Macular and Peripapillary Optical Coherence Tomography Angiography Metrics Predict Progression in Diabetic Retinopathy: A Sub-analysis of TIME-2b Study Data

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• PURPOSE: To identify optical coherence tomography angiography (OCTA)–derived vessel metrics of the macula and optic nerve head (ONH) that predict diabetic retinopathy (DR) disease progression.

• DESIGN: Secondary analysis of clinical trial data.

• METHODS: This was a sub-analysis of prospectively collected data from 73 subjects that participated in the TIME-2b study (Aerpio Pharmaceuticals), a multicenter clinical trial for patients with moderate-to-severe DR treated with AKB-9778 and followed over a 12-month period. Eligible subjects were tested every 3 months with color fundus photography, spectral-domain OCT, and slit-lamp biomicroscopy. OCTA of the macula and ONH was obtained for a subset of patients enrolled at participating sites. En face, full-depth retinal projections centered at the macula were analyzed for multiple metrics including foveal avascular zone (FAZ) area and perimeter, nonperfusion area, vessel density (VD), and presence of intraretinal microvascular abnormalities (IRMA). VD of the radial peripapillary capillaries was evaluated in 4 quadrants surrounding the optic disc for ONH images. Progression was defined as a ≥2-step increase in DR severity scale score or development of diabetic macular edema.

• RESULTS: Over a follow-up period of 12 months, 15 of 73 (20.5%) subjects progressed. At pretreatment baseline, larger FAZ area, presence of IRMA, and reduced peripapillary VD in the superior temporal and inferior temporal regions were significantly associated with increased odds of progression.

• CONCLUSIONS: FAZ area and temporal peripapillary VD are predictors of DR progression. OCTA metrics may improve progression risk assessment in DR when compared to established risk factors alone. (Am J Ophthalmol 2020;219:66–76. © 2020 Published by Elsevier Inc.)

DIABETIC RETINOPATHY (DR) IS THE MOST COMMON complication of diabetes mellitus.[1] Natural progression of the disease leads to sight-threatening complications of macular edema and vascular proliferation, making DR the leading cause of blindness among working-age adults in the developed world.[2] Owing to the disease's devastating complications, patients with DR require close surveillance by eye care providers.[3] Currently, DR severity established by color fundus photography (CFP) is used to assess progression risk.[4] However, sight-threatening complications of proliferative diabetic retinopathy (PDR) and diabetic macular edema (DME) also occur in patients with more benign fundus appearance. Identifying patients at increased risk of progression would allow health care resources to be focused on this group and would reduce the follow-up burden for patients with more stable disease.

Beyond risk stratification through CFP, vessel visualization with fluorescein angiography (FA) has also identified microvascular risk factors associated with DR disease progression.[5,6] Currently, FA is the primary imaging modality for vascular visualization in DR.[7] However, this procedure is time consuming and invasive, requiring the use of dye injection. Although mostly benign, FA has risks, including nausea, vomiting, and, rarely, anaphylaxis.[8] In recent years, optical coherence tomography angiography (OCTA) has emerged as a noninvasive, depth-resolved modality for imaging the ocular microvasculature.[9] OCTA uses intrinsic motion contrast generated by flowing blood cells, obviating the need for dye injection. Moreover, because OCTA images are derived from repeated OCT B-scans, OCTA is inherently 3-dimensional, thereby allowing for the depth-resolved evaluation of the chorioretinal microvasculature.

A substantial body of literature has emerged to describe DR microvascular changes seen on OCTA. Increases in ischemic area, enlargement of the foveal avascular zone (FAZ), reduced vessel density, and the presence of microaneurysms have all been associated with DR.[10,11] Studies

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have also shown that more severe stages of DR are associated with greater ischemic changes and microvascular abnormalities on OCTA.[12,13] More recently, investigators have identified OCTA changes in the retinal vasculature, such as FAZ area, vessel density, and fractal dimension, that could be predictive of DR progression.[14] The current study expands upon these findings and evaluates both the macula and optic nerve head for OCTA parameters predictive of DR progression.

METHODS

• **DESIGN:** This is a sub-analysis of prospectively collected OCTA data from the TIME-2b study (Aerpio Pharmaceuticals; ClinicalTrials.gov identifier: NCT03197870). Exploration of OCTA metrics was prespecified as part of the study protocol and analysis plan. The TIME-2b study was a prospective, randomized, double-masked, placebo-controlled trial. It evaluated the efficacy and safety of 48 weeks of subcutaneous AKB-9778 (15 mg daily and 15 mg twice daily) when compared to placebo in subjects with moderate-to-severe nonproliferative diabetic retinopathy (NPDR). The study was conducted in 53 sites in the United States in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization.

• **PARTICIPANTS:** Patients aged 18 years or older with type 1 or type 2 diabetes mellitus with moderate-to-severe NPDR (ETDRS level 43-53) as confirmed by the image reading center, no evidence of center-involved DME on spectral-domain OCT (SDOCT) as confirmed by the reading center, and a best-corrected visual acuity (BCVA) letter score ≥70 (ETDRS chart) in the study eye were enrolled in the trial. All inclusion criteria needed to be met in at least 1 eye for enrollment. If both eyes met inclusion criteria, the eye with the highest Diabetic Retinopathy Severity Scale (DRSS) score was designated the study eye.

