina (NFLP and SVC) than in the deeper layers. Reflectance-compensated CD and VD parameters for both NFLP and SVC could be useful in the clinical management of glaucoma. (Am J Ophthalmol 2019;207:99–109. © 2019 Elsevier Inc. All rights reserved.)

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GLAUCOMA IS THE SECOND LEADING CAUSE OF blindness in the world and a major source of morbidity and disability in the United States.[1–4] Optical coherence tomography (OCT) of the optic disc,[5,6] peripapillary retinal nerve fiber layer (NFL),[6–8] and macular ganglion cell structures[8–12] has provided precise objective measurements for the diagnosis and monitoring of glaucoma. However, structural OCT has significant limitations. Structural OCT cannot detect the earliest stages of glaucoma, when ganglion cells may be dysfunctional but have not yet died and led to loss of tissue thickness. In the later stages, residual scar tissue provides a floor for the NFL and ganglion cell-related tissue thickness, thus limiting the usefulness of structural OCT in monitoring advanced glaucoma.[13–15] This limited dynamic range of structural changes also manifests as poor correlation between structural OCT and visual field (VF) parameters.[13,16,17]

Several years ago, this group introduced OCT angiography (OCTA) as a further refinement of OCT in glaucoma evaluation.[18–21] Glaucoma has been shown to reduce vessel density (VD) in the optic disc,[20] the peripapillary retina,[21–24] and the macular retina.[25,26] OCTA VD parameters show less floor effect and better correlation with VF parameters.[14,21] These early encouraging results showed that OCTA has the potential to complement VF and structural OCT in the assessment of glaucoma damage.

OCTA is a rapidly advancing technology, and its optimal use in glaucoma management is still being explored. Several recent investigators have focused on the peripapillary NFL plexus (NFLP), also known as the radial peripapillary capillaries. However, it is not clear how glaucoma damages deeper vascular plexi at the peripapillary retina. One technical difficulty that prevented plexus-specific analysis of the retinal circulation is that the flow projection artifact[12,27] projects superficial vascular patterns onto the deeper layers, making it impossible to cleanly quantify the vessel density in deeper vascular plexi. The present authors developed a projection-resolved OCTA (PR-OCTA) algorithm to effectively remove the projection artifact and provide clean angiograms of deeper vascular plexi.[12,28,29] Recently, the PR-OCTA algorithm was used to show the superficial vascular complex (SVC) to be the best slab for detecting

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glaucomatous changes in the macular region.[25] This study examined the performance of the PR-OCTA algorithm in the peripapillary region to answer the question of whether the NFLP may be the peripapillary retinal plexus of choice in glaucoma evaluation. In addition, the question of whether capillary density (CD), which is VD minus the larger retinal vessels, provides advantages over VD in either diagnostic accuracy or measurement precision, was also investigated.

METHODS

• **STUDY POPULATION:** This prospective observational study was performed from March 6, 2015, to May 31, 2017, at the Casey Eye Institute, Oregon Health and Science University (Longitudinal Observational Study Using Functional and Structural Optical Coherence Tomography to Diagnose and Guide Treatment of Glaucoma [FSOCT]; NCT01957267). The research protocols were approved by the Institutional Review Board at OHSU and carried out in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from each participant.

All participants were part of the Functional and Structural Optical Coherence Tomography for Glaucoma study. The inclusion criteria for the perimetric glaucoma group were 1) an optic disc rim defect (thinning or notching) or NFL defect visible on slit-lamp biomicroscopy; and 2) a consistent glaucomatous pattern on both qualifying Humphrey Swedish Interactive Thresholding Algorithm (SITA) 24-2 VF (Zeiss Meditec, Dublin, California), meeting at least 1 of the following criteria: pattern standard deviation outside normal limits ($P < 0.05$) and glaucoma hemifield test results outside normal limits.

For the normal group, the inclusion criteria were: 1) no evidence of retinal pathology or glaucoma; 2) a normal Humphrey 24-2 visual field; 3) intraocular pressure <21 mm Hg; 4) central corneal pachymetry >500 μm; 5) no chronic ocular or systemic corticosteroid use; 6) an open angle on gonioscopy examination; 7) a normal appearing optic nerve head and NFL; and 8) symmetrical optic nerve head between left and right eyes.

The exclusion criteria for both groups were: 1) best-corrected visual acuity less than 20/40; (2) age <30 or >80 years old; 3) refractive error of > +3.00 D or <−7.00 D; 4) previous intraocular surgery except for an uncomplicated cataract extraction with posterior chamber intraocular lens implantation; 5) any diseases that may cause VF loss or optic disc abnormalities; or 6) inability to perform reliably on automated VF testing. One eye from each participant was scanned and analyzed. For normal eyes, the eye was randomly selected. For the glaucoma group, the eye with the worse VF was selected.

• **VISUAL FIELD TESTING:** VF tests were performed using the Humphrey field analyzer II set for the 24-2 threshold test, size III white stimulus, using the SITA standard algorithm.

• **OPTICAL COHERENCE TOMOGRAPHY:** A 70-kHz, 840-nm wavelength spectral-domain OCT system (Avanti RTVue-XR, Optovue Inc., Fremont, California) was used.

• **IMAGE ACQUISITION AND PROCESSING:** The peripapillary retinal region was scanned using a 4.5- × 4.5-mm volumetric angiography scan centered on fixation. Each volume consisted of 304 line-scan locations at which 2 consecutive B-scans were obtained. Each B-scan contained 304 A-scans. The AngioVue software used the split-spectrum amplitude-decorrelation angiography algorithm, which compared the consecutive B-scans at the same location to detect flow using motion contrast.[18] Each scan set consisted of 2 volumetric scans: 1 vertical priority raster and 1 horizontal priority raster. The AngioVue software used an orthogonal registration algorithm to register the 2 raster volumes to produce a merged 3-dimensional (3D) OCT angiogram.[30] Two sets of scans were performed during 1 visit. The OCTA parameters from the 2 scans were then averaged for further analysis.

