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Quantification of Retinal Nonperfusion and Neovascularization With Ultrawidefield Fluorescein Angiography in Patients With Diabetes and Associated Characteristics of Advanced Disease

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IMPORTANCE Quantification of nonperfusion (NP) and neovascularization (NV) in diabetic retinopathy (DR) may identify better biomarkers of disease progression.

OBJECTIVE To identify demographic risk factors and markers of advanced DR that are associated with increased areas of NP and NV in eyes with disease ranging from no DR but diagnosed as having diabetes to proliferative DR (PDR) and to calculate a threshold total area of NP that may be associated with an increased risk of PDR.

DESIGN, SETTING, AND PARTICIPANTS This retrospective case series was performed on ultrawidefield fluorescein angiography (UWF FA) images from January 2009 to May 2018 at the University of Michigan Kellogg Eye Center. A total of 363 participants (651 eyes) diagnosed as having type 1 or 2 diabetes receiving UWF FA were included. Exclusion criteria included previous panretinal photocoagulation (PRP) and poor-quality images (eg, vitreous hemorrhage and significant cataract).

MAIN OUTCOMES AND MEASURES The surface areas in millimeters squared of the foveal avascular zone; total NP; NP at posterior pole, midperiphery, and far periphery; total NV; NV at posterior pole, midperiphery, and far periphery were measured.

RESULTS Of 363 patients, most were male (205 patients [56.5%]) and white (247 [68%]) or black (77 [21.2%]). The mean (SD) age was 59.4 (13.7) years. Seventy-six eyes with no DR, 92 with mild NPDR, 144 with moderate NPDR, 101 with severe NPDR, 220 with PDR, and 18 with DR of unknown severity were included. Male sex had a positive association with total NP (difference, 15.72; 95% CI, 4.83-26.61; $P = .005$); black race/ethnicity with total NV (difference, 2.32; 95% CI, 0.09-4.55; $P = .04$); and vitreous hemorrhage with total NP (difference, 30.00; 95% CI, 5.26-54.75; $P = .02$). A threshold total NP area of 77.48 mm^2 (95% CI, 54.24-92.66 mm^2) was identified, at greater than which patients may have an increased risk of developing PDR (sensitivity of 59.5% and specificity of 73.6%).

CONCLUSIONS AND RELEVANCE Our results indicate NP and NV can be quantified on UWF FA. These biomarkers interpreted with demographic risk factors may help predict disease progression. Conclusions are limited by ascertainment and information biases because the results are from retrospective data.

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Supplemental content

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Diabetic retinopathy (DR) is the leading cause of new cases of vision loss in adults aged 20 to 74 years.[1-3] The criterion standard for grading DR severity is defined by the Early Treatment Diabetic Retinopathy Study (ETDRS).[4] Through the evaluation of 7 standard retinal fields, a patient's risk of DR progression can be calculated.[5] Ultrawidefield (UWF) scanning laser ophthalmoscopy allows for imaging of a larger area of the retina not captured previously.[6,7] Studies comparing UWF imaging and ETDRS demonstrate significantly higher severity of DR in UWF images and more areas of nonperfusion (NP) and neovascularization (NV).[8-19]

With ETDRS, increasing DR severity only requires the presence of certain pathology, such as intraretinal microvascular abnormality (IRMA) and venous beading, which disregards potentially valuable quantifiable information. Quantifiable measurements, such as total surface area, are difficult to measure in ETDRS for several reasons: the imaging does not include more peripheral retina, there could be distortions in the areas connecting fields, and the montage creates an anatomically inaccurate border that may capture only part of a biomarker.[4]

Better calculation of areas may improve analyses of associations. For example, despite demographic risk factors showing a strong association with DR prevalence within clinical practice, no quantifiable association linking pathologic retinal areas to demographics has been established. However, the clinical relevance of areas, such as the foveal avascular zone (FAZ), NP, and NV, as biomarkers for DR has been shown.[20] Identifying a quantifiable association is particularly pertinent for peripheral NP and NV because they may be associated with increased risk of progression.[8] Similarly, quantifying the association between advanced DR characteristics, such as vitreous hemorrhage (VH) and diabetic macular edema (DME), with biomarkers could provide insight for associations with visually significant pathology.