Baseline exclusion criteria included (1) evidence of center-involved DME, (2) history of DME or DR treatment within 12 months, (3) a decrease in visual acuity due to any ocular pathology other than DR, (4) evidence of active ocular infection, (5) high myopia (≥−8 diopters of correction), (6) history of panretinal photocoagulation, (7) evidence of neovascularization on clinical examination, (8) ocular surgery within 3 months, (9) uncontrolled glaucoma (intraocular pressure [IOP] ≥30 mm Hg on maximum IOP reduction therapy), (10) glycated hemoglobin (HbA1c) ≥12.0%, and (11) uncontrolled hypertension (systolic blood pressure ≥180 mm Hg or diastolic blood pressure ≥100 mm Hg).

Patients were followed prospectively over a 12-month period. ETDRS BCVA, slit-lamp biomicroscopy, IOP, dilated indirect ophthalmoscopy, SDOCT, and CFP were completed at 3-month intervals. BCVA scores were reported as letter scores of 0-100 based on the ETDRS scale.[15] Fundus photographs were graded based on the ETDRS DRSS by at least 2 graders at the Boston Imaging Reading Center (BIRC) and the Wisconsin Fundus Photograph Reading Center. A subset of 73 patients at 18 sites received OCTA imaging of the macula and optic nerve head at each visit. All macular OCTA imaging was graded by 2 independent expert readers at the BIRC; image analysis is described below.

• **ENDPOINTS:** The primary endpoint of the TIME-2b study was ≥2-step improvement in DRSS score at 48 weeks. The trial did not meet the primary endpoint; progression and improvement rates were similar between treatment groups. Thus, progressors were considered as a group regardless of assigned treatment arm in this sub-analysis. The current study uses data collected during the trial to assess if pretreatment, baseline OCTA metrics correlate with DR disease progression.

DR progression was defined as either DRSS progression or development of DME at the end of a 12-month follow-up period.

DRSS progression was defined as a ≥2-step increase from baseline DRSS score, as scored on CFP by at least 2 expert reading center graders.

The TIME-2b study did not strictly define DME. Progression to DME was determined by retina specialists at each participating site and confirmed by the reading center on SDOCT. Example OCTA images of patients who developed DME are displayed in Figure 1 for clarity (SDOCT images were not available to the investigators at the time of publication).

• **OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY IMAGING:** Three OCTA imaging devices were used: Zeiss Cirrus 5000 HD-OCT (Zeiss Meditec, Inc, Dublin, California, USA), Angiovue RTVue XR Avanti (Optovue, Inc, Fremont, California, USA), and Topcon DRI Triton Swept Source OCT (Topcon, Tokyo, Japan). All subjects participating in the OCTA subgroup were scanned every 3 months. Macular imaging included a 3 × 3 mm and 6 × 6 mm scan centered at the fovea. Optic nerve head (ONH) imaging was performed as a 4.5 × 4.5 mm angiography scan centered on the Optovue instrument, and as a 6 × 6 mm angiography scan centered on the ONH in the Zeiss Cirrus device. ONH scans acquired on the Topcon device were excluded from analysis owing to poor fixation and image quality.

• **MACULAR VESSEL ANALYSIS:** Each macular OCTA image was graded for a number of quantitative parameters and presence of intraretinal microvascular abnormalities (IRMA). IRMA were assessed only on 6 × 6 mm scans and defined as irregular branching flow lesions consisting