The merged volumetric angiograms were then exported for customized processing using Center for Ophthalmic Optics and Lasers-Angiography Reading Toolkit software.[31] This software removed flow projection artifacts and calculated reflectance-compensated CD. The OCTA scans contained both volumetric flow (decorrelation) data and structural (reflectance) data. The PR-OCTA algorithm retained flow signal from real blood vessels while suppressing projected flow signal in deeper layers, which appeared as downward tails on cross-sectional angiograms and duplicated vascular patterns on en face angiograms.[12,28,32] PR-OCTA visualized up to 4 retinal plexi: the neve fiber layer plexus (NFLP), the ganglion cell layer plexus (GCLP), the intermediate capillary plexus (ICP), and the deep capillary plexus (DCP).[28,33–36] In the peripapillary retina, as the ICP and DCP were often very thin, they were combined to form a single measurement, the deep vascular complex (DVC). The PR-OCTA volume was segmented into NFLP, GCLP, SVC (NFLP + GCLP), DVC (ICP + DCP), and all-plexus retina slabs (Figure 1) for VD and CD measurements.[28] Segmentation of the retinal layers was performed by automated MATLAB software (MathWorks, Natick, Massachusetts) that operates on the structural OCT data. Further manual correction of the segmentation was conducted if required. An en face angiogram of each slab was obtained by maximum flow (decorrelation value) projection. The VD, defined as the percentage of area occupied by the large vessels and microvasculature, was evaluated in the 4- × 4-mm-scan area excluding the central 2-mm-diameter circle, which was manually centered on the optic disc based on

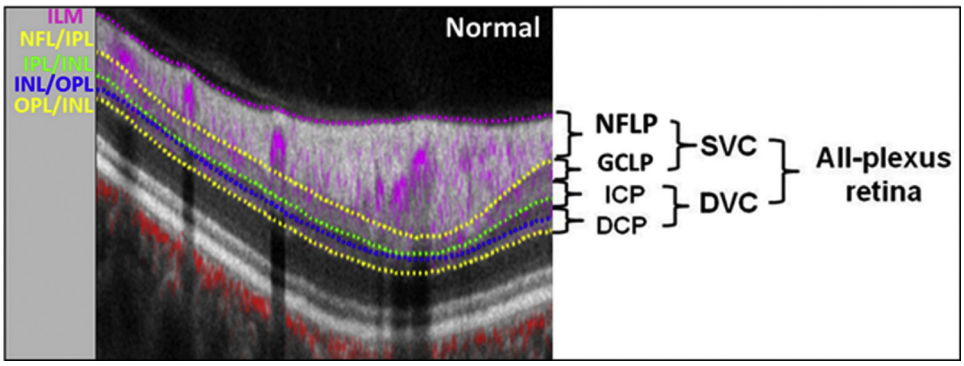

FIGURE 1. Relationship between the retinal vascular plexi and anatomic layers. Cross-sectional projection-resolved optical coherence tomography angiograms of a normal eye. The 4.5-mm section was taken 1 mm superior to the center of the disc. Flow signals showing retina (purple) and choroidal blood flow (red) were overlaid on reflectance signal (gray scale). DCP = deep capillary plexus (outer 50% of INL +OPL); DVC = deep vascular complex (ICP + DCP); GCL = ganglion cell layer; GCLP = ganglion cell layer plexus (inner 80% of GCC excluding NFLP); ICP = intermediate capillary plexus (outer 20% of GCC + inner 50% of INL); ILM = inner limiting membrane; INL = inner nuclear layer; IPL = inner plexiform layer; NFL = nerve fiber layer; NFLP = nerve fiber layer plexus; ONL = outer nuclear layer; OPL = outer plexiform layer; SVC = superficial vascular complex (inner 80% of GCC).

the enface reflectance image. Arterioles and venules (larger vessels) were automatically identified by thresholding the en face mean projection of OCT reflectance within the all-plexus slab. After these larger vessels were excluded, the remaining angiogram was used to compute CD.

Because OCTA measurements were found to be strongly correlated with signal strength index in previous studies, a reflectance-adjustment method was developed that corrected the artifactually lower flow signal in regions of reduced reflectance (eg, due to medium opacity or pupil vignetting).[37] The method is based on statistical analysis of the relationship between the flow noise in the foveal avascular zone, with reflectance in retinal tissue, in which the reflectance was manipulated by simulated medium opacity (optical filters). In the extrafoveal retina, the average reflectance in the inner nuclear, outer plexiform, and outer nuclear layers was used to adjust the threshold of the flow signal value to classify vessel versus static tissue on en face OCTA. Clinical validation showed that this algorithm was able to remove the dependence of retinal OCTA measurements on the signal strength index and reduce population variation.[37]

Image quality was assessed for all OCTA scans. Poor quality scans with signal strength index (SSI) below 50 or registered image sets with residual motion artifacts (discontinuous vessel patterns) were excluded from analysis. Two image sets, each meeting the quality criteria, were required in order to provide data for an assessment of within-visit repeatability. These sets were then averaged for further analysis. Within-visit repeatability was assessed by using the pooled standard deviation (SD), the intraclass correlation coefficient (ICC). Population variation was assessed by SD.

• **STRUCTURAL OCT ANALYSIS:** The global average NFL thickness was measured from a 3.45-mm-diameter circular scan centered on the optic disc. The result for each participant was the average value of 2 sets of images obtained during 1 visit.