The objective of this study was to analyze whether certain demographic features and characteristics of advanced DR may be associated with certain biomarkers, namely FAZ, NP, and NV, as segmented on UWF fluorescein angiography (FA). In addition, we determined a threshold area of NP with the highest sensitivity and specificity for association for risk of progression to PDR. By comparing a quantifiable metric to potential risk factors, more objective patterns and trends may be established, which may aid in more effective follow-up and treatment for high-risk populations.

Key Points

Question Which biomarkers calculated from ultrawidefield fluorescein images of patients with diabetes are most associated with demographic risk factors and retinopathy progression?

Findings In this case series, male sex, black race/ethnicity, and presence of vitreous hemorrhage were most strongly associated with greater areas of nonperfusion and neovascularization, and a retinal nonperfusion threshold of 77.48 mm^2 may be associated with increased risk for progression. Given the study's design, statistical significance could not be established.

Meaning These results suggest which factors may indicate higher risk of severe disease progression, and eyes with at least 77.48 mm^2 nonperfusion are at risk of proliferative retinopathy.

Methods

A retrospective medical record review of patients with type 2 diabetes who received UWF FA from January 2009 to May 2018 at the University of Michigan W. K. Kellogg Eye Center was conducted after approval of the University of Michigan institutional review board and adhering to the tenets of the Declaration of Helsinki. Because this study was completed retrospectively, a Health Insurance Portability and Accountability Act waiver of consent was obtained from the University of Michigan institutional review board.

Inclusion and Exclusion Criteria

Eligible participants met the following inclusion criteria: 18 years or older, diagnosed as having type 1 or type 2 diabetes, and evaluated with a dilated fundus examination and UWF FA at Ann Arbor, Michigan, or the Grand Blanc satellite. Images were excluded if there was evidence of panretinal photocoagulation treatment, indistinguishable areas of NV and NP owing to poor image quality, or abundant media opacity (eg, vitreous hemorrhage or cataracts).

Ultrawidefield FA

Images were obtained with an UWF scanning laser ophthalmoscopy (Optos 200Tx or California; Optos PLC). To minimize warping, images were projected onto a curved surface with a nominal eye diameter of 24 mm using proprietary Optos research software that may be available to clinicians in the near future.[9,15,21]

