Correlation of Diabetic Disease Severity to Degree of Quadrant Asymmetry in En Face OCTA Metrics

Jesse J. Jung,[1,2] Shen Yi Lim,[3] Xavier Chan,[3] Srinivas R. Sadda,[4,5] and Quan V. Hoang[3,6]

[1]East Bay Retina Consultants Inc., Oakland, California, United States
[2]Department of Ophthalmology, University of California, San Francisco, San Francisco, California, United States
[3]Singapore Eye Research Institute, Singapore National Eye Centre, Duke-NUS Medical School, Singapore
[4]Doheny Eye Institute, Los Angeles, California, United States
[5]Department of Ophthalmology, David Geffen School of Medicine at UCLA, Los Angeles, California, United States
[6]Department of Ophthalmology, Edward S. Harkness Eye Institute, Columbia University College of Physicians and Surgeons, New York, New York, United States

Correspondence: Jesse J. Jung, East Bay Retina Consultants, Inc., 3300 Telegraph Ave, Oakland, CA 94609, USA;
jung.jesse@gmail.com.

JJJ and SYL contributed equally to this work.

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Purpose. To determine if diabetic retinopathy (DR) severity affects quadrant asymmetry (QA) of optical coherence tomography angiography (OCTA) metrics differentially.

Methods. Ninety eyes (60 patients) with no diabetes mellitus (DM) ($n = 39$) or varying levels of DR ($n = 51$) had OCTA images (3 × 3 mm, Cirrus5000) acquired five times and averaged. The vessel length density (VLD) and perfusion density (PD) of the superficial retinal layer (SRL) and deep retinal layer (DRL) were measured. QA was defined as the maximum minus minimum value among four parafoveal Early Treatment Diabetic Retinopathy quadrants, and compared with DR severity by linear regression including fixed effects for each individual and eye.

Results. The mean patient age was 55.5 years (range, 24–88 years) and 60% were male. Comparing severe nonproliferative DR or proliferative DR versus no DM/DR eyes, QA was significantly higher for SRL VLD, and PD (+0.67 ± 0.16 and +0.014 ± 0.003; $P < 0.001$) and DRL VLD, and PD (+1.25 ± 0.16 and +0.032 ± 0.003; $P < 0.001$). When comparing mild or moderate nonproliferative DR versus no DM/DR, the DRL VLD, and PD were significantly higher (+0.51 ± 0.13 and +0.015 ± 0.003; $P < 0.001$). For every step increase in DR severity, there was a +0.20 QA for SRL VLD, +0.004 SRL PD, +0.33 DRL VLD and +0.009 DRL PD ($P < 0.001$). Regression analysis comparing intraquadrant effect on DR severity demonstrated that the superior quadrant was most affected for all OCTA metrics.

Conclusions. DR severity affects VLD and PD more asymmetrically across Early Treatment Diabetic Retinopathy quadrants with a linear increase in QA for each worsening level of DR. Individual intraeye metrics such as QA can accurately quantify DR severity without concerns for intereye variabilities that could affect the reproducibility and reliability of OCTA quantification.

Keywords: averaging, diabetic retinopathy, intereye, optical coherence tomography angiography, quadrant asymmetry

Diabetic retinopathy (DR) is one of the leading microvascular complications and cause of preventable vision loss in diabetes mellitus (DM) attributed to diabetic macular edema (DME) and ischemia in the adult working population, with cases anticipated to rise from 103.12 million in 2019 to 160.50 million in 2045.[1] Early screening, detection, and treatment of DR is of vital importance as it may improve prognosis and prevent permanent visual impairment. Current imaging methods for DR include fundus photography (using the Early Treatment Diabetic Retinopathy Study [ETDRS] Group standard fields), fluorescein angiography, and optical coherence tomography (OCT). Fluorescein angiography is a sensitive method for detection of microangiopathy based on observations from the ETDRS Group,[2] but is invasive, time consuming, costly, holds a risk for anaphylaxis, and has limited axial resolution for distinguishing the various capillary plexuses of the retina and choroid.[3–6] OCT is a noninvasive imaging technique that uses high-resolution cross-sectional images of the retinal layers and choroid.[7]

OCT angiography (OCTA) is an extension of OCT that can readily segment and image the central subfield and parafoveal macular microvasculature.[8] Using motion contrast by comparing multiple B-scans obtained at the same location, vessel density metrics obtained from OCTA images have been used to discriminate different ETDRS severity levels in the diabetic retina.[9–11] However, limitations of OCTA include motion artifacts attributed to poor fixation, projection artifacts, segmentation errors, lack of information regarding vascular leakage or permeability, difficulties in distinguishing pseudoflow from true flow, and a comparatively smaller field of view.[12,13] To address some of these limitations, montaging of multiple images, averaging of

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multiple en face OCTA images, and patient guidance to avoid decentration and defocus have been used.[14,15] However, when comparing quantitative OCTA metrics from the superficial capillary plexus (SCP), deep capillary plexus (DCP) and choroid, intereye, or intersubject variabilities including age,[16] axial length,[17] refractive error,[18] and astigmatism,[19] may result in difficulties comparing across a cohort of eyes requiring mathematical calculations to account for these variabilities.[19] Additionally, studies have shown significant differences in quantitative metrics across multiple OCTA devices[20] and limited reproducibility of quantitative metrics with different postprocessing algorithms.[21] Given these concerns with intereye quantitative metric comparisons, evaluating intraeye characteristics and the impact of retinal disease on quantitative measurements in different quadrants within a single eye with quadrant asymmetry (QA) analysis may be a potential solution. For instance, quadrant analyses were conducted in optic disc drusen patients.[22] Additionally, QA was also used by our group to show the presence of asymmetric outflow with wide field indocyanine green angiography among the vortex veins in central serous chorioretinopathy and pachychoroid diseased eyes.[23]

Based on the potential utility of this intraeye metric, herein, we sought to determine if the level of DR severity could be accurately assessed with QA of OCTA metrics in both the SCP and DCP.

Methods

This observational, cross-sectional cohort study received institutional review board approval from the Salus Institutional Review Board (Austin, TX). This study complied with the Health Insurance Portability and Accountability Act of 1996 and followed the tenets of the Declaration of Helsinki. All individuals signed a written informed consent before participating in the study.

