# Effect of algorithms and covariates in glaucoma diagnosis with optical coherence tomography angiography

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## ABSTRACT

**Purpose** To assess the effects of algorithms and covariates in glaucoma diagnosis with optical coherence tomography angiography (OCTA).

**Methods** In this prospective cross-sectional study, one eye each of 36 normal controls and 64 patients with glaucoma underwent 4.5 mm disc-centred and 6 mm macula-centred OCTA scans. The peripapillary nerve fibre layer plexus capillary density (NFLP-CD) and macular superficial vascular complex vessel density (SVC-VD) were measured using both a commercial algorithm (AngioAnalytics) and a custom algorithm (Center for Ophthalmic Optics & Lasers Angiography Reading Toolkit (COOL-ART)). The nerve fibre layer and ganglion cell complex thicknesses were measured on structural OCT.

**Results** The overall peripapillary NFLP-CD and macular SVC-VD measured with the two algorithms were highly correlated but poorly agreed. Among the normal controls, the perfusion measurements made by both algorithms were significantly correlated with age. AngioAnalytics measurements were also correlated with signal strength index, while COOL-ART measurements were not. These covariates were adjusted. The diagnostic accuracy, measured as the area under the receiver operating characteristic curve for glaucoma detection, was not significantly different between algorithms, between structural and perfusion parameters and between the peripapillary and macular regions (All p>0.05). The macular SVC-VD in the 6 mm square had a significantly higher diagnostic accuracy than that of the central 3 mm square area (p=0.005).

**Conclusions** AngioAnalytics and COOL-ART vessel density measurements are not interchangeable but potentially interconvertible. Age and signal strength are significant covariates that need to be considered. Both algorithms and both peripapillary and macular perfusion parameters have similarly good diagnostic accuracy comparable to structural OCT. A larger macular analytic area provides higher diagnostic accuracy.



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Glaucoma is the leading cause of irreversible blindness worldwide.[1 2] It is estimated to affect 76 million populations in 2020 and increase to 112 million in 2040.[2] The pathogenesis of glaucoma remains unclear. Although elevated intraocular pressure (IOP) is a known risk factor, 59%–92% of patients with glaucoma have normal tension,[3] suggesting that other risk factors exist. Vascular dysfunction has been suggested to play a role in glaucoma pathogenesis.[4 5] Thus, measurement of ocular circulation in glaucomatous eyes is of great interest.

Optical coherence tomography angiography (OCTA) is a novel noninvasive method for in vivo evaluation of retinal blood flow.[6] Previous studies demonstrated good repeatability and reproducibility of OCTA for measuring vessel density (VD) in the optic disc, peripapillary retina and macula.[7 8] Recent studies on glaucomatous eyes with OCTA have shown microcirculation changes in these regions, however, with different results.[9–15]

Our earliest pilot studies of OCTA in glaucoma showed good diagnostic accuracy using disc VD,[9] peripapillary retinal VD and peripapillary flow index.[10] These were followed by larger studies using projection-resolved OCTA. Takusagawa *et al* assessed VD in 30 perimetric glaucoma (PG) and 30 age-matched normal participants and demonstrated that glaucoma primarily affected VD in the superficial vascular complex (SVC) using 6 mm macular OCTA. Using SVC-VD in the worse hemisphere (superior or inferior) to differentiate glaucoma from healthy controls, the area under the receiver operating characteristic curve (AROC) was 0.983 with a sensitivity of 96.7% at a fixed specificity of 95%.[12] Liu reported, with 4.5 mm OCTA disc scans, a significant lower peripapillary retinal capillary density (CD, VD measured after excluding large vessels) in PG in nerve fibre layer plexus (NFLP, also known as radial peripapillary capillaries) and SVC, but not in ganglion cell layer plexus and deep vascular complex. Both NFLP-CD and SVC-CD had excellent diagnostic accuracy, with AROC of 0.981 and 0.976, respectively.[16]

Other investigators have also found good glaucoma diagnostic accuracy with OCTA. Using a customised algorithm, Geyman *et al* compared peripapillary NFLP-CD of 62 PG eyes with 24 controls on 4.5 mm disc OCTA scans. The AROC of overall NFLP-CD for differentiating PG from control is 0.900.[17] Using AngioVue AngioAnalytics (Optovue) software, Yarmohammadi *et al* reported an AROC of 0.94 using whole-image NFLP-VD from 4.5 mm disc scan for differentiating PG from healthy control.[13 18] These authors' work suggest that OCTA is a very useful tool with high diagnostic accuracy for clinical evaluation of glaucoma.