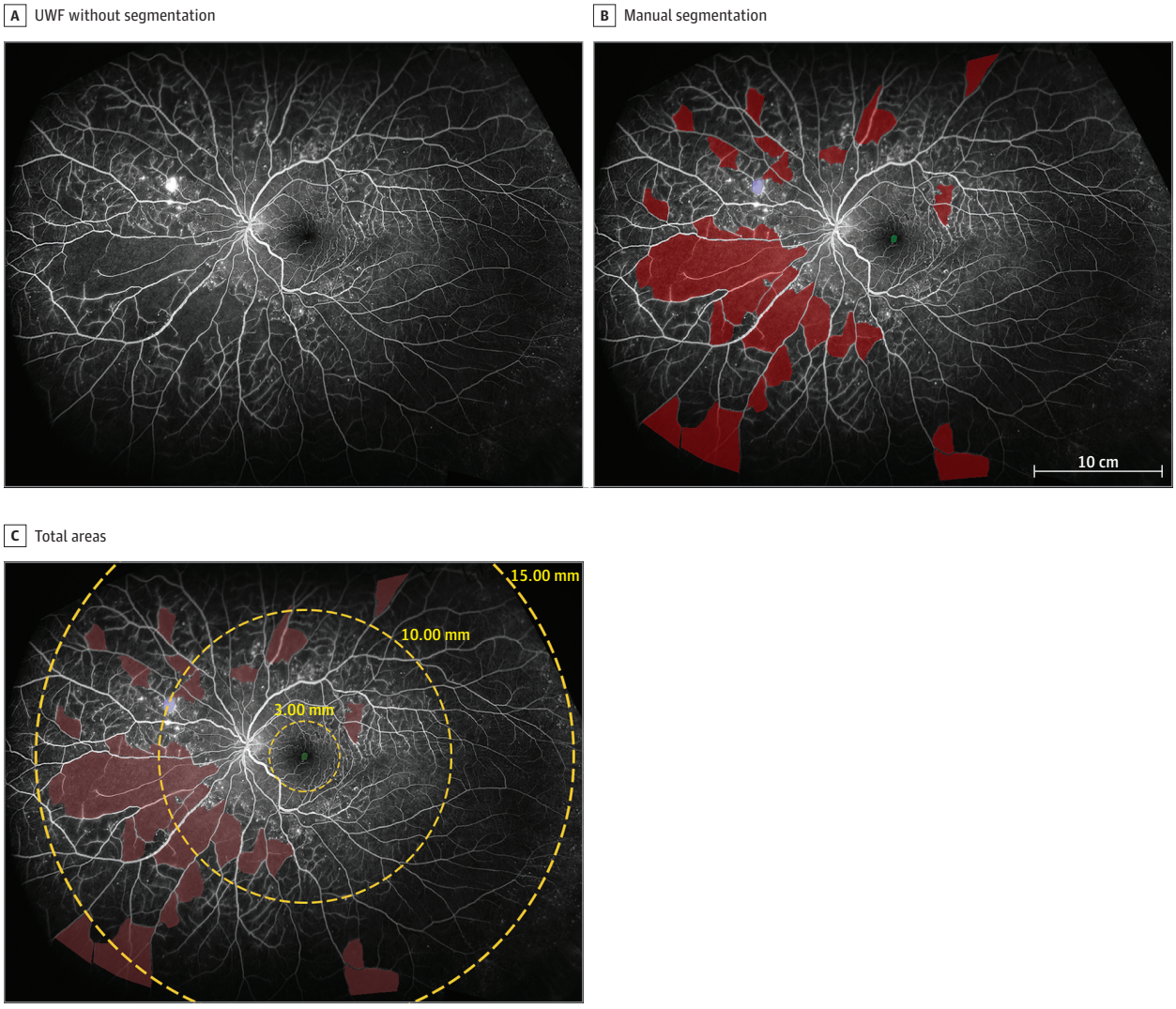
Image Segmentation

All image segmentations were completed using Insight Toolkit SNAP (ITK-SNAP), an open-source application that allows level set active contour segmentation of 3-dimensional images (University of North Carolina Chapel Hill, Insight Toolkit).[22] Areas were delineated manually and color-coded to annotate FAZ, NP, and NV (**Figure**). All graders were masked of all patient data. There were 4 graders (1 postundergraduate researcher who performed most segmentation [more than 85% of images], 2 medical students, and 1 ophthalmology resident). Each grader underwent an extensive training with the first 20 images reviewed in detail for accuracy, verification, and standardization by a single retina specialist experienced in analyzing UWF images. Each image was segmented by 1 grader, and segmentations were considered final unless there were unclear areas, which would then require adjudication by the retinal specialist. Regions with image artifacts like eyelashes were excluded.

Surface Area Quantification

The mechanics of the Optos research software used have previously been described.[21] Foveal avascular zone, NP, and NV surface areas were calculated for each image in millimeters squared. Neovascularization and NP were grouped by region

Figure. Representative Image Segmentation



Patient with proliferative diabetic retinopathy. A, Ultrawidefield (UWF) without segmentation. B, Manual segmentation of nonperfusion (red), neovascularization (purple), and foveal avascular zone (green). C, Areas were totaled using the following radii (*r*) from the center of the foveal avascular zone: posterior pole, *r* 3.00 mm or less; midperiphery, *r* between 3.00 and 10.00 mm; far periphery, *r* between 10.00 mm and 5.00 mm.

depending on their distance from the FAZ: the region within and including 3.00 mm of the identified FAZ was classified as posterior pole; between 3.00 mm and including 10.00 mm was classified as midperiphery; and between 10.00 mm and including 15.00 mm was classified as far periphery (Figure, C).

Statistical Analysis

All statistical analyses were conducted in R (R Core Team, 2019).[23] For qualitative variables (eg, sex), frequencies and relative frequencies (in percentage) were calculated; for continuous variables (eg, age), mean, standard deviation, and ranges were calculated (**Table 1**). Both parametric and nonparametric statistics were used in the statistical comparisons. Generalized estimating equations models were implemented using the geeglm function from the R package geepack available at the Comprehensive R Archive Network (CRAN) to determine *P* values, with *P* less than .05 warranting further testing in a more robust study.[24] These models were used to compute linear multivariate regressions with the area segmentations as outcomes, accounting for correlations between left and right eyes of a single patient. Demographic factors (eye laterality, sex, race/ethnicity, age, and type of diabetes) and features of advanced DR (VH, DME, requirement of PPV) were x variables. Each race/ethnicity was compared with white race/ethnicity for regression analysis. The *P* values are unadjusted given the scope of this study as more hypothesis-generating instead of confirmatory, especially because there are limited previous studies evaluating the associations between demographics and areas segmented from UWF imaging. Because no statistical analysis plan was drafted prior to data collection, statistical significance cannot be established from these results. Instead, an exploratory analysis with consideration of which characteristics

should be studied further within a robust study (ie, factors with $P < .05$) was completed.