• **STATISTICAL ANALYSIS:** The Student t test was used to compare normal and glaucoma groups. Pearson correlation was used to investigate the effects of age, mean ocular perfusion pressure (MOPP), and intraocular pressure (IOP) on CD measurements: MOPP = 2/3 × MAP − IOP, where mean arterial pressure (MAP) = 2/3 × diastolic blood pressure + 1/3 × systolic blood pressure. Pearson correlation was also used to determine the relationships between peripapillary retinal CD and the conventional glaucoma measurements of function and structure, such as the VF mean deviation and circumpapillary retinal nerve fiber layer thickness. The correlation coefficients were also compared.[38] A generalized linear model was used to evaluate the effect of eye drops and systemic medications on the OCTA parameters. A multivariate analysis was used to demonstrate which OCT and OCTA parameters dominated the correlation with VF. The area under the receiver operating characteristic curve (AROC), sensitivity and specificity, were used to evaluate diagnostic accuracy. The estimated sensitivities for fixed specificities were calculated by using MedCalc software (Ostend, Belgium), using the method of Zhou and associates.[39] The McNemar test and the method of Delong and associates[40] were used to compare sensitivity and AROC of different parameters. All statistical analyses were performed using SPSS version 20.0 software (Chicago, Illinois) and MedCalc version 10.1.3.0 software. The statistical significance was assumed at a P level of

TABLE 1. Participants' Characteristics

Parameter	Normal	Glaucoma	Mean Difference (95% CI, P value)
n Participants	34	41	
n Eyes	34	41	
Age (y)	65.3 ± 8.9	64.9 ± 9.3	−0.4 (−4.8 to 4.0, 0.853)
Glaucoma drops	0 (0%)	1.9 ± 0.8,41 (100%)	
Intraocular pressure (mm Hg)	14.9 ± 3.6	15.1 ± 2.7	0.2 (−1.2 to 1.7, 0.767)
Axial length (mm)	23.9 ± 1.2	24.3 ± 1.1	0.4 (−0.1 to 1.0, 0.830)
Diastolic blood pressure (mm Hg)	76.1 ± 16.0	77.4 ± 10.9	1.3 (−5.4 to 7.9, 0.702)
Systolic blood pressure (mm Hg)	121.9 ± 25.2	126.2 ± 15.9	4.3 (−6.1 to 14.5, 0.415)
Mean ocular perfusion Pressure (mm Hg)	46.1 ± 10.2	47.3 ± 6.9	1.3 (−3.0 to 5.4, 0.562)
Visual field			
MD (dB)	0.06 ± 1.16 (−3.22 to 1.87)	−4.77 ± 3.70 (−12.56 to −0.12)	−4.83 (−6.3 to −3.7, <0.001)
PSD (dB)	1.42 ± 0.25 (1.02 to 2.17)	6.20± 3.90 (1.51 to 14.24)	4.78 (3.7 to 6.4, <0.001)
Structural OCT thickness Measurement			
NFL (μm)	97.9 ± 7.0 (85-111)	77.5 ± 11.8 (57-100)	−20.4 (−25.0 to −15.8, <0.001)
OCT angiography measurements (% area)			
Signal Strength Index	69 ± 8 (54-89)	64 ± 8 (50-84)	−5 (−9 to −1, <0.001)
NFLP-CD	69.4 ± 4.8 (59.9-79.6)	48.5 ± 12.9 (19.1-76.1)	−20.9 (−25.2 to −16.2, <0.001)
GCLP-CD	49.4 ± 5.6 (39.5-62.1)	48.5 ± 5.5 (37.5-60.1)	−0.9 (−3.5 to 1.7, 0.477)
SVC-CD	77.4 ± 4.3 (69.7-86.3)	62.4 ± 9.9 (37.4-85.5)	−15.0 (−18.6 to −11.5, <0.001)
DVC-CD	48.4 ± 9.3 (29.4-65.1)	48.5 ± 9.8 (29.6-66.8)	0.1 (−4.3 to 4.4, 0.977)
All-Plexus-CD	89.3 ± 4.0 (83.0-95.8)	79.4 ± 7.4 (58.7-96.6)	−9.9 (−12.6 to −6.9, <0.001)
NFLP-VD	75.7 ± 4.2 (67.0-83.9)	57.6 ± 11.7 (33.0-82.1)	−18.1 (−22.4 to −13.9, <0.001)
SVC-VD	82.5 ± 3.7 (75.8-89.5)	70.2 ± 8.6 (49.2-89.8)	−12.3 (−15.5 to −9.2, <0.001)

Values are mean ± SD (range); n (%); or CI = confidence interval.
CD = capillary density; DVC = deep vascular complex; GCLP = ganglion cell layer plexus; NFL = nerve fiber layer; NFLP = nerve fiber layer plexus; OCT = optical coherence tomography; PSD = pattern standard deviation; SVC = superficial vascular complex; VD = vessel density.

<0.05. However, for multiple comparisons of OCT and OCTA parameters between normal and glaucoma groups, the Bonferroni correction was applied with resultant significance level set at a P level of <0.008.

RESULTS

• **STUDY POPULATION:** Peripapillary retinal perfusion was studied in 37 normal and 45 perimetric glaucoma participants. Three normal and 4 glaucoma participants were not analyzed due to poor OCTA scan quality, leaving 34 normal and 41 glaucoma participants for statistical analysis. In the glaucoma group, 29 participants had early glaucoma, 11 had moderate glaucoma, and 1 had advanced glaucoma, according to the Hodapp-Parrish-Anderson classification system.[41] All 41 glaucoma patients were using at least 1 of 4 classes of glaucoma drops (Table 1). Prostaglandin analogs were used in 33 patients; beta-blockers were used in 19 patients; alpha agonists were used in 7 patients; and carbonic anhydrase inhibitors were used in 19 patients. There were no significant differences in NFLP or SVC parameters associated with these eye drops. Among the 41 glaucoma patients, 14 patients were using aspirin, and 10 patients were using blood pressure medications. Among the 34 normal participants, 5 participants were using aspirin, and 8 participants were using blood pressure medications. There were no significant differences in NFLP or SVC parameters associated with these glaucoma and systemic medications. There were no statistically significant differences between the normal and glaucoma groups for age, IOP, axial length, MOPP, and systolic/diastolic blood pressures (Table 1).