By contrast, other investigators found relatively poor diagnostic ability of OCTA. Rao *et al* assessed 78 eyes of 53 controls and 64 eyes of 39 patients with primary open-angle glaucoma using

AngioAnalytics. The AROC of optic disc VD was 0.59–0.73, of peripapillary NFLP-VD (without excluding large vessels) was 0.70–0.89, and that of 3 mm macular SVC-VD was 0.56–0.64.[18] Using a customised software, Wan *et al* also found a moderate diagnostic accuracy with AROC 0.54–0.83 using SVC-CD of macular 3 mm OCTA scans to differentiate glaucoma from control.[19]

The apparent discrepancy in the diagnostic performance of OCTA in the above-mentioned studies could be due to differences in the population characteristics, OCT hardware, OCTA scan patterns (region, size, density), scan quality and algorithms used to generate diagnostic parameters. The purpose of the current study was to analyse the effect of algorithms and clinical covariates to gain insight into how to optimise the use of OCTA in glaucoma evaluation and avoid pitfalls that could lead to false diagnosis.

## METHODS

The institutional review board of Oregon Health Science University approved this prospective cross-sectional study. The study complied with Helsinki declaration and the Health Insurance Portability and Accountability Act. Written informed consent was obtained from each participant.

All the participants were from the *Functional and Structural Optical Coherence Tomography for Glaucoma* study (NIH R01 EY023285-05). One eye of each participant was included. The inclusion and exclusion criteria have been reported previously.[12 16] In brief, PG was diagnosed if the following criteria were fulfilled: (1) an optic disc rim defect (thinning or notching) or NFL defect detected on slit-lamp biomicroscopy and (2) a consistent glaucomatous visual field (VF) loss on both qualifying VF tests. Glaucomatous VF loss was defined as pattern SD outside normal limits ($p<0.05$) or glaucoma hemifield test outside normal limits ($p<0.01$). Pre-PG (PPG) was defined as similar disc/NFL defects as in PG but without the consistent VF loss. For the normal group, the inclusion criteria were IOP <21 mm Hg; a normal appearing optic nerve head and NFL without evidence of retinal pathology; a normal Humphrey 24–2 VF and no chronic ocular or systemic corticosteroid use. The exclusion criteria for both glaucoma and control groups were (1) age <18 or >80 years, (2) previous intraocular surgery except for an uncomplicated cataract surgery, (3) any other diseases that may cause VF loss or optic disc abnormalities, (4) best-corrected visual acuity <20/40, (5) spherical equivalent refractive error >+3.00 diopters or <−6.00 diopters or (6) inability to perform reliable automated perimetry.

All the participants underwent comprehensive clinical examinations including systemic blood pressure, IOP measured with Goldman applanation tonometry, slit-lamp biomicroscopy, perimetry and OCTA. The VF test was performed using Humphrey Field Analyzer II (Carl Zeiss Meditec Dublin, California) set for the 24–2 threshold test, size III white stimulus, using the Swedish Interactive Thresholding Algorithm. The mean ocular perfusion pressure (MOPP) was calculated as 2/3 × (mean arterial pressure-IOP), where mean arterial pressure was calculated as diastolic blood pressure +1/3 × (systolic blood pressure–diastolic blood pressure).

The OCTA scans were acquired using a 70 KHz 840 nm wavelength spectral domain OCT system (RTVue-XR Avanti, Optovue, Fremont, California). The optic disc and macular regions were imaged with 4.5 mm and 6 mm OCTA scan patterns, respectively. Two raster scans, one vertical-priority and one horizontal-priority, were obtained in each region. The two orthogonal raster scans were registered and merged into an OCTA volume. Each OCT volume was composed of 400 locations at which two consecutive B-scans were performed. Each B-scan contains 400 A-lines. OCTA was calculated by commercial version of split-spectrum amplitude-decorrelation angiography algorithm.[6] Poor-quality scans with signal strength index (SSI) below 50 or scan quality index (Q) below 6 were excluded from the analysis. Human graders also inspected both the OCT and OCTA en face images and rejected those with significant residual motion artefacts, cropping and decentration.