Participants

A total of 90 eyes (60 patients) with varying degrees of DR (including eyes with no DR or no DM), were chosen for this study from a single, vitreoretinal referral practice, East Bay Retina Consultants Inc. Patients who were either type 1 or 2 diabetics with varying levels of nonproliferative DR (NPDR) or proliferative DR (PDR) and healthy, normal, age-matched controls without DM or DR underwent 3 × 3-mm OCTA scans with a 245 × 245 resolution and a mean distance of 12.2 µm between each scan on the Zeiss Cirrus 5000 with AngioPlex, which images at 68,000 A-scans per second (Carl Zeiss Meditec, Inc., Dublin, CA) with a light source with a central wavelength of 840 nm and a full width at half maximum bandwidth of 90 nm. The A-scan depth was 2 mm with an axial resolution of 5 µm and a transverse resolution of 15 µm.[24] Owing to concerns of potential segmentation errors, which could impact measurements of the superficial retinal capillary layer (SRL) and deep retinal capillary layer (DRL), diabetic eyes with DME involving the central 3 × 3-mm area (of the scan pattern) were excluded from the study. Ultra-widefield color fundus photography and/or fluorescein angiography (Optos California, Dunfermline, UK) were also obtained and grading of DR was completed by an experienced retinal specialist based on the clinical exam and ancillary imaging according to the International Clinical Diabetic Retinopathy Disease Severity Scale.[25] Diabetic eyes, which had undergone prior noncentral (outside of the parafoveal 3 × 3-mm area of interest), focal laser therapy more than 6 months ago or eyes with prior or current/ongoing treatment with anti-VEGF injections such as aflibercept (Regeneron, Tarrytown, NJ), bevacizumab (Genentech, South San Francisco, CA), or ranibizumab (Genentech, South San Francisco, CA) were included in this study. All eyes included in this study were well-controlled on maintenance treatment without exhibiting signs of active DME or vitreous hemorrhage at the time of imaging.

OCT Imaging

All eye images were captured consecutively at a single timepoint before analysis was performed as previously described.[26] In short, a spectral domain OCTA (Zeiss Cirrus 5000 with AngioPlex, Carl Zeiss Meditec, Inc.) using an angiography 3 × 3-mm scan pattern was used to capture all OCTA images. OCTA acquisition was repeated four more times to facilitate image averaging. The optical microangiography (OMAG, Carl Zeiss Meditec, Inc.) algorithm was used to produce en face OCTA images and standard OCTA tracking software, along with centering images on the fovea, was performed to minimize motion artifacts. Additionally, the correct identification of the SRL and DRL was ensured by stringent examination of each segmentation, to ensure the default segmentation boundaries of the device were accurate. Each OCTA scan obtained had a signal strength of greater than 7 (normal scale 1–10), was centered on the fovea, autosegmented for the SRL/DRL, had uniform illumination without areas of darkness, and had good centration of the fovea, as well as no significant motion artifacts (evidenced via vessel segment misalignment). In a previous study, we established that the optimal number of repeated images for use in averaging was five scans.[27] Uji et al.[15] also demonstrated that for the SRL, the largest difference in vessel length density (VLD) occurred in the first level of averaging and diminished in magnitude after five frames of averaging; and for the DRL, the ideal number of averaged images was more than three but no significant differences were found after six averaged images. Finally, the Cirrus AngioPlex software (version 10.0; clearance by the US Food and Drug Administration pending, Carl Zeiss Meditec, Inc.) was used to eliminate projection artifacts and exported at a size of 1024 × 1024 pixels for further analysis of OCTA VLD and perfusion density (PD) metrics.

Multiple En Face Imaging Averaging

As previously published by Uji et al.,[15] postprocessing image averaging of the five en face images was performed using ImageJ (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html). By identifying features of the fundus, an 819 × 819 pixels central square area was stacked to produce a five-frame video of the SRL and DRL images, before averaging and stitching to obtain a single averaged image.

Quantitative Measurements

The Cirrus AngioPlex software (version 10.0; clearance by the US Food and Drug Administration pending, Carl Zeiss Meditec, Inc.) was used to export and process OCTA en face images. The ETDRS inner ring is defined as a concentric ring

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with an inner diameter of 1000 µm and an outer diameter of 3000 µm centered at the fovea. This inner ring can be divided into superior, inferior, nasal, and temporal subfields which constitute the four quadrants assessed in our analysis. The parafoveal VLD, defined as the total length of perfused vasculature per unit area (mm^{-1}) and PD, defined as the total area covered by perfused vasculature per unit area (%). The VLD and PD were computed in each of these ETDRS subfields or quadrants for both the SRL and DRL.

QA Analysis

The QA for the SRL and DRL metrics was calculated by subtracting the minimum value from the maximum value (max–min) among the four ETDRS quadrants of a given eye, with fixed effects for each individual left and right eye, and was used as a measure of the asymmetry and nonuniformity among quadrants. We included fixed effects for each individual eye to use variation within an individual eye to estimate the relationship between QA and DR. By using a univariate linear regression, and accounting for patient eye–specific variability, specific quadrants with the greatest quantitative VLD or PD metric in DR eyes could be determined. This analysis was performed on averaged values within a quadrant on dummy variables to analyze individual quadrants. Therefore, we used within-eye variation versus variation across eyes. By using fixed effects for our linear regression, we account for variations in between eyes such as laterality, refractive error, or axial length, which may affect the initial quantification of the SRL and DRL quantitative OCTA metrics.

The main analyses determined the QA for worsening DR severity with the cohort stratified into three groups: (1) control (no DM and no DR combined), (2) DR that does not typically require treatment (mild and moderate NPDR), and (3) DR severity levels that typically require treatment (severe NPDR and PDR). A secondary analysis using the Mann–Whitney *U* test was also performed to quantify the mean max–min difference in values between all DR eyes versus control eyes (no DM and no DR) via plotting of the cumulative distribution function.

All data were analysed using the Stata 13.0 statistical package (StataCorp LP, College Station, TX). All quantitative values were expressed as the mean with standard deviation. A difference was considered significant when the *P* values was less than 0.05.

Results

Ninety eyes from 60 patients, consisting of normal controls and varying levels of DR, who met the inclusion criteria, were chosen for this study. Thirty-nine eyes (28 patients) were controls including 27 eyes with no DM (19 patients) and 12 eyes from diabetics without DR (9 patients), 11 eyes (8 patients) had mild NPDR, 10 eyes (7 patients) had moderate NPDR, 7 eyes (5 patients) had severe NPDR, and 23 eyes (16 patients) had PDR. At the time of image acquisition for the cross-sectional study, among the 51 diabetic eyes, 34 were treatment naïve. Eleven eyes were undergoing treatment with anti-VEGF (five for center-involved DME, but at the time of imaging, did not have active edema); two eyes had been or were subsequently treated with panretinal photocoagulation (PRP), two eyes with focal laser, seven eyes with a combination of anti-VEGF and PRP, four eyes with anti-VEGF and focal laser, one eye with PRP and focal laser, and two eyes with anti-VEGF, PRP, and focal laser. Baseline characteristics of included subjects are summarized in Table 1.