• **QUALITATIVE ASSESSMENT OF FOCAL CAPILLARY DROPOUT:** In the normal eye (Figure 2), the en face PR-OCTA of the NFLP showed that the radial peripapillary CD was greater along the superotemporal and inferotemporal arcuate nerve fiber bundles. The SVC showed the same distribution pattern, but because the SVC contained both NFLP and GCLP, the CD in the SVC was higher than in the NFLP. A perimetric glaucoma eye was chosen to demonstrate the loss of retinal capillaries in the

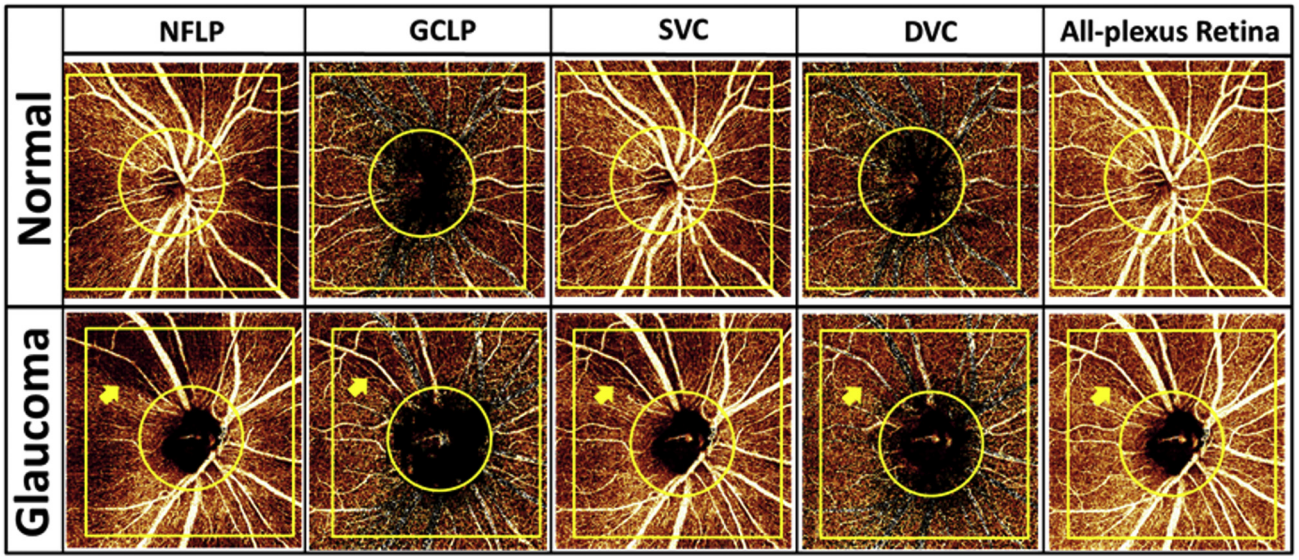

FIGURE 2. Comparison between a normal eye (top row) and a perimetric glaucoma eye (bottom row). The angiograms shown are 4.5- × 4.5-mm en face projection-resolved optical coherence tomography angiography. The focal capillary dropout in the glaucomatous eye (arrow) could be visualized more clearly in the nerve fiber layer plexus (NFLP) slab than on the ganglion cell layer plexus (GCLP), superficial vascular complex (SVC), and all-plexus retinal angiograms, whereas the deep vascular complex (DVC) appeared unaffected. The capillary density was calculated in the 4- × 4-mm yellow square excluding a 2-mm diameter circle centered on the optic disc.

superotemporal region in comparison with a normal eye (Figure 2). In the glaucomatous eye, severe capillary dropout in a wedge pattern could be visualized in the NFLP and SVC. The GCLP and all-plexus angiograms also showed decreased CD in the same region but to a lesser degree. No capillary dropout was found in the DVC (Figure 2). Overall, the NFLP slab provided the best contrast for visualizing the focal glaucomatous defect.

• **COMPARISON BETWEEN PR-OCTA AND NON-PR-OCTA:** En face non-PR-OCTA images of the deeper slabs (GCLP and DVC) contained projection artifacts from the most superficial slab (NFLP). These projected patterns could be recognized as large vessels and radial capillaries (Figure 3). The non-PR-OCTA DVC angiogram showed projected NFLP perfusion defects in the glaucoma eye (Figure 3). PR-OCTA successfully removed these projection artifacts.

Because the NFLP is the most superficial vascular slab, it does not contain any projection artifacts. Therefore, the non-PR-OCTA images had the same appearance as the PR-OCTA images for this slab.

• **QUANTITATIVE ASSESSMENT OF OCTA PARAMETERS:** The CD in the NFLP, SVC and all-plexus slabs of the glaucomatous eyes were lower than in the normal eyes (all $P < 0.001$). There were no significant differences between the GCLP-CD and DVC-CD in the glaucoma eyes and those in the normal groups (Table 1).

In the normal group, when both age and SSI are included in a multivariate linear regression analysis, the GCLP-CD, DVC-CD, and all-plexus-CD were correlated with SSI (scale of 100) and age (years). Higher CD in these slabs were correlated with stronger signal and younger age, as follows:

GCLP-CD = $-0.16\% \times$ age $+ 0.47\% \times$ SSI $+ 27.1\%$ ($R^2 = 0.63$; $P = 0.064$ for age and $P < 0.001$ for SSI), and DVC-CD = $-0.22\% \times$ age $+ 0.87\% \times$ SSI $+2.7\%$ ($R^2 = 0.78$; $P = 0.037$ for age and $P < 0.001$ for SSI), and all-plexus-CD = $-0.12\% \times$ age $+ 0.30\% \times$ SSI $+ 76.1\%$ ($R^2 = 0.57$; $P = 0.059$ for age and $P < 0.001$ for SSI).

The NFLP-CD and SVC-CD were not significantly correlated with age or SSI ($P > 0.13$). There was no correlation between MOPP or IOP with the CD in any slabs in the normal or glaucoma groups.