The volumetric OCTA scans were processed with the commercial AngioAnalytics software (V.2018.0.0.18) and a custom software, the Center for Ophthalmic Optics & Lasers Angiography Reading Toolkit (COOL-ART), as described in detail previously.[12 16 20 21] For both algorithms VD was defined as percentage of measured area occupied by blood vessels.

For analysis of the peripapillary circulation, the central 2 mm diameter circle centred on the optic disc was excluded and the region was divided into eight sectors according to an extension[22] of the Garway-Heath scheme.[23] VD was measured in the NFLP, which is also known as the radial peripapillary capillaries. The NFLP-CD was also calculated. CD was simply the VD measured after excluding large vessels.

VD was measured in the SVC slab for macular scans. The SVC includes two components that are both affected by glaucoma—the NFLP and the ganglion cell layer plexus. The central 0.6 mm (COOL-ART) or 1.0 mm (AngioAnalytics) circle were excluded to avoid the impact of foveal avascular zone area. VD was averaged over the entire macular region as well as superior and inferior hemispheres divided by a horizontal line crossing the foveal centre. To analyse the potential effect of scan size in macular region on diagnostic accuracy, VD on central 3 mm and 4.5 mm square macular region was also calculated for COOL-ART measurement.

COOL-ART and AngioAnalytics differed in two steps: segmentation and VD calculation. AngioAnalytics segmented retinal layer boundaries and disc boundary automatically to generate the NFLP and SVC slabs. COOL-ART added a manual review step that the automatically segmented boundaries could be manually corrected where necessary by a certified grader. Both the COOL-ART and AngioAnalytics algorithms calculated

**Table 1** Demographic and clinical characteristics of study participants

| Parameters | Control (n=36) | PPG (n=30) | PG (n=34) | P value |
|---|---|---|---|---|
| Mean age (SD), years | 60 (11) | 65 (9) | 67 (9) | 0.01 |
| Sex female, n (%) | 28 (78%) | 18 (60%) | 14 (41%) | 0.008 |
| SBP (SD), mm Hg | 117 (13) | 127 (16) | 126 (19) | 0.02 |
| DBP (SD), mm Hg | 71 (9) | 76 (12) | 77 (14) | 0.07 |
| MABP (SD), mm Hg | 86 (9) | 93 (13) | 93 (13) | 0.02 |
| Disc scan SSI (SD) | 70 (7) | 66 (8) | 61 (7) | <0.001 |
| Macular scan SSI (SD) | 66 (6) | 64 (7) | 61 (7) | 0.01 |
| IOP (SD), mm Hg | 14.5 (2.6) | 14.1 (2.7) | 14.3 (3.4) | 0.86 |
| MOPP (SD), mm Hg | 47.6 (5.4) | 52.3 (8.1) | 52.5 (9.0) | 0.01 |
| Glaucoma eye drops (types ) | 0 (0) | 0.8 (1.0) | 2.0 (0.9) | <0.001 |
| MD (SD), DB | 0.2 (1.2) | -0.6 (1.9) | -6.0 (5.1) | <0.001 |
| PSD (SD), DB | 1.5 (0.3) | 1.8 (0.6) | 7.5 (4.5) | <0.001 |

DBP, diastolic blood pressure; IOP, intraocular pressure; MABP, mean arterial blood pressure; MD, mean deviation of visual field test; MOPP, mean ocular perfusion pressure; PG, perimetric glaucoma; PPG, pre-PPG; PSD, pattern SD of visual field test; SBP, systolic blood pressure.