The Youden index was calculated to determine the total area of NP that would provide the best sensitivity and specificity in predicting PDR, using a similar technique previously published.[12] The boot package used the adjusted bootstrap percentile method to calculate a 95% confidence interval for the threshold based on 10 000 resamples.[25,26] The R package AUC was used for calculating sensitivity and specificity, and the package pROC was used for the area under the receiving operating characteristic (AUC) value and 95% confidence intervals using the DeLong method.[27] *P* values were 2-tailed and unadjusted.

Results

Demographic Characteristics of Patients

A total of 651 eyes from 363 patients (205 men and 158 women) with no DR (76 eyes), mild NPDR (92 eyes), moderate NPDR (144 eyes), severe NPDR (101 eyes), PDR (220 eyes), or unknown severity (18 eyes) were analyzed (Table 1). The mean (SD) age was 59.4 (13.7) years. Mean (SD) best-corrected visual acuity logMAR was 0.34 (0.31) (Snellen equivalent 20/43.7). The "other" race/ethnicity group included the following races/ethnicities: Native Hawaiian, Pacific Islander, American Indian, and Alaska Native.

Comparison of Demographic Characteristics

The strongest association in demographics and biomarkers (**Table 2**) was the difference in the total area of NP between men and women (difference, 15.72; 95% CI, 4.83-26.61; $P = .005$). Other associations with *P* less than .05 included the difference in NP found at the midperiphery between men and women (difference, 7.12; 95% CI, 1.98-12.26; $P = .01$) and NP far periphery between Hispanic and white (difference, −16.65; 95% CI, −30.87 to −2.44; $P = .02$).

For NV, the difference in NV far periphery between black and white (difference, 0.64; 95% CI, 0.18-1.10; $P = .01$) and other race/ethnicity and white (difference, −0.17; 95% CI, −0.30 to −0.4; $P = .01$) had the strongest associations. Total areas are provided as supplementary material (eTable in the Supplement).

Comparison of Advanced DR Features

In multiple linear regression models accounting for demographic factors, VH had the strongest association with biomarkers (**Table 3**), with a positive association between VH and total NP (difference, 30.00; 95% CI, 5.26-54.75; $P = .02$) and between VH and NP in the midperiphery (difference, 12.85; 95% CI, 2.98-22.72; $P = .01$).

Identifying Threshold Total Area of NP for PDR

To determine the threshold total area of NP with the highest sensitivity and specificity for association with increased PDR risk, the total areas of NP in mild NPDR (mean, 49.2 mm^2; 95% CI, 40.6-57.8), moderate NPDR (mean, 53.9 mm^2; 95% CI, 46.8-60.9), severe NPDR (mean, 67.7 mm^2; 95% CI, 56.3-79.1), and PDR (mean, 101.0 mm^2; 95% CI, 91.0-110.7) were calculated. The threshold total area of NP that maximizes the Youden statistic is 77.48 mm^2 (95% CI, 54.24-92.66), with a sensitivity of 59.5% and specificity of 73.6%. The AUC was 0.7 (95% CI, 0.66-0.75; $P < .001$) and graphed in the supplementary material (eFigure 1 in the Supplement). Additional analysis attempting to achieve a higher AUC was also performed (eFigure 2 in the Supplement).