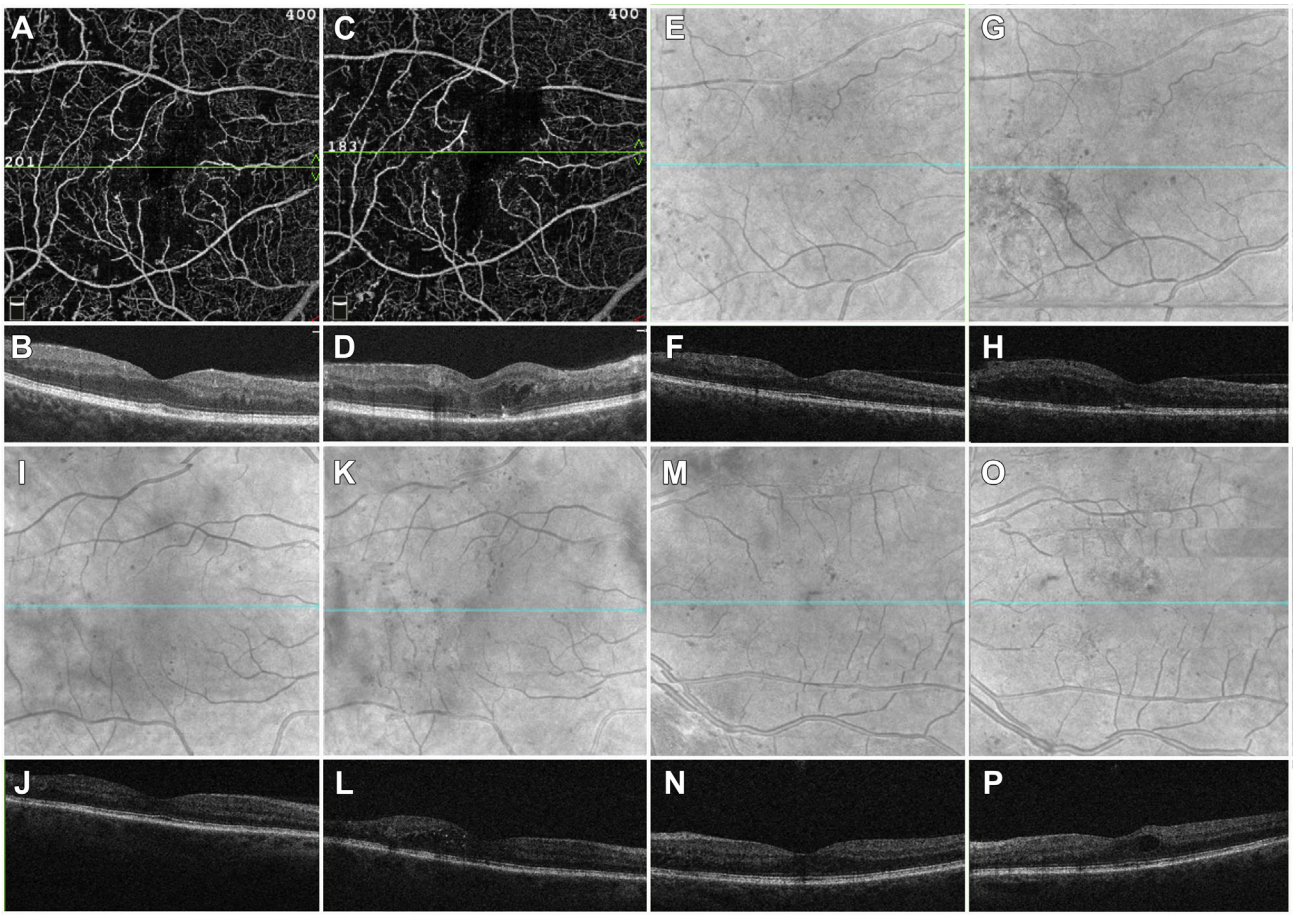

FIGURE 1. Examples of diabetic macular edema (DME) progressors. En face image and associated B-scan at baseline and time of progression are shown for 4 DME progressors. Subject 1: (A, B) Baseline images. (C, D) Progression images. Subject 2: (E, F) Baseline images. (G, H) Progression images. Subject 3: (I, J) Baseline images. (K, L) Progression images. Subject 4: (M, N) Baseline images. (O, P) Progression images.

solely of flow internal to the internal limiting membrane on cross-sectional B-scan (Figure 2). This definition is consistent with previous structural OCT and histopathologic studies.[16]

Quantitative macular metrics were assessed on the full-depth retinal projection. All quantitative macular metrics were calculated using the BIRC OCTA Analysis Toolbox software. This software allows for manual delineation of the FAZ, as well as custom image thresholding optimized for each device. A global binarization threshold was used to process all macular images. As the name implies, global binarization applies the same binarization threshold across the entire image, and is a reliable method for full-depth retinal projection analysis.[17]

All FAZ metrics including FAZ area, FAZ perimeter, and FAZ acircularity index were assessed only on 3 × 3 mm scans. The following metrics were assessed on both 6 × 6 and 3 × 3 mm scans: vessel density, nonperfusion, vessel length, vessel skeleton density, and fractal dimension. To calculate vessel length, vessel skeleton density, and fractal dimension, images were skeletonized after binarization. Skeletonization thins all vessels to a single pixel in thickness, allowing vessel length to be assessed independently of vessel caliber. All metrics and units are defined in Table 1.

• **OPTIC NERVE HEAD ANALYSIS:** Vessel metrics of the radial peripapillary capillary (RPC) (located between the internal limiting membrane and the posterior boundary of the retinal nerve fiber layer [RNFL]) were performed for ONH images acquired on the Optovue and Cirrus devices.

Custom slabs were created to isolate the RPC in each device. A technique published by Rodrigues and associates[18] was used for peripapillary vessel density analysis. In brief, this Fiji (SciJava Consortium)[19] custom macro masks the large branches of the central retinal artery and vein to better assess the peripapillary microvasculature (Figure 3B). Images are binarized with a global default threshold, previously shown to be reliable for peripapillary vessel density