**Table 2** Vessel density parameters measured with AngioAnalytics and COOL-ART algorithms

| | | AngioAnalytics (% area) | | | | COOL-ART (% area) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Glaucoma (n=64) | | Control (n=36) | | Glaucoma (n=64) | | Control (n=36) | |
| Locations | | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Peripapillary overall | | 43.3 | 7.9 | 51.3 | 2.6 | 44.2 | 15.8 | 62.9 | 7.5 |
| Peripapillary sectors | Nasal superior | 40.5 | 9.4 | 48.6 | 4.0 | 37.4 | 20.5 | 57.9 | 11.9 |
| | Nasal inferior | 39.3 | 8.7 | 47.5 | 4.1 | 32.7 | 17.0 | 49.2 | 11.7 |
| | Inferior nasal | 40.9 | 10.6 | 49.3 | 5.0 | 49.1 | 20.7 | 69.0 | 11.8 |
| | Inferior temporal | 43.6 | 14.3 | 56.8 | 4.0 | 54.6 | 30.1 | 84.0 | 8.8 |
| | Temporal inferior | 47.1 | 7.1 | 52.1 | 4.5 | 40.7 | 16.8 | 54.9 | 14.4 |
| | Temporal superior | 50.3 | 7.6 | 55.1 | 4.0 | 50.0 | 19.3 | 63.5 | 11.1 |
| | Superior temporal | 45.5 | 11.8 | 54.7 | 4.1 | 57.4 | 24.6 | 80.1 | 10.6 |
| | Superior nasal | 40.5 | 9.3 | 49.0 | 3.8 | 52.3 | 19.3 | 72.4 | 10.3 |
| Macula overall | | 42.3 | 5.5 | 48.3 | 2.9 | 44.8 | 9.3 | 55.9 | 5.9 |
| Macula superior hemisphere | | 42.8 | 5.6 | 48.4 | 3.1 | 46.1 | 10.2 | 56.0 | 5.8 |
| Macula inferior hemisphere | | 41.7 | 5.9 | 48.3 | 3.0 | 42.7 | 10.6 | 54.9 | 6.5 |

SD, population SD within group; vessel density in superficial vascular complex is shown for macular region and capillary density is shown for peripapillary region.
COOL-ART, Center for Ophthalmic Optics & Lasers Angiography Reading Toolkit.

VD using adaptive thresholding that adjusted the cut-off value separating flow and nonflow pixels on en face OCTA according to local image characteristic. For COOL-ART, the threshold was adjusted for local reflectance signal strength. This prevented media opacity or pupil vignetting from artifactually decreasing the measured VD.[20] The algorithm had been validated previously, showing the ability to remove the dependence of VD on SSI and to reduce the variation in measured VD in a normal population.[20] AngioAnalytics was a commercial algorithm and its inner workings were proprietary.

Statistical analysis was conducted using Statistical Package for Social Sciences software (SPSS for Windows, V.25.0; IBM SPSS, Chicago) and MedCalc V.10.1.3.0 (MedCalc Software, Ostend, Belgium, www.medcalc.be). The Shapiro-Wilk test was used to evaluate normality of distributions. Descriptive statistics included mean, SD, range and percentages were presented where appropriate. The Student t-test was used to compare normally distributed variables between normal and glaucoma groups. Pearson correlation was used to evaluate the correlations of the measurements between the two algorithms. Paired-sample t-test and Bland-Altman plots were used to evaluate the agreement of the VD measurements between the two algorithms. The AROC, sensitivity and specificity were calculated to evaluate diagnostic accuracy. Or-logic was used to combine results when a region is divided into sectors. In or-logic, the values of each sector parameter were transformed into the standardised deviate using the measured value minus the mean value of the normal group, and then divided by the SD of the normal group. For each eye, the sector with the lowest deviate value was used in the AROC calculation. The DeLong method[24] was used to compare AROC values and the McNemar test was used to compare sensitivity values. The dependence of VD on age and SSI in normal control group was tested using Pearson correlation and linear regression analysis. All p values were two sided and considered statistically significant if the value was less than 0.05.

## RESULTS

The study excluded 12 glaucoma participants (11%) due to poor image quality and included one eye each of 100 participants, including 34 patients with PG, 30 with PPG and 36 normal controls. Compared with the controls, patients with glaucoma (PG or PPG) were significantly older, were less likely to be women, had lower SSI in disc and macular scans, had higher MOPP and worse VF. There were no significant differences in IOP (table 1).

The automatic segmentation of OptoVue system was accurate overall. None of the scans needs major manual corrections. Minimal manual adjustment (focal adjustment for fewer than 10% of B scans of each volumetric scan) of the segmentation was performed for 36% of the disc scans and 53% of the macular scans. For disc scans, the eyes that needed minimal manual corrections had significantly worse VF mean deviation (−3.9 vs −1.2 DB, p=0.002), worse pattern SD (5.2 vs 2.7 DB, p=0.001) and lower SSI (63.0 vs 67.6, p=0.007) but had no significant difference in axial length (24.32 vs 24.23 mm, p=0.75) or age (64.6 vs 63.4 years old, p=0.58). For macular scans, those eyes that needed manual corrections had a significantly worse VF pattern SD (4.3 vs 2.8 DB, p=0.04) but had no difference in VF mean deviation, SSI, axial length or age. We also compared the AROCs for glaucoma diagnosis between those needing manual correction and those not needing manual corrections, and we found no significant difference between the AROCs of overall peripapillary NFLP-CD (0.929 vs 0.821, p=0.12) and macular SVC-VD (0.810 vs 0.817, p=0.42) of AngioAnalytics.