Table 1. Patient Characteristics

Variable	No. (%)
Patients, No.	
Total	363
Eyes	651
Age, y	
18-44	43 (11.8)
45-54	70 (19.4)
55-64	127 (34.9)
>65	123 (33.9)
Sex	
Male	205 (56.5)
Female	158 (43.5)
Race/ethnicity	
White	247 (68.0)
Black	77 (21.2)
Asian	10 (2.8)
Hispanic	6 (1.7)
Other[a]	12 (3.3)
Unknown	11 (3.0)
Diabetes	
Type 1	61 (16.8)
Type 2	299 (82.3)
Unknown	3 (0.8)
Eyes	
Severity of diabetic retinopathy	
None	76 (11.7)
Mild NPDR	92 (14.1)
Moderate NPDR	144 (22.1)
Severe NPDR	101 (15.5)
PDR	220 (33.8)
Unknown	18 (2.8)
VH	
Yes	18 (2.8)
No	633 (97.2)
DME	
Yes	374 (57.4)
No	277 (42.5)
PPV, No.	
0	616 (94.6)
≥1	35 (5.4)

Abbreviations: DME, diabetic macular edema; NPDR, nonproliferative diabetic retinopthy; PDR proliferative diabetic retinopathy; PPV, pars plana vitrectomy; VH, vitreous hemorrhage.

[a] Other races/ethnicities included Native Hawaiian, Pacific Islander, American Indian, and Alaska Native.

Table 2. Influence of Demographics on Biomarkers as Explored by Multiple Linear Regression Analysis

Confounding factor	Biomarker	Coefficient (95% CI)	*P* value
Sex (male/female)	FAZ	0.00 (−0.13 to 0.12)	.96
	NP total	15.72 (4.83 to 26.61)	.005
	NP posterior	0.30 (−0.16 to 0.76)	.20
	NP mid-periphery	7.12 (1.98 to 12.26)	.01
	NP far-periphery	8.72 (1.61 to 15.82)	.02
	NV total	−0.38 (−1.44 to 0.67)	.48
	NV posterior	−0.07 (−0.25 to −0.10)	.41
	NV mid-periphery	−0.09 (−0.92 to 0.75)	.84
	NV far-periphery	−0.23 (−0.47 to 0.02)	.07
Patient age at time of image, y	FAZ	0.00 (−0.01 to 0.00)	.91
	NP total	−0.30 (−0.71 to 0.10)	.14
	NP posterior	−0.02 (−0.04 to 0.00)	.07
	NP mid-periphery	−0.11 (−0.31 to 0.09)	.27
	NP far-periphery	−0.22 (−0.48 to 0.04)	.09
	NV total	−0.04 (−0.08 to 0.00)	.06
	NV posterior	0.00 (−0.01 to 0.00)	.16
	NV mid-periphery	−0.03 (−0.07 to 0.00)	.06
	NV far-periphery	0.00 (−0.01 to 0.01)	.41
Diabetes type (2/1)	FAZ	0.11 (−0.03 to 0.25)	.12
	NP total	−0.48 (−15.57 to 14.62)	.95
	NP posterior	0.63 (−0.11 to 1.37)	.10
	NP mid-periphery	4.45 (−2.71 to 11.61)	.22
	NP far-periphery	−4.19 (−14.58 to 6.19)	.43
	NV total	−1.59 (−3.73 to 0.56)	.15
	NV posterior	−0.30 (−0.70 to 0.11)	.15
	NV mid-periphery	−1.20 (−2.89 to 0.50)	.17
	NV far-periphery	−0.08 (−0.43 to 0.27)	.66
Race			
Black/white	FAZ	0.05 (−0.07 to 0.17)	.44
	NP total	11.09 (−3.95 to 26.13)	.15
	NP posterior	0.03 (−0.63 to 0.70)	.92
	NP mid-periphery	−0.03 (−6.22 to 6.16)	.99
	NP far-periphery	10.48 (−0.02 to 20.99)	.05
	NV total	2.32 (0.09 to 4.55)	.04
	NV posterior	0.18 (−0.13 to 0.49)	.26
	NV mid-periphery	1.50 (−0.31 to 3.32)	.11
	NV far-periphery	0.64 (0.18 to 1.10)	.01
Asian/white	FAZ	0.24 (−0.19 to 0.68)	.28
	NP total	2.64 (−20.14 to 25.41)	.82
	NP posterior	0.60 (−0.74 to 1.93)	.38
	NP mid-periphery	4.77 (−10.06 to 19.59)	.53
	NP far-periphery	−2.80 (−14.81 to 9.21)	.65
	NV total	−0.01 (−0.66 to 0.64)	.98
	NV posterior	0.02 (−0.08 to 0.12)	.67
	NV mid-periphery	0.11 (−0.41 to 0.64)	.67
	NV far-periphery	−0.14 (−0.28 to 0.00)	.05
Hispanic/white	FAZ	−0.01 (−0.46 to 0.44)	.97
	NP total	−25.93 (−52.46 to 0.61)	.06
	NP posterior	−0.63 (−1.56 to 0.29)	.18
	NP mid-periphery	−10.38 (−24.33 to 3.57)	.14
	NP far-periphery	−16.65 (−30.87 to −2.44)	.02
	NV total	0.12 (−1.36 to 1.60)	.87
	NV posterior	−0.11 (−0.27 to 0.04)	.13
	NV mid-periphery	0.32 (−1.07 to 1.71)	.65
	NV far-periphery	−0.07 (−0.32 to 0.18)	.57