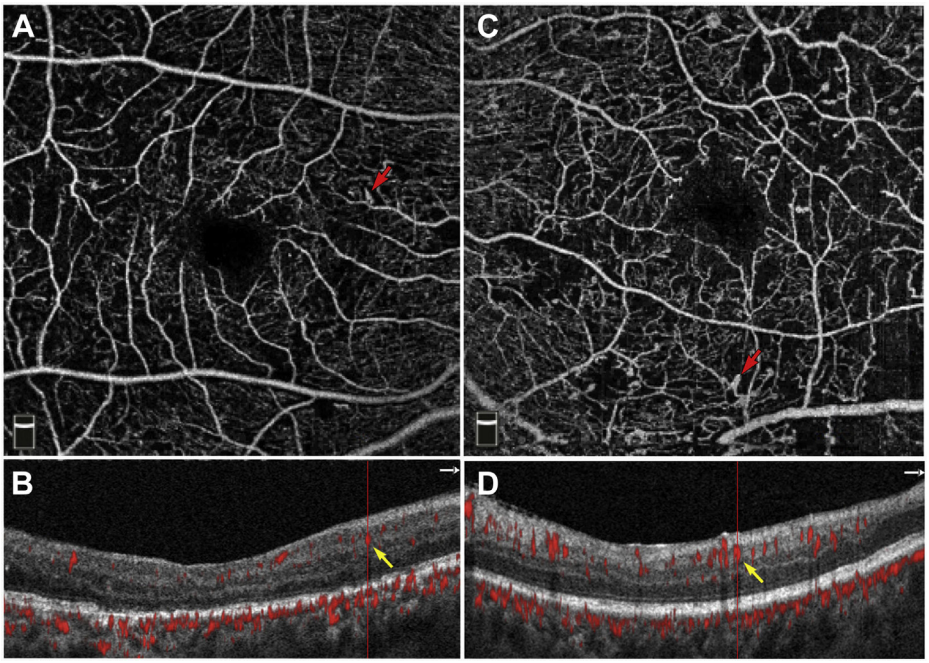

FIGURE 2. En face superficial retina projection and associated B-scan for 2 examples of intraretinal microvascular abnormalities (IRMA). Red arrows point to IRMA on en face (A & C); yellow arrows show flow below the internal limiting membrane on corresponding B-scan (B & D).

analysis.[20] To ensure the same region of interest is analyzed in all images, the macro defines an annular peripapillary region delineated by 2 concentric circles. The inner circle of 1.95 mm in diameter is manually centered around the ONH and is encompassed by a concentric 3.45-mm-diameter outer circle. The annular region is defined as the area between these 2 circles. The macro was modified to divide the annular region into 4 equal quadrants: superior temporal, superior nasal, inferior temporal, and inferior nasal (Figure 3C). This allowed the investigators to assess if microvascular changes in a single peripapillary region were more predictive of DR progression.

RNFL thickness was recorded for each subject at baseline and 12 months. For subjects imaged on the Optovue device, RNFL thickness was determined by the proprietary device software. Cirrus angiography scans do not automatically measure RNFL thickness. Collaborators at Zeiss extracted OCT volume data from ONH OCTA scans. The algorithm used to measure RNFL thickness on the Zeiss Cirrus SDOCT device was modified to process these volume data and generate an RNFL thickness measure. Segmentation lines for all volumes were checked to ensure correct RNFL layer identification.

• **STATISTICAL ANALYSIS:** Multivariate linear regression analysis adjusting for imaging device, refractive error, pre-existing glaucoma diagnoses, and baseline HbA1c was performed to explore associations between baseline OCTA metrics and DRSS scores at baseline and at 12 months. To ensure glaucomatous changes did not account for variations in peripapillary vasculature, paired Student *t* tests were conducted to compare RNFL thickness at baseline and at 12 months for the entire cohort, per device, and for progressors alone. Further, logistic regression analysis (described below) was also performed after exclusion of patients with glaucoma diagnoses and magnification error of >3 diopters, as high myopia increases risk of glaucoma and can affect FAZ measurements.[21–23]

Logistic regression modeling was used to assess the relationship between baseline OCTA metrics and odds of DR progression at 12 months when adjusting for imaging device, treatment group, pre-existing glaucoma diagnoses, and baseline HbA1c as a measure of baseline DR severity. No adjustment was made for baseline DRSS scores, as these were restricted (ETDRS level 43-53) by study design. Similar analyses were performed for DRSS progression alone, for DME development alone, and for each device individually. However, these were restricted to univariate models owing to reduction in progression events and cohort size. Analysis of variance (ANOVA) tests were performed to compare the means of significant metrics across devices.

Receiver operating characteristic curves were plotted for OCTA metrics found to be predictive of DR progression in the overall cohort. These curves plot true-positive rate against false-positive rate, elucidating a model's sensitivity and specificity. Area under the curve measurements were used to quantify this graphical analysis. A standard model was developed based on established risk factors alone (age, diabetes duration, HbA1c, and baseline DR severity

TABLE 1. Definition of Optical Coherence Tomography Angiography Quantitative Metrics

Metric	Unit	Definition
FAZ area	mm^2	Area within manually traced FAZ
FAZ perimeter	mm	Perimeter of manually traced FAZ
FAZ acircularity index	Unitless	Irregularity of the FAZ perimeter when compared to the perimeter of a perfect circle of equal area. Mathematically, it is defined by the formula: $FAZ\ perimeter/\sqrt{4\pi \times FAZ\ area}$
Vessel density	Unitless	Ratio of white pixels (vessels/flow) to total pixels in an image
Nonperfusion	Unitless	The inverse of vessel density in an entire image, excluding the FAZ. It is defined by the formula: $1 - VD$
Vessel length	mm	Sum of the pixels occupied by a skeletonized vessel segment multiplied by the pixel width (mm). All segments are summed to produce a total vessel length measure.
Vessel skeleton density	mm^{-1}	Ratio of the vessel length (mm) over the total area of the image (mm^2)
Fractal dimension	Unitless	Measure of the complexity of a vascular pattern. Fractal dimension, also known as the box-counting dimension, refers to the number of boxes needed to cover a pattern, and is dependent on the size of the box selected.

FAZ = foveal avascular zone.

score). Significant OCTA metrics were individually added to the standard model to assess if a metric's inclusion had a significant effect on the model's predictive power. Models were compared using the bootstrapping method suggested by Steyerberg and associates for internal validation of logistic regression models.[24]

All statistical analyses were conducted on RStudio version 1.1.463.[25]

RESULTS

OF 167 SUBJECTS ENROLLED IN THE TIME-2B TRIAL, A TOTAL of 73 subjects were eligible for this study. Each eligible subject had baseline and 12-month DRSS scores, as well as baseline OCTA imaging. Only study eyes, as designated by the trial, were used in this analysis, for a total of 73 study eyes from 73 eligible patients. Of all subjects included in the study, 39 (53.4%) were imaged on the Zeiss Cirrus device, 23 (31.5%) were imaged on the Optovue Avanti device, and 12 (15.1%) were imaged on the Topcon Triton device. Table 2 summarizes baseline characteristics of included subjects. Of the 73 subjects included, 12 (16.4%) had a ≥2-step increase in DRSS score and 5 (6.9%) developed DME. Two patients overlapped in both DRSS progression and DME development.