QA values for the control (no DM and no DR) and each DR severity level (mild NPDR, moderate NPDR, severe NPDR, PDR) (Figs. 1 and 2) are presented as mean ± standard deviation (Table 2). A Mann–Whitney *U* test showed significant differences between QA among eyes with no DM and eyes with no DR only for DRL VLD (2.27 ± 0.080 vs. 1.86 ± 0.15; $P = 0.037$), but not for SRL VLD, SRL PD, or DRL PD (all $P > 0.05$). Therefore, in this analysis, eyes with no DM and no DR were merged as a single group and used as the control and compared with eyes with various DR severity levels (as a categorical variable with levels of mild NPDR, moderate NPDR, severe NPDR, and PDR).

In our initial analysis, we stratified the cohort into three groups: (1) control (no DM and no DR combined), (2) DR that does not typically require treatment (mild and moderate NPDR), and (3) DR severity levels that typically require treatment (severe NPDR and PDR). Comparing (2) mild and moderate NPDR against (1) no DM and no DR, the mean difference in QA significantly differed for DRL VLD and DRL PD (+0.51 and +0.015, respectively; $P < 0.001$ for both), but not for SRL VLD and SRL PD ($P > 0.05$ for both). Comparing (3) severe NPDR and PDR against (1) no DM and no DR, the mean difference in QA for SRL VLD, SRL PD, DRL VLD, and DRL PD were +0.67, +0.014, +1.25, and +0.032, respectively ($P < 0.001$ for all) (Table 3).

A univariate linear regression analysis was used to compare the QA quantitative metrics for averaged images, including the VLD and PD measurements of both the SRL and DRL, and fixed effects for each individual and for each eye (Table 4). For all the averaged image types, for every step increase in DR severity (mild NPDR, moderate NPDR, severe NPDR, PDR), QA increased by 0.20, 0.004, 0.33, and 0.009 for SRL VLD, SRL PD, DRL VLD,

Table 1. Demographics

Characteristic	Control (No DM and No DR) ($n = 156$)	Mild NPDR ($n = 44$)	Moderate NPDR ($n = 40$)	Severe NPDR ($n = 28$)	PDR ($n = 100$)
Age, y	53.6 ± 14.5	58.4 ± 9.8	61.5 ± 9.9	51.4 ± 15.3	55.96 ± 11.7
Laterality (right eye)	68 (43.6)	28 (63.6)	20 (50.0)	20 (71.4)	36 (36.0)
Gender (male)	80 (51.3)	28 (63.6)	16 (40.0)	20 (71.4)	76 (76.0)
Lens status (phakic)	152 (97.4)	44 (100.0)	36 (90.0)	24 (85.7)	76 (76.0)
logMAR BCVA	0.041 ± 0.058	0.051 ± 0.061	0.16 ± 0.11	0.096 ± 0.11	0.11 ± 0.13

Baseline demographics comparing 90 eyes of 60 patients of control (no DM and no DR) and DR severity group (mild NPDR, moderate NPDR, severe NPDR, and PDR).

Data are presented as mean ± standard deviation or number (%).

BCVA, best-corrected visual acuity; logMAR, logarithm of the minimum angle of resolution.

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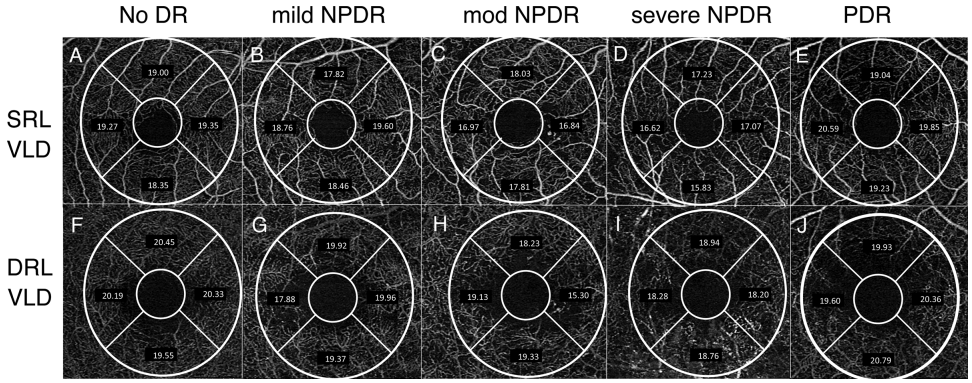

Figure 1. Quantitative measurements of VLD in the parafoveal ETDRS subfields in spectral domain OCTA images of the SRL (*top*, **A–E**) and DRL (*bottom*, **F–J**). The QA increased with DR severity comparing eyes with no DR (*left-most column*; **A** and **F**) to mild NPDR (*second column*; **B** and **G**), moderate NPDR (*third column*; **C** and **H**), severe NPDR (*fourth column*; **D** and **I**), and PDR (*fifth column*; **E** and **J**).

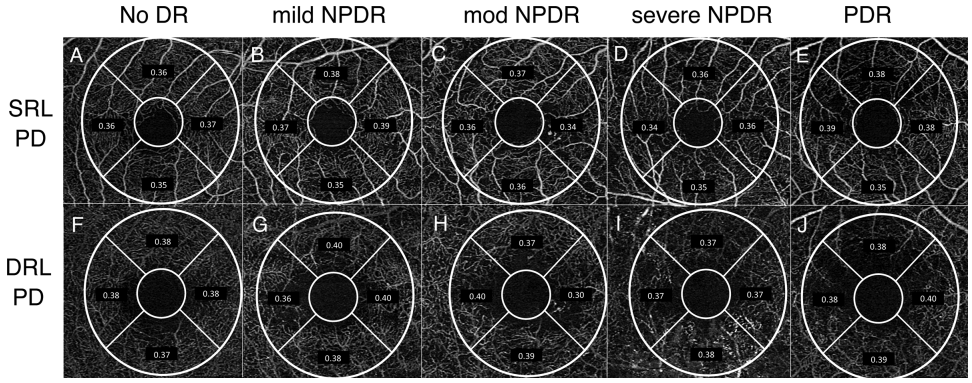

Figure 2. Quantitative measurements of PD in the parafoveal Early Treatment Study (ETDRS) subfields in spectral-domain OCTA images of the SRL (*top*; **A–E**) and DRL (*bottom*; **F–J**). The QA increased with DR severity comparing eyes with no DR (*left-most column*; **A** and **F**) to mild NPDR (*second column*; **B** and **G**), moderate NPDR (*third column*; **C** and **H**), severe NPDR (*fourth column*; **D** and **I**), and PDR (*fifth column*; **E** and **J**).

and DRL PD, respectively ($P < 0.001$ for these image metrics).