(continued)

Table 2. Influence of Demographics on Biomarkers as Explored by Multiple Linear Regression Analysis (continued)

Confounding factor	Biomarker	Coefficient (95% CI)	*P* value
Other/white	FAZ	0.21 (−0.19 to 0.60)	.30
	NP total	−0.91 (−39.90 to 38.09)	.96
	NP posterior	−0.35 (−1.10 to 0.40)	.35
	NP mid-periphery	3.11 (−16.97 to 23.19)	.76
	NP far-periphery	−4.57 (−24.49 to 15.36)	.65
	NV total	−0.58 (−1.20 to 0.03)	.06
	NV posterior	−0.04 (−0.13 to 0.06)	.43
	NV mid-periphery	−0.37 (−0.83 to 0.09)	.12
	NV far-periphery	−0.17 (−0.30 to −0.04)	.01
Unknown/white	FAZ	0.15 (−0.26 to 0.55)	.48
	NP total	26.57 (−1.53 to 54.67)	.06
	NP posterior	−0.24 (−0.85 to 0.37)	.44
	NP mid-periphery	11.73 (−5.70 to 29.17)	.19
	NP far-periphery	14.81 (−2.83 to 32.46)	.10
	NV total	0.03 (−0.68 to 0.74)	.93
	NV posterior	−0.06 (−0.18 to 0.06)	.31
	NV mid-periphery	0.01 (−0.44 to 0.46)	.96
	NV far-periphery	0.08 (−0.24 to 0.41)	.61

Abbreviations: FAZ, foveal avascular zone; NP, nonperfusion; NV, neovascularization.

Discussion

This study analyzed UWF FA biomarkers for patients with diabetes and compared surface areas of NP and NV to demographic factors and characteristics of advanced DR. It also identified a threshold total area of NP that may be associated with increased PDR risk. The results of this study show male sex, black race/ethnicity, and presence or development of VH are most associated with greater areas of NP and NV in patients with diabetes. Also, eyes with at least 77.48 mm^2 of total NP may be associated with an increased risk of developing PDR. There are numerous studies and surveys describing the demographic characteristics of patients diagnosed as having DR,[28-30] but they are limited by analyses that only compare presence or absence of disease and not incremental, quantifiable data such as the areas of NP and NV at specific radii. With quantifiable data, associations can be calculated, and the potential practicality of the associations may be better understood.

The study's results on sex and race/ethnicity match current literature in that men and black individuals were most strongly associated with having more advanced DR.[29,30] Confounding factors could have contributed to these associations. For example, hypertension is associated with larger biomarkers, especially NP and NV, and is also more prevalent in men and black individuals.[31,32] These unidentified comorbidities will need to be considered in future analyses of this data set. National demographic data also indicate Hispanic patients tend to have more advanced disease.[29,30] Although the results of this study did not corroborate this finding, this may be owing to the limited number of Hispanic patients. Despite a greater proportion of black patients than previous studies,[8,11,13,17-19] there was a disproportionately low percentage of Hispanic patients. Thus, the negative association between Hispanic race/ethnicity and NP area in the far periphery demonstrated by this study must be interpreted accordingly.

Race/ethnicity were more strongly associated with NP area in the midperiphery and far periphery radii compared with total NP area. Similarly, there were stronger associations between race/ethnicity and NV in the far periphery. This was a surprising observation and may argue for the need to examine the far periphery, instead of using a holistic judgment based on the total retina observed. Our results were similar to those of Silva et al,[8,17,18] with far-peripheral pathology associated with increased risk for worse DR severity.