• **LINEAR REGRESSION:** Multivariate linear regression analysis showed a significant positive correlation between FAZ area and 12-month DRSS scores (estimate (EST) = 3.71, $P = .009$). The presence of IRMA was also significantly positively correlated with DRSS scores at 12 months (EST = 1.87, $P < .0001$). A significant negative association was found between baseline inferior temporal vessel density and 12-month DRSS scores (EST = −0.06, $P = .02$). Strong associations between baseline vessel density in the annular peripapillary area and superior temporal peripapillary area were noted, though significance was not reached ($P = .09$ and $P = .07$ respectively). No significant associations were found between baseline OCTA metrics and baseline DRSS scores, likely owing to restriction of baseline DRSS scores by study design.

• **GLAUCOMATOUS CHANGES:** Paired Student t tests showed no significant differences between baseline and 12-month RNFL thickness for the overall cohort, per device or among progressors. Further, linear regression analysis showed no significant association between FAZ area and refractive error in this cohort.

• **LOGISTIC REGRESSION:** Table 3 shows the relationship between baseline OCTA metrics and progression at 12 months for the entire cohort on univariate and multivariate analysis. There was a significant positive relationship between FAZ area and progression (β = 3.00, odds ratio [OR] = 20, $P = .038$). Similarly, there was a significant positive relationship between IRMA at baseline and progression (β = 1.74, OR = 5.70, $P = .017$). Both superior temporal peripapillary vessel density (β = −0.14, OR = 0.87, $P = .021$) and inferior temporal peripapillary vessel density (β = −0.16, OR = 0.85, $P = .019$) showed a significant negative relationship with progression. Of note, all significant relationships held true after excluding patients with >3 diopters of magnification error and a history of glaucoma (15 subjects excluded).

Logistic regression analysis for overall progression was performed per device (results not shown). Baseline FAZ area (β = 5.38, OR = 216.81, $P = .023$), FAZ perimeter (β = 1.07, OR = 2.92, $P = .033$), and presence of IRMA (β = 2.77, OR = 16, $P = .035$) were significantly correlated

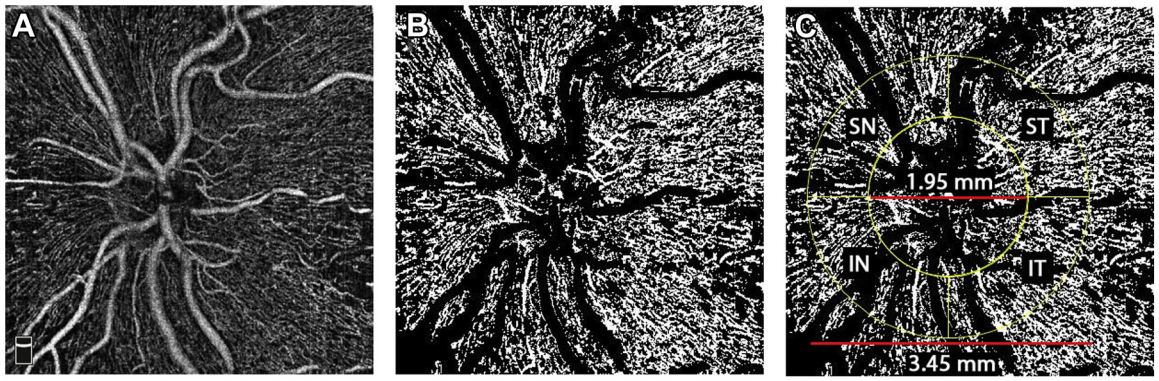

FIGURE 3. Optic nerve head (ONH) optical coherence tomography angiography image processing. (A) En face radial peripapillary capillary (RPC) slab of the ONH as exported from the imaging device (Optovue pictured). (B) En face ONH image after global thresholding and masking of large vessels and device insignia (lower left corner). (C) En face ONH image showing central 1.95 mm circle centered around ONH; this area was excluded from all analyses. Annular area is encompassed by 1.95 mm circle and 3.45 mm ring, further divided into 4 quadrants: IT = inferior temporal; IN = inferior nasal; SN = superior nasal; ST = superior temporal.

TABLE 2. Demographic Data for Subjects at Baseline Visit

Demographic Characteristic	N	Mean (SD) or %
Age (y)	73	59.59 (11.06)
Sex		
Female	37	50.68%
Male	36	49.32%
Race		
White	62	84.93%
African American	7	9.68%
Native American	3	4.11%
Other	1	1.37%
Ethnicity		
Not Hispanic	40	54.80%
Hispanic	33	45.20%
DM type		
Type 1	7	9.59%
Type 2	66	90.41%
Duration of DM (y)	73	16 (10.15)
HbA1c (%)	73	8.40 (1.79)
BMI, kg/m^2	73	30.24 (4.83)

BMI = body mass index; DM = diabetes mellitus; HbA1c = glycated hemoglobin.

with disease progression in the Optovue device, but not on the Cirrus or Topcon devices. Decreased baseline annular vessel density ($\beta = -0.14$, OR = 0.87, $P = .046$), superior temporal ($\beta = -0.16$, OR = 0.86, $P = .015$), and inferior temporal vessel density ($\beta = -0.15$, OR = 0.86, $P = .025$) showed a significant correlation with progression on the Cirrus device, but not the Optovue device. There were no significant associations within the small cohort of patients imaged on the Topcon device. Despite these differences, ANOVA testing showed no significant difference between FAZ area, presence of IRMA, superior temporal vessel density, and inferior temporal vessel density metrics across imaging devices in the overall cohort.