• **REPEATABILITY AND POPULATION VARIATION:** In both normal and glaucoma participants (Table 2), the NFLP-CD, SVC-CD, and all-plexus-CD had excellent within-visit repeatability as measured by pooled SD and ICC. They also had tight population variations of less than 5%. In comparison, the GCLP-CD and DVC-CD had worse ICC and wider population variation.

• **GLAUCOMA DIAGNOSTIC ACCURACY OF CD AND THICKNESS PARAMETERS:** The NFLP-CD had significantly higher sensitivity among the OCTA parameters (Table 3). Its sensitivity was significantly ($P = 0.016$) higher than all-plexus-CD but not significantly higher than NFL thickness ($P = 0.25$). The sensitivity of SVC-CD was close to that of NFLP-CD but not significantly higher than all-plexus-CD or NFL thickness ($P > 0.063$). The NFLP-CD and SVC-

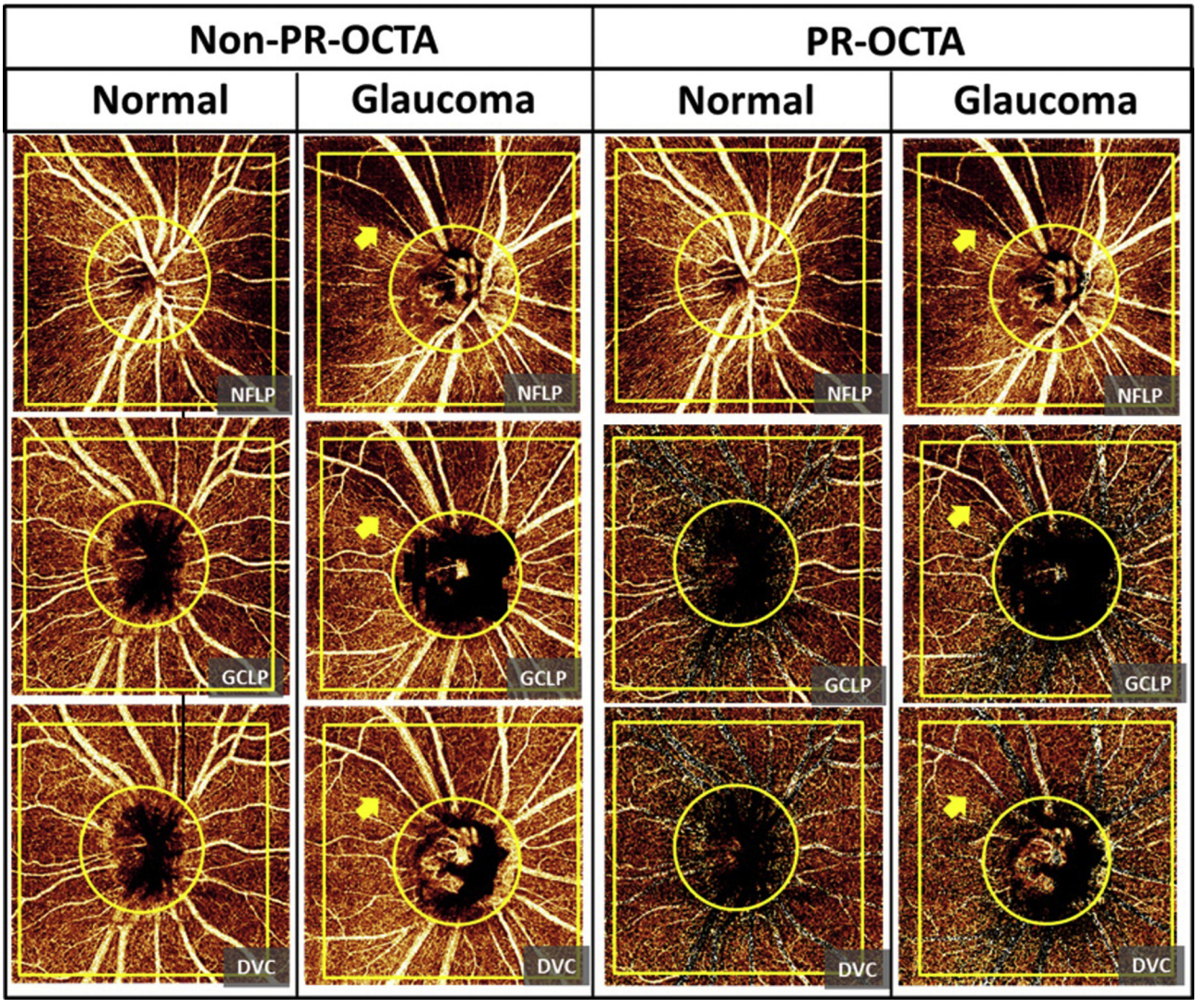

FIGURE 3. Comparison of the ganglion cell layer plexus (GCLP) and the deep vascular complex (DVC) angiograms between non projection-resolved optical coherence tomography angiography (non-PR-OCTA) and PR-OCTA from a normal eye and a perimetric glaucoma eye. The vessel pattern in the nerve fiber layer plexus (NFLP), large vessels and radial capillaries, were projected in the non-PR-OCTA GCLP and DVC angiograms in both normal and glaucoma eyes. PR-OCTA removed these projected patterns in the GCLP and DVC angiograms. The non-PR-OCTA DVC angiogram showed projected perfusion defects (arrow) in the glaucoma eye. The PR-OCTA showed that the DVC was unaffected by glaucoma.

CD AROCs did not differ significantly from those of the all-plexus-CD and NFL thickness AROCs ($P > 0.099$ for all pairwise comparisons). The GCLP-CD and DVC-CD had significantly worse sensitivity and AROC than the other parameters.