Age had no consistent associations with any of the biomarkers. A similar finding was observed in a study by Chatziralli et al,[33] which showed a significant positive association between DR severity and age on univariate analysis in a study with 120 eyes that lost significance on multivariate analysis. The researchers hypothesized this result was likely because of the strong association between increased age and longer duration of diabetes, making age a confounding variable. Diabetes type also had no consistent associations, except type 1 diabetes was generally associated with larger biomarkers, especially NV. Studies indicate different VEGF gene polymorphisms are associated with each diabetes type, and type 1 diabetes may be associated with a more aggressive gene.[34]

Characteristics of advanced DR were also analyzed because relating these visually significant markers of advanced disease with quantifiable biomarkers could help explain the pathogenesis of PDR. In our study, there was a positive association between VH and NP at the midperiphery. In some patients with diabetes, retinal ischemia can initiate NV and vitreous hemorrhages. Although the far periphery is often associated with the greatest areas of NP and subsequent NV, the association demonstrated in the midperiphery, which includes the optic disc, matches initial RECOVERY study results.[14] The lack of association between VH and NV might be owing

Table 3. Association of Advanced Diabetic Retinopathy Indicators With Biomarkers as Explored by Multiple Linear Regression Analysis

Confounding factor	Biomarker	Coefficient (95% CI)	*P* value
VH (yes/no)	FAZ	0.41 (−0.84 to 1.65)	.52
	NP total	30.00 (5.26 to 54.74)	.02
	NP posterior	2.82 (−0.36 to 6.01)	.08
	NP mid-periphery	12.85 (2.98 to 22.72)	.01
	NP far-periphery	15.34 (−2.62 to 33.30)	.09
	NV total	−0.46 (−2.41 to 1.49)	.64
	NV posterior	−0.23 (−0.48 to 0.01)	.06
	NV mid-periphery	0.02 (−1.63 to 1.68)	.98
	NV far-periphery	−0.33 (−0.69 to 0.03)	.07
DME (yes/no)	FAZ	−0.13 (−0.29 to 0.03)	.11
	NP total	−0.83 (−10.58 to 8.91)	.87
	NP posterior	0.23 (−0.25 to 0.71)	.35
	NP mid-periphery	0.20 (−3.84 to 4.24)	.92
	NP far-periphery	−0.36 (−7.01 to 6.30)	.92
	NV total	−0.86 (−2.06 to 0.33)	.16
	NV posterior	−0.08 (0.22 to 0.07)	.29
	NV mid-periphery	−0.53 (−1.53 to 0.47)	.30
	NV far-periphery	−0.23 (−0.48 to 0.02)	.08
At least 1 PPV	FAZ	0.16 (−0.40 to 0.73)	.57
	NP total	20.10 (−2.55 to 42.76)	.08
	NP posterior	−0.02 (−1.06 to 1.02)	.97
	NP mid-periphery	5.29 (−2.42 to 13.01)	.18
	NP far-periphery	13.33 (−2.78 to 29.43)	.10
	NV total	5.30 (−0.36 to 10.96)	.07
	NV posterior	0.49 (−0.16 to 1.14)	.14
	NV mid-periphery	4.28 (−0.70 to 9.27)	.09
	NV far-periphery	0.52 (−0.24 to 1.28)	.18

Abbreviations: DME, diabetic macular edema; FAZ, foveal avascular zone; NP, nonperfusion; NV, neovascularization; PPV, pars plana vitrectomy; VH, vitreous hemorrhage.

to the relatively small number of patients with VH (18 eyes total) that also presented with areas of NV (10 eyes). Our results showed no association between macular edema and NP or NV, which is in contrast to published studies that show far-periphery ischemia to be significantly associated.[8,11,13] Analyses with larger cohorts of patients with advanced DR characteristics are necessary to elicit a better understanding of their association with biomarkers.

Although the FAZ showed no strong association to any of the variables considered, previous work has shown its clinical significance. Balaratnasingam et al[35] studied the FAZ in 95 eyes and found it to be significantly associated with visual acuity. Sim et al[36] found an enlargement of FAZ between 5% to 10% compared with baseline per year in eyes with DR. With one of the greatest areas of oxygen consumption per gram of tissue in humans, the FAZ is one of the most susceptible areas to ischemic pathology. The contradiction between the presented results and previous results might be because the previous studies analyzed FAZ using optical coherence tomography and 7 standard retinal fields, while our study analyzed FAZ using UWF FA. On UWF FA, the FAZ makes up a much smaller area, so fine differences in FAZ may not be as effectively detected via manual segmentation.