Logistic regression sub-analysis was performed for DME development alone and DRSS progression alone (results not shown). There was a significant positive association between DME development and baseline FAZ area ($\beta = 3.78$, OR = 44, $P = .037$) and presence of IRMA ($\beta = 4.16$, OR = 64, $P = .023$). Sub-analysis for DRSS progression alone showed a significant negative association between DRSS progression and baseline temporal peripapillary vessel density in the inferior and superior quadrants ($\beta = -0.13$, OR = 0.87, $P = .028$). There was also a significant association between DRSS progression and baseline presence of IRMA ($\beta = 1.54$, OR = 4.67, $P = .043$).

• **RECEIVER OPERATING CHARACTERISTIC/AREA UNDER THE CURVE ANALYSIS:** To further assess logistic regression findings, receiver operating characteristic/area under the curve analysis was performed for metrics significantly predictive of DR progression in the overall cohort (FAZ area, superior temporal peripapillary vessel density, and inferior temporal peripapillary vessel density). Figure 4 graphically displays the effect of adding each metric to a standard model based on established risk factors (age, diabetes duration, HbA1c, and baseline DRSS score). All 3 OCTA metrics significantly improved the model's ability to predict DR progression, corroborating our initial findings (Table 4).

DISCUSSION

RECENT LITERATURE SUPPORTS THE USE OF OCTA METRICS as biomarkers for determining risk of progression in DR.[14]

TABLE 3. Disease Progression vs Baseline Optical Coherence Tomography Angiography Metrics

	Univariate Analysis			Multivariate Analysis			
Parameter	Estimates	OR	*P* Value	Estimates	OR	*P* Value	N
3 × 3 Image Metrics							
FAZ acircularity index	0.0029	1.00	.21	0.006	1.01	.178	58
FAZ area	3.00	20.09	.038*	4.75	115.6	.019*	59
FAZ perimeter	−0.006	0.99	.73	−0.007	0.99	.73	59
Nonperfusion	−0.011	0.99	.99	5.69	295.9	.36	60
Total vessel length	−13.89	NA	.69	−13.2	NA	.58	59
Vessel density	0.008	1.00	.83	−0.06	0.94	.47	59
Vessel skeleton density	−0.011	0.99	.88	−0.22	0.80	.17	59
Fractal dimension	4.28	72.24	.68	−4.97	0.007	.70	63
6 × 6 Image Metrics							
Nonperfusion	−1.20	0.30	.42	−3.90	0.02	.35	67
Total vessel length	38.49	NA	.27	20.84	NA	.39	66
Vessel density	0.02	1.02	.25	0.04	1.04	.35	66
Vessel skeleton density	0.002	1.00	.97	0.02	1.02	.86	66
Fractal dimension	−4.30	0.014	.39	−6.66	0.001	.37	63
IRMA	1.74	5.70	.017*	3.00	20.09	.008*	68
Optic Nerve Head Metrics							
Annular vessel density	−0.12	0.89	.10	−0.12	0.89	.13	56
Superior nasal vessel density	−0.02	0.98	.67	−0.06	0.94	.39	56
Superior temporal vessel density	−0.14	0.87	.021*	−0.13	0.88	.044*	56
Inferior nasal vessel density	−0.009	0.99	.87	−0.01	0.99	.85	56
Inferior temporal vessel density	−0.16	0.85	.019*	−0.16	0.85	.045*	56

FAZ = foveal avascular zone; IRMA = intraretinal microvascular abnormalities.

Univariate and multivariate logistic regression analysis comparing diabetic retinopathy progression at 12 months and baseline optical coherence tomography angiography metrics. Multivariate analysis adjusts for baseline HbA1c, imaging device, treatment group, and pre-existing glaucoma diagnoses.

Asterisk indicates statistically significant *P* values.

To our knowledge, this is the first study to assess the association between quantitative OCTA metrics in both the macula and optic nerve head and DR disease progression. Disease progression was defined as either a change in DRSS score greater than 2 steps or DME development. This definition of progression was chosen owing to its increased clinical utility, as both outcomes affect follow-up interval and treatment options.[26–28]

To summarize, macular analysis showed that both a larger FAZ area and the presence of IRMA at baseline were significantly associated with increased odds of progression at 12 months. ONH analysis suggested that lower vessel density in the superior temporal and inferior temporal peripapillary regions at baseline were significantly associated with increased odds of progression at 12 months. The lack of significant change in RNFL thickness throughout the study period suggests peripapillary changes were in fact due to DR progression and not glaucomatous changes. When added to a model based on established risk factors, each of these quantitative OCTA metrics significantly improved the model's predictive ability.