The perfusion parameters measured with COOL-ART in the normal control group were significantly higher (p<0.001 paired

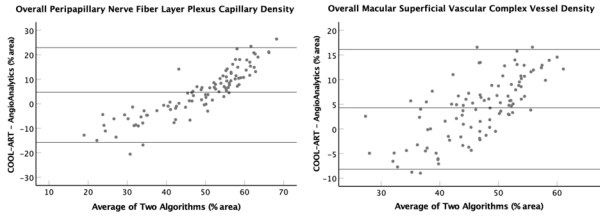


**Figure 1** Bland-Altman plots of regional OCTA parameters measured with AngioAnalytics and COOL-ART. COOL-ART, Center for Ophthalmic Optics & Lasers Angiography Reading Toolkit; OCTA, optical coherence tomography angiography.

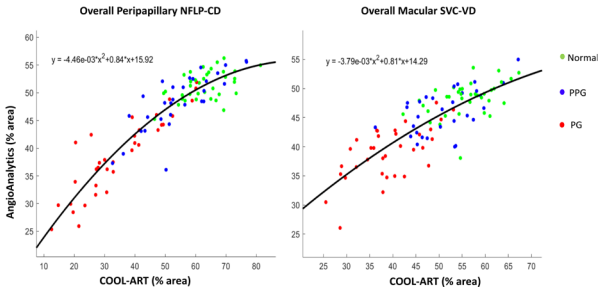


**Figure 2** Interconvertibility of regional OCTA parameters measured with AngioAnalytics and COOL-ART. COOL-ART,Center for Ophthalmic Optics & LasersAngiography Reading Toolkit; NFLP-CD,nerve fibre layer plexuscapillary density; PG,perimetric glaucoma; PPG,pre-PG; SVC-VD,superficial vascular complex vessel density.

t-test) than those measured with AngioAnalytics (table 2) except for the nasal inferior and temporal inferior peripapillary sectors. The perfusion parameters in the glaucoma group were not significantly different between the algorithms. The mean difference between glaucoma and normal groups was markedly higher when measured with COOL-ART than with AngioAnalytics for both overall peripapillary NFLP-CD (18.7% vs 8.0%) and macular SVC-VD (11.1% vs 6.0%) as well as all other perfusion parameters. However, the population SD in the normal group was also significantly greater when measured with COOL-ART ($p<0.001$ F-test) compared with AngioAnalytics, for all perfusion parameters. Both algorithms were able to demonstrate significantly lower values in the glaucoma group compared with the normal group ($p<0.001$, t-test) for all peripapillary and macular parameters.

The Bland-Altman plots (figure 1) demonstrated poor agreement between the two algorithms. The mean difference of the two algorithms for overall peripapillary NFLP-CD and macular SVC-VD was 4.7% (95% CI −15.8% to 22.9%) and 4.3% (95% CI −8.2% to 16.1%), respectively. The 95% CI for the difference between algorithms was wide for both overall peripapillary NFLP-CD (38.7%) and macular SVC-VD (24.3%). The Pearson correlation coefficient between AngioAnalytics and COOL-ART was 0.910 ($p<0.001$) and 0.802 ($p<0.001$) for

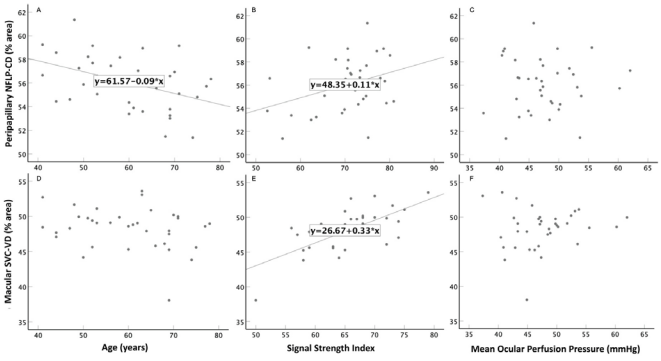


**Figure 3** The clinical covariates of AngioAnalytics measurements in the normal control group. (A) The peripapillary NFLP-CD significantly decreased with age (p=0.004) and (B) increased with signal strength index (p=0.03), (C) but not correlated with mean ocular perfusion pressure (p=0.94). (E) The macular SVC-VD significantly increased with signal strength index (p<0.001), (D) but not significantly associated with age (p=0.22) and (F) mean ocular perfusion pressure (p=0.59). NFLP-CD, nerve fibre layer plexuscapillary density; SVC-VD,superficial vascular complex vessel density.