• **CORRELATION WITH TRADITIONAL GLAUCOMA DIAGNOSTIC MEASUREMENTS:** The NFLP-CD, SVC-CD and all-plexus-CD had excellent correlation with the NFL thickness and good correlation with the VF-MD (Table 4, Figure 4). These correlations were highly significant. The NFLP-CD had significantly ($P < 0.02$) higher linear correlation with VF-MD than the correlation between NFL thickness with VF-MD, suggesting the visual function is better correlated with perfusion than structure. In the multivariate analysis where the VF-MD was the dependent variable and the 2 independent variables were NFLP-CD and NFL thickness, NFLP-CD remain significant ($P < 0.0001$), whereas NFL thickness was no longer significant ($P = 0.92$).

• **COMPARISON OF CAPILLARY DENSITY AND VESSEL DENSITY IN OCTA MEASUREMENTS:** The 2 slabs with the highest diagnostic power, NFLP and SVC, were used to compare the capillary and vessel densities. The 4 OCTA measurements, NFLP-CD, NFLP-VD, SVC-CD, and

TABLE 2. Repeatability and Normal Population Variability of Peripapillary Retinal Optical Coherence Tomography Angiographic Parameters

Group	Normal (n = 34)		Glaucoma (n = 41)	All (N = 75)
Parameter	Within-Visit Repeatability (Pooled SD)	Population Variation (SD)	Within-Visit Repeatability (Pooled SD)	Intraclass Correlation Coefficient
NFLP-CD	2.0% area	4.8% area	2.2% area	0.981
GCLP-CD	2.0% area	5.7% area	2.1% area	0.880
SVC-CD	1.7% area	4.3% area	1.9% area	0.976
DVC-CD	3.2% area	8.9% area	3.2% area	0.889
All-plexus-CD	1.0% area	3.9% area	1.7% area	0.974

CD = capillary density; DVC = deep vascular complex; GCLP = ganglion cell layer plexus; NFLP = nerve fiber layer plexus; SD = standard deviation; SVC = superficial vascular complex.

TABLE 3. Diagnostic Accuracy of Peripapillary Retinal Optical Coherence Tomography Angiographic and Structural Parameters

Parameters	AROC	Sensitivity (95% confidence interval)[a]
NFLP-CD	0.947	90.2% (76.9%-97.2%)
GCLP-CD	0.549	9.8% (2.8%-23.1%)
SVC-CD	0.942	85.3% (70.8%-94.4%)
DVC-CD	0.509	4.9% (0.7%-16.6%)
All-Plexus-CD	0.893	73.2% (57.1%-85.8%)
NFL thickness	0.918	82.9% (67.9%-92.8%)

AROC = area under the receiver operating characteristic curve; CD = capillary density; NFL = nerve fiber layer; NFLP = nerve fiber layer plexus; DVC = deep vascular complex; GCLP = ganglion cell layer plexus; SVC = superficial vascular complex.

[a]Sensitivity levels at 95% specificity were evaluated.

SVC-VD, had excellent within-visit repeatability as measured by the pooled SD and ICC in both the normal and the glaucoma groups (Table 5). All 4 measurements had tight population variations, high correlation with VF-MD, and high diagnostic power as measured by AROC. The CD parameters had slightly better AROC than the VD parameters, and the NFLP parameters had slightly better AROC than the SVC parameters, but the differences were not statistically significant.

• **GANGLION CELL LAYER PLEXUS IN MORE ADVANCED GLAUCOMA:** A surprise finding was that GCLP-CD was not significantly lower in the glaucoma group than in the normal group. This might have been because the glaucoma group consisted mostly of patients with mild glaucoma. Therefore, the hypothesis that GCLP-CD was reduced in the combined moderate (11 participants) and advanced (1 participant) glaucoma subgroup was tested further. The GCLP-CD was 46.5% ± 5.6% in the moderate/advanced glaucoma subgroup (12 participants) and 49.4% ± 5.6% in the normal group (34 participants). The difference was of borderline significance ($P = 0.064$, 1-tailed *t*-test).

DISCUSSION

STRUCTURAL OCT EVALUATION OF GLAUCOMA HAS focused on the tissue layers that are primarily affected by glaucoma, which include the NFL in the peripapillary region and the ganglion cell complex in the macula. The expectation is that OCTA evaluation should focus on the blood vessels supplying these layers in order to optimize glaucoma diagnosis and monitoring. However, it is wise to confirm this theoretical expectation with actual clinical data. An impediment to layer-by-layer analysis in OCT is the projection artifact,[12] which projects duplicate superficial blood vessels in deeper layers. Fortunately, it is possible to resolve the ambiguity between in situ and projected vessels in deeper layers in postprocessing by using the PR-OCTA algorithm.[12,28,29,42,43] In a recent publication, the present authors reported that, in the macula, glaucoma primarily affects the SVC and using the SVC slab, as opposed to the other deeper slabs, provides the best diagnostic accuracy and correlation with VF.[25] In the present study, the same technique was used to investigate the peripapillary region.

Based on previous findings in the macular region, it was anticipated that in the peripapillary region, both the NFLP and the GCLP would be affected by glaucoma. A surprising finding was that the CD was significantly reduced only in the NFLP and not in the GCLP. Because it is known that glaucoma causes loss of ganglion cells and that the GCLP supplies the ganglion cell bodies,[33,34] the disease should eventually affect the GCLP not only in the macula but in the peripapillary region as well. Moreover, a recent study showed the progressive macular ganglion cell-inner plexiform layer thinning was detected most frequently in the inferotemporal region and then extended toward the fovea and optic disc.[44] Thus, the most likely explanation for the present finding is that the ganglion cell layer in the peripapillary region is affected primarily in the later stages of glaucoma. This explanation is also supported by the fact that the moderate-to-advanced glaucoma subgroup had lower GCLP-CD that was almost statistically significant; a larger sample of advanced glaucoma subjects is needed to test this hypothesis. Combining NFLP and GCLP into the SVC provided similar diagnostic accuracy and VF correlation as NFLP alone, and as the GCLP was often very thin, combining the 2 seemed more practical and served well for the analysis of glaucoma parameters in the peripapillary region. Therefore, both the NFLP and the SVC are