The above results held true when patients with greater than 3 diopters of magnification error and glaucoma diagnoses were excluded from the cohort. However, when logistic regression analysis was performed separately for each device, correlations were found in some devices and not others. Though this could be due to differing properties between imaging devices, breaking up the cohort into 3 different subsets greatly decreases the sample size tested. ANOVA testing showed no significant differences existed between device means for FAZ area, presence of IRMA, and inferior or superior temporal peripapillary vessel density. These results do not confer repeatability across devices; however, they do suggest that metric values are comparable between devices. Clustering device data into a single cohort increases the sample size and power of this study, at the cost of capturing the inherent variability between OCTA instruments.

This study suggests that microvascular changes detected on OCTA are predictive of DR disease progression. Previous FA studies have shown that the presence of IRMA and vascular ischemic changes, such as fluorescein leakage and arteriolar abnormalities, are associated with DR disease progression.[5,6,29,30] Further, a positive association between FAZ area and DRSS score has been well described in both FA and OCTA literature.[31–33] The current study supports

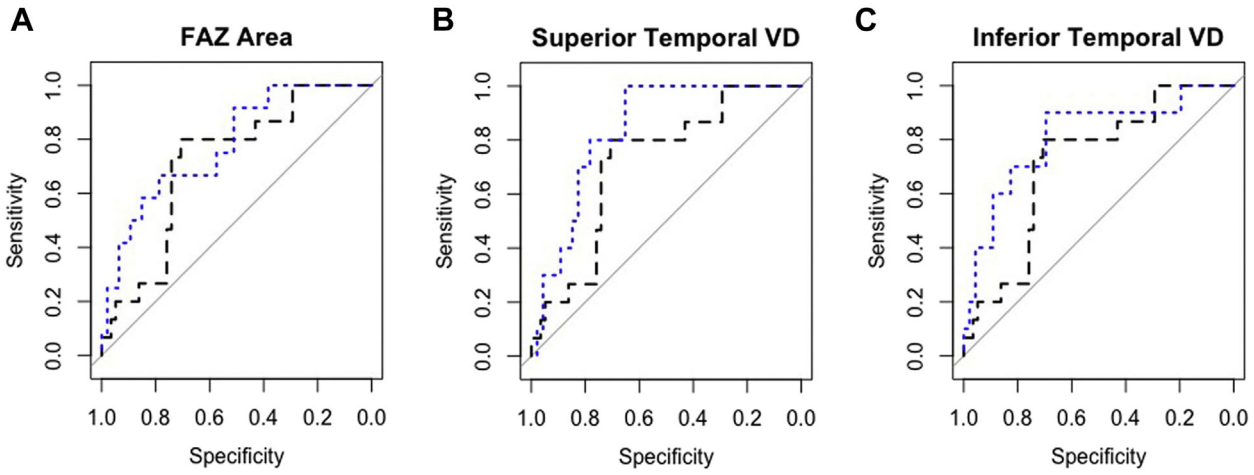

FIGURE 4. Receiver operating characteristic curves for predictive models with 3 different optical coherence tomography angiography metrics. In all images (A-C) the black dashed line represents the receiver operating characteristic (ROC) curve for the standard model based solely on established risk factors (age, diabetes duration, HbA1c, and baseline diabetic retinopathy severity score). (A) Blue dotted line shows ROC of standard model with addition of baseline foveal avascular zone (FAZ) area. (B) Blue dotted line shows ROC of standard model with addition of baseline superior temporal vessel density (VD). (C) Blue dotted line shows ROC of standard model with addition of baseline inferior temporal VD.

these findings—FAZ area and presence of IRMA correlated with higher DRSS scores at 12 months in this cohort—and goes 1 step further to show that baseline presence of IRMA and a larger FAZ area on OCTA significantly increase the odds of DR disease progression.

Recent work by Sun and associates[14] shows that a larger baseline FAZ area in the deep capillary plexus (DCP) correlates with a 2-step progression in DRSS score, and that a larger baseline FAZ area in the superficial capillary plexus (SCP) correlates with increased risk of DME development. The study reported on a prospective cohort of patients with a wide range of disease, including patients with no DR, mild DR, and DME at baseline. Different from our analysis, which focused on the full-depth retinal projection of both 3×3 mm and 6×6 mm scans, Sun and associates analyzed the SCP and DCP separately on 3×3 mm scans using built-in device segmentation. Previous work from our group has shown increased reproducibility of vessel metrics across devices on the full-depth retinal projection, as compared to metrics performed on the DCP or SCP, which are subject to segmentation errors.[34] Use of the full retinal projection is repeatable and reproducible in eyes with and without diabetic macular edema as well as across different devices and is, therefore, more easily and accurately reproduced in both a clinical and reading center setting.

Owing to the known interplay between diabetes and microvascular ischemia, peripapillary vessel metrics have become of interest in recent years. Current evidence points to reduced peripapillary vessel density in diabetic subjects as compared to controls.[18,35,36] Recent work by Liu and associates[22] reported an inverse relationship between peripapillary vessel density and DR severity. Similarly, inferior temporal vessel density was negatively correlated with DRSS scores at 12 months in this study. Though associations between peripapillary vessel density and DR severity have been previously explored, to our knowledge we are the first to show a relationship between reduced peripapillary vessel density and DR disease progression.