This study also identified a possible threshold area of total NP of 77.48 mm^2 for PDR risk (sensitivity 59.5% and specificity 73.6%). Unlike the analysis completed by Nicholson et al,[12] which also identified a threshold area of NP for PDR progression, our work did not measure disc areas but instead measured manually segmented areas of NP in millimeters squared. Measuring in universal units could allow for more tangible interpretation. Similar to Nicholson et al,[12] we limited analysis to total NP, especially given the lack of power for NV. The finding of a threshold value that may be associated with disease progression could indicate eyes that present with larger total areas of NP may require more aggressive treatment to prevent disease progression. Although analyses of the effects of PRP or anti–vascular endothelial growth factor intravitreal injections were not completed, the threshold could be used in clinical practice as an objective marker for requiring intervention. Current practice relies on subjective clinical evaluation of a patient's eyes to determine need for treatment, which is prone to bias. Although this threshold value cannot bear clinical weight without further research, it is hoped that quantifiable biomarkers may eventually help clinicians identify tangible cutoffs to consider treatment. Also, the relatively low AUC may improve with inclusion of other features and should be a goal for subsequent analyses.

Insight Toolkit SNAP and proprietary software from Optos that adjusts for warping were used to calculate biomarkers. Other techniques to account for warping have been de-

scribed; for example, Nicholson et al[12] used a concentric ring template to estimate disc areas. Consideration of distortion is important when calculating retinal areas since as much as 10% of an area could depend on its axial length.[37] The Optos software minimizes distortion by using an on-axis UWF image of the posterior pole to transform peripheral images with elastic deformation.[21]

The study analyzed UWF FA, but there are other imaging modalities to consider that may yield similar results. Spectral-domain optical coherence tomography angiography (OCTA) or swept-source OCTA are widely available and may be a more feasible option for most clinics. However, they do not provide a widefield capture. Wide-angle OCTA does allow for a breadth of capture similar to UWF FA, but studies are inconclusive regarding its efficacy vs UWF FA.[38-40] Initial analyses indicate UWF FA has slight superiority in sensitivity and specificity for detecting NP and NV.[38] Studies published within the last year suggest wide-angle OCTA is the only follow-up testing necessary for evaluating DR progression.[39,40] Because UWF FA has been established as a valid imaging technique for robust capture of the retina, UWF FA images were used, but further analysis comparing wide-angle OCTA should be considered.

Regardless of imaging modality, the study suggests the importance of UWF in clinical treatment design because UWF captures pathologic areas associated with demographic and diabetic risk factors not captured by the standard ancillary testing. Identification of suggested higher risk populations (eg, male sex, black race, and presence of VH) from this study in clinical practice may indicate more frequent follow-up or aggressive treatment to reduce risk of progression. This study combines the more well-established tools for evaluating disease (UWF FA, demographic risk factors, and visually significant pathology) together with biomarkers, which may help determine who should be followed more closely. Also, this study may indicate an association between certain demographic features and biomarkers that could help elucidate the pathophysiology for why certain populations are associated with greater risk for progression.

Limitations

One main limitation of this study was the unrepresentative breakdown of races, although it did have a larger percentage of African American individuals than previous studies.[8,11,13,17-19] Another limitation was the use of manual graders for segmentation owing to potential variability and inaccuracy but should have been minimized with confirmation and training by the expert retinal specialist. Although images of poor quality were excluded, artifacts, such as eyelashes, still could have caused unintended variability. The quantification also assumed an eye diameter of 24 mm, which could have affected surface areas depending on actual axial lengths. Finally, a prospective study and not a cross-sectional retrospective study is warranted. These results cannot be generalized owing to these limitations.

Conclusions

Given the lack of statistical plan prior to study engagement, unadjusted *P* values, and ascertainment and information biases, no statistical significance could be established. However, the associations between visually significant pathology, demographics, and biomarkers found in this study may suggest which populations are at higher risk for disease progression. More studies evaluating peripheral surface areas are necessary to validate these findings.

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