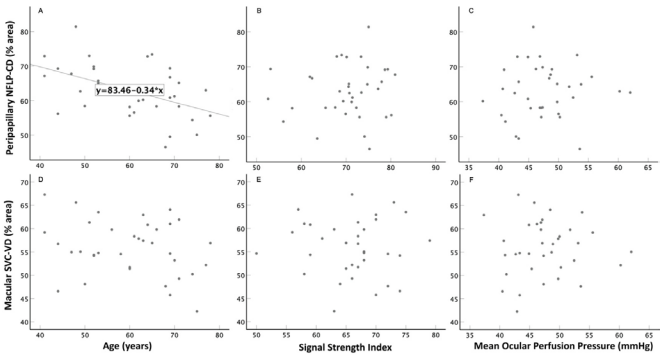


**Figure 4** The clinical covariates COOL-ART measurements in the normal control group. (A) The peripapillary NFLP-CD significantly decreased with age (p=0.006), (B) but not significantly associated with signal strength index (p=0.74) or (C) mean ocular perfusion pressure (p=0.75). (D) The macular SVC-VD was not significantly associated with age (p=0.11), (E) signal strength index (p=0.69) or (F) mean ocular perfusion pressure (p=0.87). COOL-ART, Center for Ophthalmic Optics & Lasers Angiography Reading Toolkit; NFLP-CD, nerve fibre layer plexuscapillary density; SVC-VD, superficial vascular complex vesseldensity.

overall peripapillary NFLP-CD and macular SVC-VD, respectively, showing a high correlation between the two algorithms, suggesting interconvertibility (figure 2). The quadratic terms in both regression analyses were significant (both $p<0.001$), showing that the correlations were nonlinear.

The AngioAnalytics measured peripapillary NFLP-CD (figures 3 and 4), significantly decreased with age (p=0.004) and increased with SSI (p=0.03) but was not significantly associated with MOPP (p=0.94). The COOL-ART measured peripapillary NFLP-CD significantly decreased with age (p=0.006) but was not significantly associated with SSI (p=0.74) or MOPP (p=0.75). For macula-centred scans, SSI was not significantly associated with age (slope=−0.129, p=0.18). The AngioAnalytics measured macular SVC-VD significantly increased with SSI ($p<0.001$) but was not significantly associated with age (p=0.22) and MOPP (p=0.59). The COOL-ART measured that macular SVC-VD was not significantly associated with age (p=0.11), SSI (p=0.69) and MOPP (p=0.87). In normal control structural OCT scans, the NFL thickness and GCC were not significantly associated with age, SSI or MOPP (all $p>0.05$).

After adjustment of age and SSI, the diagnostic accuracy of OCTA parameters measured with AngioAnalytics was similar to those measured with COOL-ART and structural OCT parameters in differentiating glaucoma (PG or PPG) from controls (table 3). For AngioAnalytics measurements, the best diagnostic parameters were whole-image NFLP-VD (AROC=0.851) and macular worst-hemisphere SVC-VD. For COOL-ART measurements, the best diagnostic parameters were the worst-sector NFLP-CD in peripapillary region and worse-hemisphere SVC-VD in macular region. For structural OCT measurements, the best diagnostic parameters were the worst-quadrant retinal NFL thickness and worse-hemisphere GCC thickness. With the specificity set at 95%, no significant sensitivity difference was found among OCTA parameters measured with the two algorithms and the structural OCT parameters. The most sensitive OCTA parameter for detecting PG was the worst-sector peripapillary NFLP-CD measured with AngioAnalytics. For PPG detection, the worst sector of NFL thickness had the highest sensitivity. However, there were no significant differences between any of the OCTA and structural parameters in tables 3 and 4, with the exception