A multivariate regression analysis comparing intraquadrant effect on DR severity in averaged images (Table 5) showed that the superior quadrant had lower values compared with the inferior, nasal, and temporal quadrants, with positive values for the coefficients of inferior, nasal and temporal for SRL VLD, SRL PD, DRL VLD, and DRL PD, with the only exception being the DRL PD of the temporal quadrant with a coefficient of −0.001 ($P = 0.84$). Results showed that the inferior quadrant vessel metrics were consistently greater than the superior quadrant and significantly contributed to DR severity with the values in the SRL VLD, SRL PD, DRL VLD, and DRL PD of +0.41, +0.007, +0.28, and +0.006 ($P < 0.001$, $P = 0.005$, $P = 0.021$, and $P = 0.012$), respectively. The nasal quadrant was also greater than the superior quadrant and significantly contributed to DR severity with the values in SRL VLD and SRL PD of +0.23 and +0.005 ($P = 0.024$ and $P = 0.032$), respectively.

The cumulative distribution function plot in Figure 3 shows the difference in distribution of QA values in control (the combination of two subgroups: no DM and no DR) and

Table 2. QA Values for Each Level of DR

Characteristic (No. of Images)	No DM and No DR ($n = 156$)	Mild NPDR ($n = 44$)	Moderate NPDR ($n = 40$)	Severe NPDR ($n = 28$)	PDR ($n = 100$)
SRL VLD	2.14 ± 1.23	1.71 ± 0.81	2.25 ± 1.31	2.25 ± 1.18	2.97 ± 1.44
SRL PD	0.046 ± 0.025	0.038 ± 0.014	0.043 ± 0.012	0.047 ± 0.027	0.064 ± 0.030
DRL VLD	2.14 ± 0.92	2.35 ± 0.87	2.99 ± 1.09	3.214 ± 1.75	3.435 ± 1.67
DRL PD	0.043 ± 0.018	0.049 ± 0.019	0.070 ± 0.029	0.076 ± 0.044	0.075 ± 0.036

Calculating QA values for control (no DM and no DR) and at each DR severity level (mild NPDR, moderate NPDR, severe NPDR, and PDR).

Data are presented as mean ± standard deviation.

PD is defined as the total area covered by perfused vasculature per unit area (%); VLD is defined as the total length of perfused vasculature per unit area (mm/mm^2).

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Table 3. Comparison of QA with Different Permutations of DR Severity Levels

Image Type	DR Severity (No. of Images)		Mean Difference in QA	*P* Value	DR Severity (No. of Images)		Mean Difference in QA	*P* Value
SRL VLD	Mild NPDR (44) moderate NPDR (40)	No DM (108) no DR (48)	−0.17 ± 0.16	0.31	Severe NPDR (28) PDR (100)	No DM (108) no DR (48)	0.67 ± 0.16	<0.001*
SRL PD	Mild NPDR (44) moderate NPDR (40)	No DM (108) no DR (48)	−0.005 ± 0.003	0.152	Severe NPDR (28) PDR (100)	No DM (108) no DR (48)	0.014 ± 0.003	<0.001*
DRL VLD	Mild NPDR (44) moderate NPDR (40)	No DM (108) no DR (48)	0.51 ± 0.13	<0.001*	Severe NPDR (28) PDR (100)	No DM (108) no DR (48)	1.25 ± 0.16	<0.001*
DRL PD	Mild NPDR (44) moderate NPDR (40)	No DM (108) no DR (48)	0.015 ± 0.003	<0.001*	Severe NPDR (28) PDR (100)	No DM (108) no DR (48)	0.032 ± 0.003	<0.001*

Images were compared across different permutations of DR severity levels, namely: (1) mild NPDR and moderate NPDR against no DM and no DR; (2) severe NPDR and PDR against no DM and no DR. Mean difference was calculated by subtracting the second group from the first group for each permutation.

Data are presented as mean ± standard error.

* $P < 0.05$.

PD is defined as the total area covered by perfused vasculature per unit area (%); VLD is defined as the total length of perfused vasculature per unit area (mm/mm^2).

Table 4. Univariate Regression Analysis of QA of Quantitative Metrics With DR Severity

Image Type	Coefficient	Standard Error	95% Confidence Interval		*P* Value
			Lower	Upper	
SRL VLD	0.20	0.039	0.13	0.28	<0.001*
SRL PD	0.004	0.001	0.003	0.006	<0.001*
DRL VLD	0.33	0.038	0.26	0.41	<0.001*
DRL PD	0.009	0.001	0.007	0.010	<0.001*

Univariate regression analysis was performed comparing QA at every step increase in DR severity (mild NPDR, moderate NPDR, severe NPDR, and PDR) in averaged images, controlling for patient eye specific fixed effects. More positive values correspond with increased QA.

*$P < 0.05$.

PD is defined as the total area covered by perfused vasculature per unit area (%); VLD is defined as the total length of perfused vasculature per unit area (mm/mm^2).

DR groups for both SRL and DRL VLD and PD. The distribution for the DR group (dotted line) is right shifted relative to control eyes (solid line) for SRL and DRL VLD and PD, which indicates that the DR group has QA values that are higher relative to control throughout the distribution. The distribution for the control group also showed a lower variability in QA values with a steeper cumulative distribution function plot for DRL VLD and DRL PD.

The intraquadrant comparison in Figure 4 of averaged images showed significant difference in values between control (the combination of two subgroups: no DM and no DR) and the DR group for all quadrants of SRL VLD, DRL VLD, and DRL PD ($P < 0.05$ for all comparisons, Mann–Whitney U test), whereas SRL PD showed significant difference between the QA for the control (no DM and no DR) and DR groups (mild NPDR, moderate NPDR, severe NPDR, and PDR) for superior and temporal quadrants ($P < 0.05$ for both comparisons), but not for inferior and nasal quadrants ($P > 0.05$).

Discussion

In this study, we assessed the accuracy of an intraeye comparison using QA among the EDTRS inner ring subfield OCTA metrics to predict the severity of DR. When comparing control eyes (without DM or without DR) with eyes with severe NPDR or PDR, QA of the SRL and DRL OCTA metrics were significantly different (Table 3). However, when comparing control eyes (without DM or without DR) with eyes with mild or moderate NPDR, QA of only the DRL OCTA metrics (but not SRL) were significantly different, which may be due to the susceptibility of the DCP to hypoxic insult.[28] More strikingly, a univariate regression analysis showed that QA of the SRL and DRL VLD and PD significantly increased with DR severity (Table 4).