TABLE 4. Correlation Matrix of Visual Field, Peripapillary Retinal Optical Coherence Tomography Angiographic and Structural Parameters

Parameters	NFLP-CD	GCLP-CD	SVC-CD	DVC-CD	All-plexus CD	NFL Thickness
GCLP-CD	0.218 (0.060)					
SVC-CD	**0.979 (0.000)**	**0.404 (0.000)**				
DVC-CD	−0.088 (0.455)	**0.754 (0.000)**	0.011 (0.924)			
All-plexus-CD	**0.853 (0.000)**	**0.609 (0.000)**	**0.910 (0.000)**	**0.397 (0.000)**		
NFL Thickness	**0.926 (0.000)**	**0.285 (0.012)**	**0.914 (0.000)**	0.020 (0.863)	**0.837 (0.000)**	
VF-MD	**0.819 (0.000)**	**0.275 (0.017)**	**0.831 (0.000)**	−0.029 (0.808)	**0.750 (0.000)**	**0.759 (0.000)**

Pearson's r (*P* value); statistically significant correlation ($P < 0.05$) are in bold type.

CD = capillary density; DVC = deep vascular complex; GCLP = ganglion cell layer plexus; NFL = nerve fiber layer; NFLP = nerve fiber layer plexus; SVC = superficial vascular complex; VF-MD = visual field mean deviation.

good choices for evaluating glaucoma in the peripapillary region.

By using the compensation algorithm, NFLP-CD and SVC-CD were independent of SSI, but GCLP-CD, DVC-CD, and all-plexus-CD were still positively correlated to SSI. Fortunately, the GCLP, DVC, and all-plexus slabs were not affected by glaucoma, so this limitation did not impact the glaucoma evaluation. Previous studies have shown the uncompensated VD or CD were correlated with SSI.[25,37,45] Because the glaucoma group tended to have lower SSI, the compensation was necessary. Moreover, the age attenuation effect on the CD cannot be ruled out even if there were no significant changes. With the standard deviation of the slope between age and CD, the sample size was too small to detect any significantly slow change.

The DVC, which included the ICP and DCP, was found to be minimally affected by glaucoma, which is expected given that the DVC supplies the middle retinal layers that do not include the retinal ganglion cells. These results are in agreement with PR-OCTA results in the macula, which also showed no DVC loss in glaucoma.[25]

Because the DVC is minimally affected by glaucoma, selectively measuring the SVC or NFLP slabs may be better than measuring all retinal plexi. The present results support this. SVC-CD and NFLP-CD offered better diagnostic accuracy and correlation with VF-MD than all-plexus CD. Furthermore, en face OCTA of the NFLP slab allowed the best visualization of focal glaucomatous defects. Therefore, the authors conclude that, for glaucoma evaluation, the SVC or NFLP slabs should be used when analyzing OCTA; the deeper plexi can be excluded. Because the NFLP and the SVC are the most superficial slabs, there are no projection artifacts in them, unlike the deeper ICP and DCP. Therefore, both PR-OCTA and non-PR-OCTA provide equivalent results for NFLP-CD and SVC-CD. Thus, although PR-OCTA was useful in determining which retinal plexi are affected by glaucoma, both PR-OCTA and non-PR-OCTA work well for evaluation of clinical glaucoma.

The OCTA quantitative retinal perfusion study findings agreed with those of previous case reports on the effects of retinal circulation in glaucoma. In 1968, Kornzweig and associates[46] found a postmortem eye with chronic glaucoma showing selective atrophy of the radial peripapillary capillaries (RPC), but the deeper capillaries appeared normal. Recently, by using speckle variance OCTA, Mammo and associates[47] found the density and morphologic characteristics of deeper capillary networks at the sites of RPC loss appeared normal in 1 glaucomatous eye.

The NFLP was conventionally known as the radial peripapillary capillaries (RPC). These authors are adopting the new term NFLP because wide-field OCT showed that the capillaries suppling the NFL are not limited to the immediate peripapillary region but extend past the macula along the arcuate nerve fiber bundles.[48] Along these bundles, the orientation of the capillaries is parallel to the nerve fibers, which are arcuate rather than radial. Therefore the conventional term RPC is only appropriate for the small circumferential portion of the NFLP surrounding the optic disc. The new term NFLP is, thus, more appropriate.

Further refinement of the VD measurement, which includes capillaries and the larger vessels compared to a measurement of the capillaries alone, may improve its performance. Our results showed that CD offered slightly better diagnostic accuracy than VD, but both of them had excellent performance. The comparisons in this paper were based on overall global averages of the peripapillary retinal region. The next step in the development of OCTA analysis will be to divide the peripapillary region into sectors which will provide more specific information based on the peripapillary location of glaucoma damage. Theoretically, CD would have an advantage in sectoral analysis because it removes the noise introduced by variations in the pattern of large retinal vessels. Combining these empirical and theoretical considerations, these authors believe CD may be better than VD for evaluating glaucoma. However, in their current forms, both parameters offer similar information.