Multivariate analysis showed a significant association between reduced peripapillary vessel density in the temporal quadrants and DR disease progression. We postulate 2 hypotheses that could account for this regional finding. The first hypothesis stems from the chosen analytic method. As seen in Figure 3, the temporal quadrants within the annular region are the least interrupted by large vessels, and, therefore, they have a higher fraction of analyzable microvasculature in the RPC slab. A greater area of quantifiable RPC leads to more accurate approximation of pixel density in this region. This provides increased granularity to accurately detect changes in microvascular density in this area. The second hypothesis is based on anatomic structure and known disease pathology. As previously discussed, a large FAZ area correlated with disease progression at 12 months in this cohort. Previous studies show that a large FAZ area is a known marker of diabetic macular ischemia.[37] Anatomically, temporal peripapillary vessels lie closer to the macula. It remains a possibility, therefore, that the temporal vasculature is preferentially affected in this cohort, showing a greater drop in vessel density than that observed in the nasal region. This is the first study to describe regional peripapillary vessel density loss as a marker of DR disease progression.

This work provides further evidence of the utility of OCTA metrics as predictive biomarkers for DR disease

TABLE 4. Area Under the Curve Analysis for Predictive Models

OCTA Metric Added	Standard Model AUC	Standard Model + Metric AUC	*P* Value
FAZ area	0.72	0.78	.041*
Superior temporal vessel density	0.72	0.84	.019*
Inferior temporal vessel density	0.72	0.81	.032*

AUC = area under the curve; FAZ = foveal avascular zone; OCTA = optical coherence tomography angiography; VD = vessel density.

Standard model is based solely on established risk factors (age, diabetes duration, HbA1c, and baseline diabetic retinopathy severity score). Three predictive models were developed by adding 1 of 3 OCTA metrics (foveal avascular zone area, superior temporal vessel density, inferior temporal vessel density) to the standard model. Each predictive model was compared to the standard model by the bootstrapping method of comparison based on random sampling with replacement.

Asterisk indicates statistically significant *P* values.

progression. Most DR studies to date explore only 3 x 3 mm and 6 x 6 mm regions centered at the macula. However, DR pathology is not limited to this central area of vision.[38] By assessing the optic nerve head, we identified novel markers for disease progression beyond the macula, providing a more accurate pathophysiologic picture of DR.

• **STRENGTHS AND LIMITATIONS:** The strengths of this study arise from a prospectively collected and ethnically diverse data set, with 15% of participants identifying as racial minorities and more than 40% of participants identifying as Hispanic (Table 2). All OCTA metrics were computed using a unifying platform and all images were graded by 2 expert readers. As opposed to similar studies in the past, patients had a multiplicity of scans (both 3 × 3 mm and 6 × 6 mm scans) that imaged a greater percentage of the retina and the ONH. This is essential to the study of a disease like DR that diffusely affects the retina.

Owing to the multisite nature of the TIME-2b study, 3 OCTA devices were used for imaging. This is both a limitation and a strength of this study. The limitation lies in the inherent variability seen across OCTA devices, which has been detailed by our group and others.[34,39] Though studies have shown good reproducibility of FAZ area measurements across OCTA devices, there is contradicting evidence regarding the reproducibility of macular vessel metrics.[34,40–42] Few studies have assessed the reproducibility of peripapillary vessel metrics across devices, as these measurements are not available on most commercial OCTA platforms. Though we cannot comment on the reproducibility of metrics in our cohort, as this would have required imaging a subset of individuals on all 3 devices, ANOVA testing did show the means of significant metrics were comparable between devices.

The use of 3 OCTA devices remains a great limitation of this study; however, our experimental design aimed to address inherent device variability and increase metric reproducibility. As previously mentioned, repeatability and reproducibility of OCTA metrics are improved in the full-depth retinal layer across differing devices in both diabetic subjects and healthy controls.[34] All macular data were therefore collected for the full-depth retinal layer, reducing the variability created by differences in software segmentation. Further, all ONH data were manually segmented to ensure the same layer was visualized in all devices. Both macular and ONH data were processed through standardized software to reduce differences that arise from proprietary software. Despite the limitations created by the use of different OCTA devices, reducing segmentation errors and controlling the quantification software identified predictive measures robust enough to be detected beyond inherent device variability. This adds to the clinical utility of our findings in everyday practice, where different equipment is available across ophthalmologic facilities.

Further limitations of this study include a limited follow-up period of 12 months and a relatively small sample size of 73 eyes. The selected sample size and follow-up period restricted the number of progression outcomes captured. Increasing the number of subjects enrolled and the follow-up time per subject in future studies would yield more powerful results. Further, this study uncovered 2 groups of nonoverlapping progressors: subjects who developed DME and did not progress in DRSS scores and subjects who progressed in DRSS scores but did not develop DME. This is consistent with findings from previous studies.[14] A larger sample size could discern differences in baseline OCTA metrics that predict DME or DRSS progression alone. Beyond being clinically relevant, such an investigation could provide new insight into the vascular changes that define each process. Lastly, DME was determined by retina specialists at each clinical site. Structural SDOCT data were not available to the authors at the time of publication and, therefore, DME could not be graded in a standard manner. Though specialist grading is relevant to clinical practice, a uniform DME definition would increase the repeatability of this study.

In conclusion, presence of IRMA, enlarged FAZ area, and decreased temporal peripapillary vessel density increased odds of DR progression at 12 months. The addition of FAZ area and temporal peripapillary vessel density to a model based on established risk factors significantly

improved the model's predictive ability. Future studies with longer follow-up times are needed to determine if these OCTA biomarkers could predict DR progression at even earlier time points.

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