We previously showed that both single and averaged quantitative metrics of spectral domain OCTA correlate with BCVA and level of DR. Image averaging of multiple en face OCTA images successfully enhances image quality,[15] demonstrates less discontinuous vessels and more uniformity of the capillary network,[27] and yields a lower mean VLD and PD, likely owing to the presence of less artefactual flow signal. However, there remains the inherent variability that exists between each eye that makes comparing these OCTA metrics across a population of eyes difficult. For example, age,[16] uncorrected axial length,[17] refractive status such as axial myopia,[18] astigmatism,[19] and uncorrected spherical refractive defocus[29] can affect magnification; as a result, measurements of vessel and PD can be erroneously overestimated or underestimated. Without properly correcting for these intereye confounders, comparisons of different cohorts of eyes, for example, in East Asian eyes with an increased prevalence of high myopia,[30] may be inaccurate and longitudinal quantitative OCTA data may be difficult to replicate. QA is a technique that relies on comparing intraeye variability, such as the difference between the ETDRS parafoveal inner ring quadrants. By simply comparing the difference in quadrants along with statistical adjustment for the fixed effects for each individual and eye with linear regression analysis, this quantitative metric focuses on differences within the ETDRS subfields. The use of intraeye QA avoids the confounding effects of variability in magnification owing to different axial lengths when comparing eyes that would otherwise have to be corrected with the Littman and modified Bennett

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Table 5. Multivariate Regression Analysis Comparing Intraquadrant Effect on DR Severity

	SRL VLD				SRL PD				DRL VLD				DRL PD			
Quadrant	Coefficient	Standard Error	*P* Value		Quadrant	Coefficient	Standard Error	*P* Value	Quadrant	Coefficient	Standard Error	*P* Value	Quadrant	Coefficient	Standard Error	*P* Value
Superior	Reference group	–	–		Superior	Reference group	–	–	Superior	Reference group	–	–	Superior	Reference group	–	–
Inferior	0.41	0.11	<0.001*		Inferior	0.007	0.002	0.005*	Inferior	0.28	0.12	0.021*	Inferior	0.006	0.003	0.012*
Nasal	0.23	0.10	0.024*		Nasal	0.005	0.002	0.032*	Nasal	0.16	0.12	0.198	Nasal	0.002	0.003	0.49
Temporal	0.10	0.12	0.38		Temporal	0.003	0.002	0.27	Temporal	0.084	0.12	0.503	Temporal	− 0.001	0.003	0.84

Multivariate linear regression analysis was performed comparing SRL and DRL VLD and PD between quadrants (superior, inferior, nasal, temporal) at every step increase in DR severity ([1] mild NPDR, [2] moderate NPDR, [3] severe NPDR, and [4] PDR) in averaged images, with superior quadrant set as the reference group and controlling for patient eye specific fixed effects.

* $P < 0.05$.

PD is defined as the total area covered by perfused vasculature per unit area (%); VLD is defined as the total length of perfused vasculature per unit area (mm/mm^2).

formulas.[17] Our group has previously used this type of analysis to successfully demonstrate QA with nonuniform drainage through vortex veins in eyes with pachychoroid disease[23] and in eyes with induced astigmatism.[19] Herein, we similarly used QA to demonstrate that we can accurately predict the level of DR severity based on an increasing QA with each level of DR (Table 4).

Previously, Kaizu et al.[31] performed a simpler technique using the minimum/maximum flow density ratio of four quadrants (superior, inferior, nasal, and temporal) that was arbitrarily divided with an *X* through the foveal avascular zone using image editing to analyze if there was spatial bias of macular capillary dropout in DR. With their analysis, they observed no significant difference in flow density in the four regions at the SCP and DCP. In comparison, using the quantitative metrics from the four parafoveal ETDRS subfields, excluding the foveal avascular zone, using multiple en face OCTA image averaging, using a more natural representation of spread of intraeye QA (vs. a ratio of min–max), and applying a more robust approach of regression analysis, we observed that QA significantly increased in both the VLD and PD of the SRL and DRL as the level of DR worsened (Table 4). Inherently, the min–max ratio shows the relative QA difference, but does not reveal the magnitude in difference or handle outliers well, as compared with maximum–minimum QA with fixed effects used in this study. For example, if comparing two sets of four quadrants with the following values, [10, 5, 10, 5] and [20, 10, 20, 10], the min–max ratio would be 0.5 for both, but the max–min with fixed effects would be 5 and 10.

Previous studies have shown that with increasing levels of DR severity, areas of capillary nonperfusion within the perifoveal microvasculature increase.[9,10] As DR progresses, there may be asymmetric loss of the capillary network in both the SCP and DCP, leading to increasing QA. Taylor et al.[32] demonstrated that capillary dropout in DR may occur in a nonuniform distribution, and our multivariate regression analysis comparing the SRL and DRL VLD and PD between quadrants (Table 5) identified that the superior ETRDS parafoveal field showed consistently lower measurement than the other fields with statistically significant differences compared with the inferior and nasal fields for the SRL VLD and PD, respectively, as well as for the inferior DRL VLD and PD. Anatomically, it is unclear why capillary dropout occurred more frequently in this superior quadrant, but this spatial difference may have driven the underlying QA seen in eyes with increasing DR severity. Further investigations using QA may be necessary to identify whether the superior quadrant is consistently affected in DR.

Clinically, referable DR typically is described based on the International Clinical Diabetic Retinopathy Disease Severity Scale as moderate or worse DR.[33] As telemedicine further develops, optimizing the use of noninvasive imaging such as fundus photography,[34] OCT, and OCTA,[35] with reliable quantitative metrics to monitor eyes with referrable levels of DR is essential. Laotaweerungsawat et al.[35] demonstrated that OCTA parameters such as VLD and PD correlated with DR severity, except for nondiabetic eyes versus diabetic eyes without retinopathy. Similarly, we compared different permutations of DR severity levels and identified that QA in the VLD and PD of both the SRL and DRL were inconsistent when comparing eyes with no DM, diabetes without DR, and mild NPDR. When comparing no DM and diabetes without DR, we observed significant differences in QA for averaged DRL VLD ($P = 0.04$), but not for averaged SRL