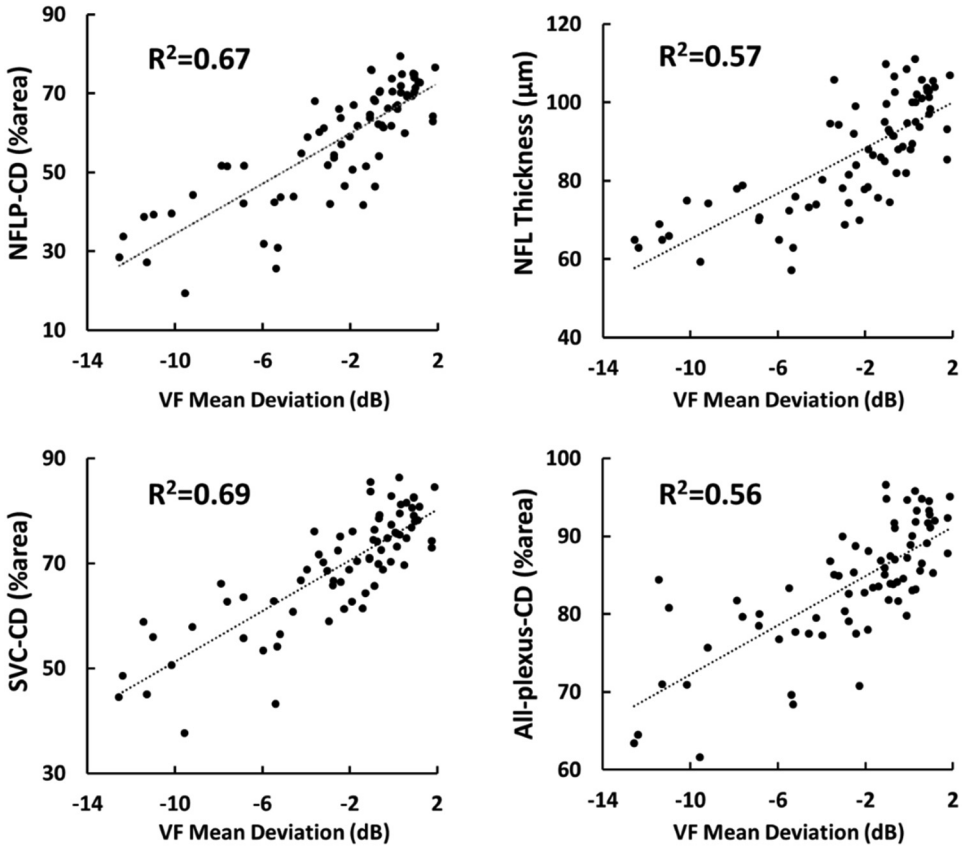

FIGURE 4. Scatterplots illustrating the linear correlation between visual field (VF) mean deviation and peripapillary retinal measurements by structural optical coherence tomography (OCT) and OCT angiography. CD = capillary density; NFLP = nerve fiber layer plexus; NFL = nerve fiber layer; SVC = superficial vascular complex.

TABLE 5. Comparison of the Performance of Capillary Density and Vessel Density in Nerve Fiber Layer Plexus and Superficial Vascular Complex Slabs

Group	Normal (N = 34)		Glaucoma (N = 41)	ALL (N = 75)		
Parameters	Within-Visit Repeatability (Pooled SD)	Population Variation (SD)	Within-Visit Repeatability (Pooled SD)	ICC	Correlation with VF-MD (dB)	AROC
NFLP-CD	2.0% area	4.8% area	2.2% area	0.981	0.819	0.947
NFLP-VD	1.9% area	4.2% area	2.3% area	0.977	0.828	0.934
SVC-CD	1.7% area	4.3% area	1.9% area	0.976	0.831	0.942
SVC-VD	1.6% area	3.7% area	1.8% area	0.970	0.830	0.919

AROC = area under the receiver operating characteristic curve; CD = capillary density; ICC = intraclass correlation coefficient; NFL = nerve fiber layer; NFLP = nerve fiber layer plexus; SVC = superficial vascular complex; VD = vessel density; VF-MD = visual field mean deviation.

Also, conventional structural measurements were compared with OCTA parameters in glaucoma evaluation. Both the NFLP-CD and the SVC-CD had significantly stronger linear correlations with VF-MD than that between NFL thickness and VF-MD (Figure 4). No significant differences were found on comparison of the diagnostic power among NFLP-CD, SVC-CD, and NFL thickness (Table 3). These findings were in agreement with previous studies. Yarmohammadi and associates[14] found peripapillary NFLP-VD had significantly stronger association with VF-MD than that between NFL thickness and VF-MD,[14] but OCTA parameters had similar diagnostic accuracy compared to NFL thickness for differentiating between healthy and glaucoma eyes.[24] In the present group's first OCTA study of the peripapillary retina, it was also shown that peripapillary retinal vessel density had better correlation with glaucoma severity than NFL thickness.[21] Overall OCTA perfusion parameters consistently showed superior

correlation with VF parameters and may be a better surrogate for VF function and a metric for monitoring glaucoma disease progression.

• **STUDY LIMITATIONS:** There are several limitations to this study. First, the relatively small sample size does not allow detection of small differences in diagnostic accuracy. Second, the cross-sectional nature of the study does not allow any direct conclusions regarding utility in monitoring or predicting glaucoma progression. Third, most of the glaucoma participants were at early to moderate stages of disease, with only 1 participant in the advanced category. Thus, conclusions could not be drawn as to which OCTA parameter might have had better correlation with VF in advanced glaucoma. Fourth, participants with preperimetric glaucoma and glaucoma suspects were not included. Thus, conclusions could not be made about the diagnostic accuracy in the earliest stages of glaucoma. Fifth, this study was not designed to contrast the potential differences between different types of glaucoma or the potential effects of medications. Results suggest that the effects of both glaucoma eye drops and systemic medications are too small to be detected with the present sample size and study design.

CONCLUSIONS

THIS STUDY SHOWED THAT EVALUATION OF THE PERIPAPILlary retina using OCTA could measure CD and VD with tight population variation and high repeatability in normal and glaucoma participants. The NFLP slab provided the best diagnostic accuracy and the best contrast for visualizing focal peripapillary perfusion defects, whereas the SVC slab provided the best correlation with VF. Further evaluation of OCTA technology for glaucoma evaluation is best served by focusing on these 2 slabs rather than on deeper slabs or overall retinal circulation. The authors again demonstrated that perfusion parameters outperformed structural parameters in terms of correlation with VF and, therefore, may possibly serve as a better surrogate for function and a better metric for monitoring glaucoma progression.

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