**Table 3** Glaucoma diagnostic accuracy of OCT angiography and structural OCT parameters

| AROC | COOL-ART | AngioAnalytics | COOL-ART vs AngioAnalytics | Structural OCT | | AngioAnalytics vs structure |
|---|---|---|---|---|---|---|
| **Peripapillary NFLP** | | | | **Peripapillary NFL thickness** | | |
| Overall CD | 0.800 | 0.785 | p=0.75 | Overall | 0.855 | p=0.13 |
| NS sector CD | 0.781 | 0.749 | p=0.47 | Nasal quadrant | 0.806 | |
| NI sector CD | 0.769 | 0.768 | p=0.99 | | | |
| IN sector CD | 0.763 | 0.707 | p=0.21 | Inferior quadrant | 0.846 | |
| IT sector CD | 0.785 | 0.743 | p=0.40 | | | |
| TI sector CD | 0.711 | 0.694 | p=0.73 | Temporal quadrant | 0.748 | |
| TS sector CD | 0.693 | 0.654 | p=0.44 | | | |
| ST sector CD | 0.788 | 0.737 | p=0.23 | Superior quadrant | 0.831 | |
| SN sector CD | 0.795 | 0.762 | p=0.50 | | | |
| Worst sector CD | 0.852 | 0.807 | p=0.20 | Worst quadrant | 0.860 | p=0.19 |
| **Macular SVC-VD** | | | | **Macular GCC thickness** | | |
| 6 mm overall | 0.842 | 0.814 | p=0.45 | Overall | 0.858 | p=0.26 |
| 4.5 mm square | 0.831 | | p=0.48* | | | |
| 3.0 mm square | 0.751 | | p=0.005 † | | | |
| Superior hemisphere | 0.795 | 0.792 | p=0.94 | Superior hemisphere | 0.811 | p=0.66 |
| Inferior hemisphere | 0.831 | 0.824 | p=0.85 | Inferior hemisphere | 0.853 | p=0.44 |
| Worse hemisphere | 0.854 | 0.829 | p=0.48 | Worse hemisphere | 0.865 | p=0.35 |

*P value for 4.5 mm vs 6 mm square measured with COOL-ART;
†P value for 3.0 mm vs 6.0 mm square measured with COOL-ART.
AROC, area under the receiver operating-characteristic curve; CD, capillary density; COOL-ART, Center for Ophthalmic Optics & Lasers Angiography Reading Toolkit; GCC, ganglion cell complex; IN, inferior nasal; IT, inferior temporal; NFL, nerve fibre layer; NFLP, nerve fibre layer plexus; NI, nasal inferior; NS, nasal superior; OCT, optical coherence tomography; SN, superior nasal; ST, superior temporal; SVC, superficial vascular complex; TI, temporal inferior; TS, temporal superior; VD, vessel density.

that the smaller 3 mm macular SVC-VD had significantly lower diagnostic sensitivity (p=0.03) and accuracy (p=0.005), compared with the 6 mm macular SVC-VD.

Or-logic combination of overall peripapillary NFLP-CD measured with AngioAnalytics and NFL thickness demonstrated an AROC of 0.943 and 0.705 for PG and PPG detection, respectively, and the corresponding sensitivity was 85.3% and 33.3%, respectively, when the specificity was fixed at 95%. The diagnostic accuracy and sensitivity were not significantly (all p>0.05) different from that of peripapillary NFLP-CD or NFL thickness alone. Similarly, or-logic combination of overall macular SVC-VD measured with AngioAnalytics and GCC thickness did not improve the diagnostic accuracy (all p>0.05), with AROC of 0.975 and 0.718 and with the corresponding sensitivity of 82.4% and 33.3% for PG and PPG detection, respectively.

## DISCUSSION

Previous studies demonstrated poor agreement of OCTA measurements among different devices.[7 25] These differences could be due to device hardware or software algorithms. The current study demonstrated that even with the same device, OCTA measurements with different algorithms were not interchangeable. The agreement between AngioAnalytics and