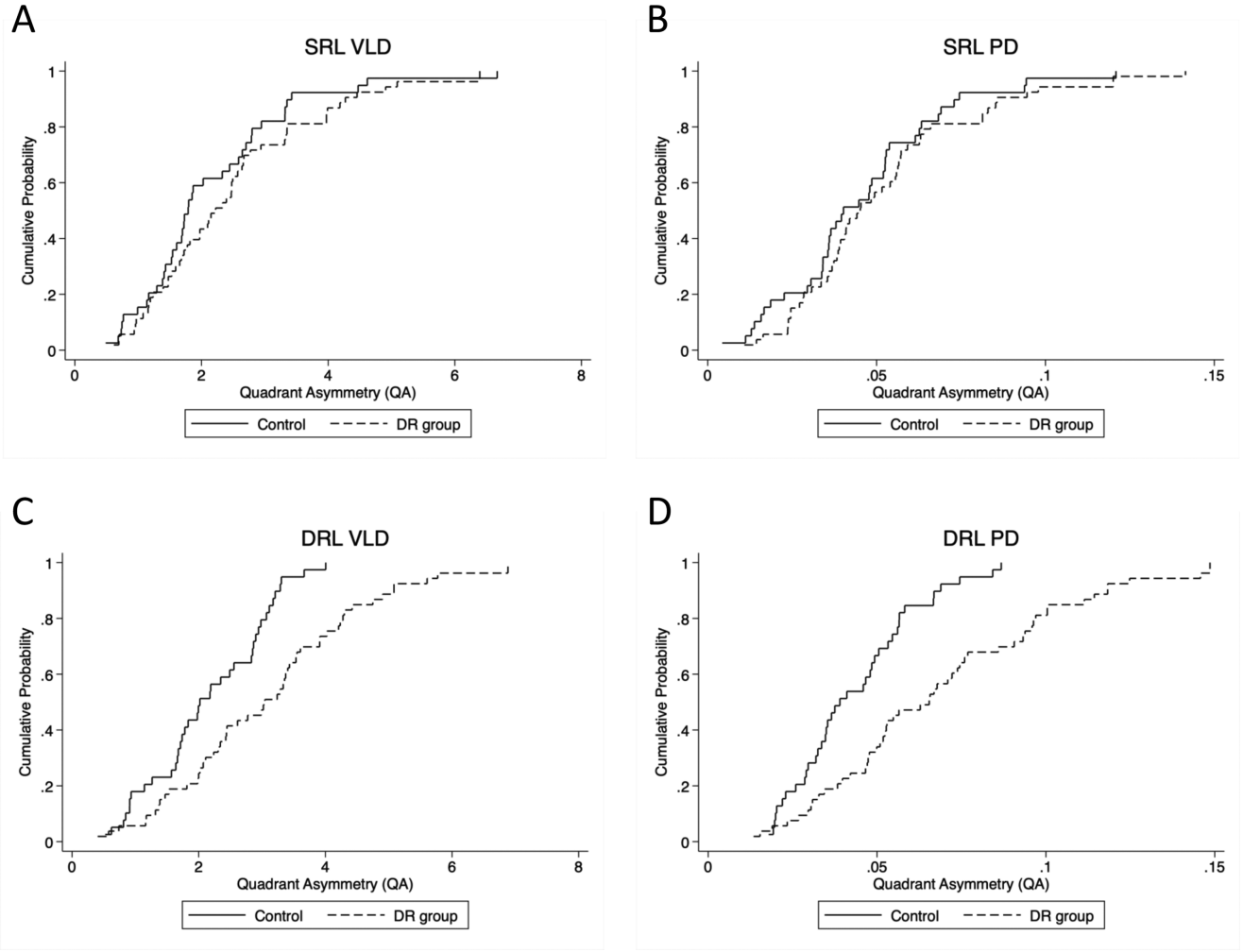

Figure 3. Graph of the cumulative distribution function (CDF) of QA for control eyes (no DM and no DR) and DR group (mild NPDR, moderate NPDR, severe NPDR, and PDR). QA was calculated by subtracting the quadrant with the minimum value from the quadrant maximum value for each individual eye (**A–D**). CDF plot showed that DR group had greater QA than control across the SRL and DRL, VLD, and PD. PD is defined as the total area covered by perfused vasculature per unit area (%); VLD is defined as the total length of perfused vasculature per unit area (mm/mm^2). Please note that the PD values are typically two orders of magnitude smaller than the VLD values, which is reflected in the *x* axis scale.

VLD, SRL PD, or DRL PD (all $P > 0.05$). Similarly, Scarinci et al.[36] previously showed parafoveal DCP loss in type 1 DM without retinopathy. QA also was significantly different in the DCP ($P < 0.001$) between control eyes and eyes with mild or moderate NPDR, which has also been seen when studying the parafoveal vessel density metrics of mild DR.[37] More important, when comparing control eyes and eyes with severe NPDR or worse (Table 3), there is a significant difference in QA for all SRL and DRL metrics ($P < 0.001$). Based on the stepwise increase in QA affecting only the DRL in DM eyes without DR and mild/moderate NPDR versus both the SRL and DRL in eyes with severe NPDR or worse disease, this metric correlated extremely well with DR severity. Using a reliable and repeatable intraeye quantitative metric such as QA may allow for more consistent grading of referable DR.

The distribution of QA values showed that control eyes were consistently lower than the combined DR group (Fig. 3). When intraquadrant comparison was performed (Fig. 4), there was a significant difference between control eyes and the DR group, with the exception of SRL PD in the inferior and nasal quadrants. This can potentially prove that analyses of DR severity can be assessed from different quadrants within a single eye, avoiding the difficulties of intereye variabilities in quantitative comparisons.

This study provides important information about the ability to analyze intraeye quantitative metrics and highlights the effectiveness of QA in predicting the level of DR severity. Specifically, for every step increase in DR severity (from no DR to mild NPDR, from mild to moderate NPDR, from moderate to severe NPDR, or from severe NPDR to PDR), there was a definitive increase in QA for all four metrics we tracked (+0.20 per step in severity for SRL VLD, +0.004 for SRL PD, +0.33 for DRL VLD, and +0.009 for DRL PD, respectively; $P < 0.001$). This implies that one could readily set thresholds based on QA to accurately predict the DR severity for a given eye, perhaps even in an automated, clinician-free fashion.

We acknowledge the study's limitations, including the relatively small sample size in each stage of DR, some of which were being actively or previously treated with anti-VEGF, focal laser, panretinal photocoagulation, or a combination of therapies. Although there may be some subtle

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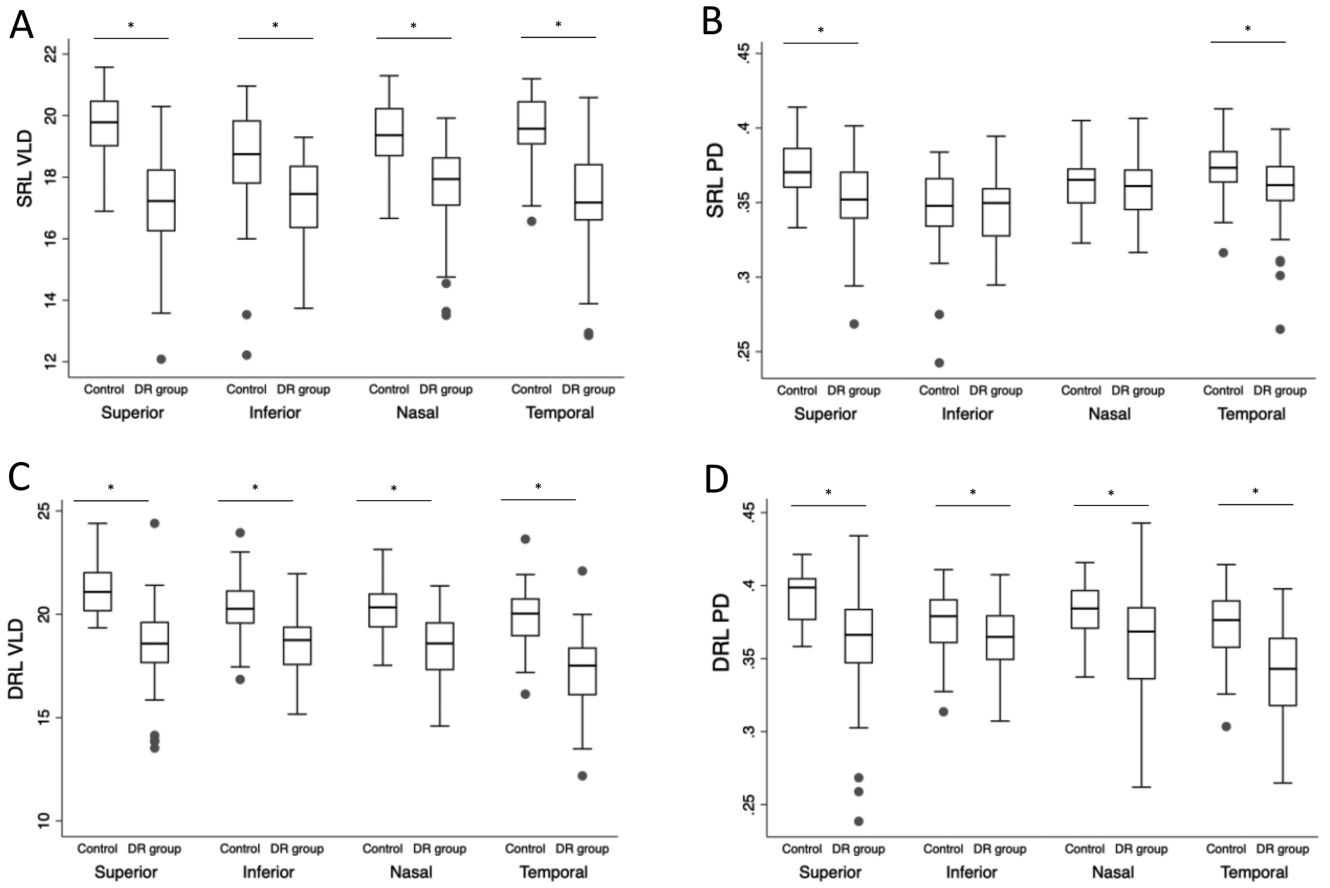

Figure 4. An intraquadrant comparison of averaged images for the SRL and DRL VLD and PD for control eyes (no DM and no DR) and DR group (mild NPDR, moderate NPDR, severe NPDR, and PDR) (**A–D**). *$P < 0.05$. PD is defined as the total area covered by perfused vasculature per unit area (%); VLD is defined as the total length of perfused vasculature per unit area (mm/mm^2).

difference between no DM and no DR eyes, we did perform a comparison between no DM and no DR eyes and found only minimal differences for only averaged DRL VLD. We, therefore, merged these two groups. Although our sample size was small for each level of DR severity, we did provide sufficient power to demonstrate a statistically significant difference in QA. Furthermore, the retrospective cross-sectional study design and the use of a single spectral-domain OCTA system (Angioplex, Carl Zeiss Meditec Inc.) may impact the ability to extrapolate our results to other commercial devices. Conceptually, however, using an intraeye calculation such as QA would allow for accurate staging of disease no matter the instrument used and future comparisons between multiple devices could confirm this hypothesis. Additionally, as noted previously, OCTA acquisition is inherently affected by artifacts that can affect the initial quantification of VLD and PD acquisition issues such as segmentation errors, projection artifacts, and poor focus; and processing issues such as not correcting for refractive error, which can affect magnification leading to incorrect values before the QA calculation is performed. Although in this study population we did not correct for or directly compare axial length or refractive error to the outcome measures, our group has previously shown that using linear regression with fixed effects in QA can normalize for these intereye variabilities,[19] and the conclusions from this study are based on this previous work and a statistical model, but not direct evidence. We also attempted to minimize the effect of these factors by only including high-quality, foveal-centered images with high signal strength and excluded eyes with center-involved DME.

Despite these limitations, our study is the first that demonstrate the reliability of an intraeye quantitative OCTA metric, QA, to accurately identify the level of DR severity. QA can be easily acquired from the ETDRS overlay in OCTA images and limits the confounding effects of intereye variables such as age, axial length, and refractive error. This form of en face OCTA image analysis may improve the reproducibility of OCTA-based DR screening and increase the reliability of disease monitoring in DR.