**Table 4** Glaucoma diagnostic sensitivity of imaging-derived perfusion and structural parameters

| Software | Region/layer | Measurements | Cut-off value* | PG sensitivity (%) | PPG sensitivity (%) |
|---|---|---|---|---|---|
| AngioAnalytics | Peripapillary NFLP | Peripapillary CD | −1.96 | 79.4 | 23.3 |
| | | Worst sector CD | −2.65 | 94.1 | 33.3 |
| | Macular SVC | Overall VD | −2.02 | 85.3 | 23.3 |
| | | Worse hemisphere | −2.14 | 88.2 | 20.0 |
| COOL-ART | Peripapillary NFLP | Overall peripapillary CD | −1.82 | 82.4 | 26.7 |
| | | Worst sector CD | −2.89 | 91.2 | 30.0 |
| | Macular SVC | 6 mm overall VD | −1.99 | 76.5 | 16.7 |
| | | Worse hemisphere | −2.18 | 88.2 | 26.7 |
| | Macular SVC | 4.5 mm overall VD | −1.78 | 70.6 | 30.0 |
| | Macular SVC | 3 mm overall VD | −1.72 | 52.9 | 16.7 |
| Structural scan | Peripapillary NFL | Overall thickness | −1.76 | 85.3 | 36.7 |
| | | Worst quadrant | −2.17 | 91.2 | 36.7 |
| | Macular GCC | Overall thickness | −1.75 | 76.5 | 33.3 |
| | | Worse hemisphere | −1.92 | 88.2 | 36.7 |

*The cut-off value is set at 95% specificity, expressed as number of SD below average of the normal control group for individual parameter.
CD, capillary density; COOL-ART, Center for Ophthalmic Optics & Lasers Angiography Reading Toolkit; GCC, ganglion cell complex; NFLP, nerve fibre layer plexus; PG, perimetric glaucoma; PPG, pre-perimetric glaucoma; RFL, retinal nerve fibre; SVC, superficial vascular complex; VD, vessel density.

COOL-ART outputs was poor. However, the correlation between the outputs of the two algorithms was good, suggesting possible interconvertibility. Despite the poor agreement, the diagnostic accuracy of the two-algorithm outputs was similar. Both algorithms' diagnostic accuracies were good for PG and fair for PPG.

The diagnostic accuracy of OCTA perfusion parameters was similar to OCT thickness parameters. This result was consistent with some previous studies, which also found OCTA and OCT parameters to have similar diagnostic performance.[11 13 26–28] Other studies showed better performance by OCTA[29] or OCT.[19 30–33] The discrepancies between studies could be due to differences in their study populations, study device or OCTA scan patterns and analysis algorithms.

It is noteworthy that for macular OCTA measurements, a larger analytic area provided a higher diagnostic accuracy. In the current study, the SVC-VD in 6 mm macular region provided a significantly greater AROC for differentiating glaucoma from control than SVC-VD in the central 3 mm square area. This could further explain the discrepancy between Takusagawa *et al*'s[12] high diagnostic accuracy using the 6 mm macular OCTA measurements and the low diagnostic accuracy of Rao *et al*'s[18] 3 mm macular OCTA measurements. It is more important to have a wider macular scan, but the width of the scan may not be as critical for the peripapillary region. Early glaucoma damage occurs most frequently in the inferior arcuate bundle,[34] which is entirely missed in a 3 mm macular scan and mostly missed in a 4.5 mm macular scan, but t would be represented in the peripapillary region no matter the size of the scan pattern. The 6 mm macular scan catches most arcuate defects and, therefore, has higher diagnostic accuracy. It is possible that even wider scans centred on the macula would further improve diagnostic accuracy.

Although the AngioAnalytics and COOL-ART had similar diagnostic accuracy, they were affected differently by clinical covariates. Poor signal strength could reduce apparent VD and CD measured by AngioAnalytics. This is a significant issue because signal strength could be affected by media opacity such as cataract and vitreous floater, common in the older population at risk for glaucoma. Therefore, regression-based compensation for SSI is helpful for AngioAnalytics. COOL-ART measurement is independent of signal strength because it uses a reflectance-compensated algorithm to measure VD and CD.[20] So, no further SSI adjustment is needed when analysing COOL-ART outputs.

For both algorithms, there was a significant association between NFLP-CD and age. This is consistent with previous reports.[35] Part of the age effect on perfusion parameters is due to its effect on signal strength. However, even the COOL-ART algorithm, which is independent of signal strength, shows strong age dependence in NFLP-CD. Therefore, the age effect is more than a measurement artefact. We propose that it could be due to an actual age-related decline in ocular circulation; therefore, an age adjustment is needed for NFLP-CD when differentiating glaucoma and normal eyes using any algorithm. The age-related change in macular perfusion seems to be smaller and is not statistically significant in the current study.

In conclusion, AngioAnalytics and COOL-ART VD measurements are not interchangeable but potentially interconvertible. Age and signal strength are significant covariates that need to be considered. Both algorithms and both peripapillary and macular perfusion parameters have similarly good diagnostic accuracy comparable to structural OCT. A larger macular analytic area provides a higher diagnostic accuracy.

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