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References

1. Teo ZL, Tham YC, Yu M, et al. Global prevalence of diabetic retinopathy and projection of burden through 2045: systematic review and meta-analysis. *Ophthalmology*. 2021;128(11):1580–1591.
2. Classification of diabetic retinopathy from fluorescein angiograms. ETDRS report number 11. Early Treatment Diabetic Retinopathy Study Research Group. *Ophthalmology*. 1991;98(5 Suppl):807–822.
3. Corcóstegui B, Durán S, González-Albarrán MO, et al. Update on diagnosis and treatment of diabetic retinopathy: a consensus guideline of the Working Group of Ocular Health (Spanish Society of Diabetes and Spanish Vitreous and Retina Society). *J Ophthalmol*. 2017;2017:8234186.
4. Yannuzzi LA, Rohrer KT, Tindel LJ, et al. Fluorescein angiography complication survey. *Ophthalmology*. 1986;93(5):611–617.
5. Spaide RF, Klancnik JM, Jr, Cooney MJ. Retinal vascular layers imaged by fluorescein angiography and optical coherence tomography angiography. *JAMA Ophthalmol*. 2015;133(1):45–50.
6. Gupta V, Al-Dhibi HA, Arevalo JF. Retinal imaging in uveitis. *Saudi J Ophthalmol*. 2014;28(2):95–103.
7. Aumann S, Donner S, Fischer J, Müller F. Optical coherence tomography (OCT): principle and technical realization. In: Bille JF , ed. *High Resolution Imaging in Microscopy and Ophthalmology: New Frontiers in Biomedical Optics*. Cham: Springer; 2019:59–85.
8. Chalam KV, Sambhav K. Optical coherence tomography angiography in retinal diseases. *J Ophthalmic Vis Res*. 2016;11(1):84–92.
9. Durbin MK, An L, Shemonski ND, et al. Quantification of retinal microvascular density in optical coherence tomographic angiography images in diabetic retinopathy. *JAMA Ophthalmol*. 2017;135(4):370–376.
10. Nesper PL, Roberts PK, Onishi AC, et al. Quantifying microvascular abnormalities with increasing severity of diabetic retinopathy using optical coherence tomography angiography. *Invest Ophthalmol Vis Sci*. 2017;58(6):BIO307–BIO315.
11. Marques IP, Alves D, Santos T, et al. Multimodal imaging of the initial stages of diabetic retinopathy: different disease pathways in different patients. *Diabetes*. 2019;68(3):648–653.
12. Spaide RF, Fujimoto JG, Waheed NK, Sadda SR, Staurenghi G. Optical coherence tomography angiography. *Prog Retin Eye Res*. 2018;64:1–55.
13. Spaide RF, Fujimoto JG, Waheed NK. Image artifacts in optical coherence tomography angiography. *Retina*. 2015;35(11):2163–2180.
14. Adhi M, Filho MA, Louzada RN, et al. Retinal capillary network and foveal avascular zone in eyes with vein occlusion and fellow eyes analyzed with optical coherence tomography angiography. *Invest Ophthalmol Vis Sci*. 2016;57(9):OCT486–OCT494.
15. Uji A, Balasubramanian S, Lei J, Baghdasaryan E, Al-Sheikh M, Sadda SR. Impact of multiple en face image averaging on quantitative assessment from optical coherence tomography angiography images. *Ophthalmology*. 2017(7);124:944–952.
16. Iafe NA, Phasukkijwatana N, Chen X, Sarraf D. Retinal capillary density and foveal avascular zone area are age-dependent: quantitative analysis using optical coherence tomography angiography. *Invest Ophthalmol Vis Sci*. 2016;57(13):5780–5787.
17. Sampson DM, Gong P, An D, et al. Axial length variation impacts on superficial retinal vessel density and foveal avascular zone area measurements using optical coherence tomography angiography. *Invest Ophthalmol Vis Sci*. 2017;58(7):3065–3072.
18. Leng Y, Tam EK, Falavarjani KG, Tsui I. Effect of age and myopia on retinal microvasculature. *Ophthalmic Surg Lasers Imaging Retina*. 2018;49(12):925–931.
19. Jung JJ, Soh YQ, Sha P, Yu S, Durbin MK, Hoang QV. Effects of induced astigmatism on spectral domain-OCT angiography quantitative metrics. *Am J Ophthalmol*. 2020;219:49–58.
20. Corvi F, Pellegrini M, Erba S, Cozzi M, Staurenghi G, Giani A. Reproducibility of vessel density, fractal dimension, and foveal avascular zone using 7 different optical coherence tomography angiography devices. *Am J Ophthalmol*. 2018;186:25–31.
21. Pedinielli A, Bonnin S, Sanharawi ME, et al. three different optical coherence tomography angiography measurement methods for assessing capillary density changes in diabetic retinopathy. *Ophthalmic Surg Lasers Imaging Retina*. 2017;48(5):378–384.
22. Yan Y, Zhou X, Chu Z, et al. Topographic quadrant analysis of peripapillary superficial microvasculature in optic disc drusen. *Front Neurol*. 2021;12:666359.
23. Jung JJ, Yu DJG, Ito K, Rofagha S, Lee SS, Hoang QV. Quantitative assessment of asymmetric choroidal outflow in pachychoroid eyes on ultra-widefield indocyanine green angiography. *Invest Ophthalmol Vis Sci*. 2020;61(8):50.
24. Rosenfeld PJ, Durbin MK, Roisman L, et al. ZEISS Angioplex spectral domain optical coherence tomography angiography: technical aspects. *Dev Ophthalmol*. 2016;56:18–29.
25. Wilkinson CP, Ferris FL, Klein RE, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology*. 2003;110(9):1677–1682.
26. Jung JJ, Chen MH, Shi Y, et al. Correlation of en face optical coherence tomography angiography averaging versus single-image quantitative measurements with retinal vein occlusion visual outcomes. *Retina*. 2020;40(4):786–794.
27. Jung JJ, Yu DJG, Zeng A, et al. Correlation of quantitative measurements with diabetic disease severity using multiple en face OCT angiography image averaging. *Ophthalmol Retina*. 2020;4(11):1069–1082.
28. Sousa DC, Leal I, Moreira S, et al. Retinal vascular reactivity in type 1 diabetes patients without retinopathy using optical coherence tomography angiography. *Invest Ophthalmol Vis Sci*. 2020;61(6):49.
29. Tomlinson A, Hasan B, Lujan BJ. Importance of focus in OCT angiography. *Ophthalmol Retina* 2018;2(7):748–749.
30. Ang M, Wong CW, Hoang QV, et al. Imaging in myopia: potential biomarkers, current challenges and future developments. *Br J Ophthalmol*. 2019;103(6):855–862.
31. Kaizu Y, Nakao S, Yoshida S, et al. Optical coherence tomography angiography reveals spatial bias of macular capillary dropout in diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2017;58(11):4889–4897.
32. Taylor E, Dobree JH. Proliferative diabetic retinopathy. Site and size of initial lesions. *Br J Ophthalmol*. 1970;54(1):11–18.
33. Abramoff MD, Folk JC, Han DP, et al. Automated analysis of retinal images for detection of referable diabetic retinopathy. *JAMA Ophthalmology*. 2013;131(3):351–357.
34. Jani PD, Forbes L, Choudhury A, et al. Evaluation of diabetic retinal screening and factors for ophthalmology referral in a telemedicine network. *JAMA Ophthalmology*. 2017;135(7):706–714.

35. Laotaweerungsawat S, Psaras C, Liu X, Stewart JM. OCT angiography assessment of retinal microvascular changes in diabetic eyes in an urban safety-net hospital. *Ophthalmol Retina*. 2020;4(4):425–432.
36. Scarinci F, Picconi F, Giorno P, et al. Deep capillary plexus impairment in patients with type 1 diabetes mellitus with no signs of diabetic retinopathy revealed using optical coherence tomography angiography. *Acta Ophthalmol*. 2018;96(2):e264–e265.
37. Simonett JM, Scarinci F, Picconi F, et al. Early microvascular retinal changes in optical coherence tomography angiography in patients with type 1 diabetes mellitus. *Acta Ophthalmol*. 2017;95(8):e7510755.