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Quantification of Retinal Nonperfusion Associated With Posterior Segment Neovascularization in Diabetic Retinopathy Using Ultra-Widefield Fluorescein Angiography

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Abstract

BACKGROUND AND OBJECTIVE: To quantify the size and location of nonperfusion associated with posterior segment neovascularization (NV) in proliferative diabetic retinopathy (PDR) using ultra-wide- field fluorescein angiography.

PATIENTS AND METHODS: Cross-sectional study of 18 eyes with PDR. The total image area, areas of nonperfusion, buds of posterior segment neovascularization (either neovascularization of the disc or elsewhere), and the distances from each bud to the nearest area of nonperfusion and to the disc were measured.

RESULTS: Nonperfused areas with associated neo- vascularization were significantly larger than areas without neovascularization ($32.0\% \pm 5.24\%$ of the retinal image vs. $3.3\% \pm 0.92\%$; $P < .001$) and were more likely to be posteriorly located. Nonperfusion encompassing greater than 23% of the total angiographic image had more associated neovascular buds (9.64 ± 2.16 vs. 0.86 ± 0.29 ; $P < .0001$), which were closer to the disc ($7.53 \text{ mm} \pm 0.27 \text{ mm}$ vs. $9.24 \text{ mm} \pm 0.64 \text{ mm}$; $P = .014$).

CONCLUSION: A threshold size of nonperfusion greater than 23% of the retinal image is associated with posterior segment neovascularization and may serve as an indicator of risk for the development of PDR.

INTRODUCTION

Proliferative diabetic retinopathy (PDR) is a prevalent source of morbidity among patients with diabetes and ranks as one of the leading causes of blindness in the United States.¹ Timely diagnosis and treatment are critical for preventing vision loss. The Early Treatment of Diabetic Retinopathy Study (ETDRS) established a convention for imaging the retina using seven standard fields (7SF) protocol, which captures the central posterior 90° of the retina.² Ultra-widefield fluorescein angiography (UWFA) has emerged as a powerful imaging tool due to its ability to capture a greater amount of the peripheral retina compared to the 7SF protocol, imaging approximately 200° of the retina in a single image.³ Prior

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studies have demonstrated that UWFA captures more total retinal surface area, areas of nonperfusion, areas of retinal neovascularization, and peripheral lesions.^{4,5} Current practice guidelines for diabetic retinopathy were developed using 7SF, but multiple recent reports suggest that UWFA may significantly alter the management of many retinal conditions, including diabetic retinopathy.⁴⁻⁶ One of the proposed implications has been that earlier identification of peripheral areas of nonperfusion may promote earlier treatment with laser photocoagulation prophylactically prior to the development of neovascularization or its complications.⁶

Retinal capillary nonperfusion on fluorescein angiography has been shown to be associated with neovascularization.^{5,7,8} However, the precise size of nonperfusion that accompanies neovascularization is unknown. The purpose of this pilot study was to analyze UWFA retinal images using prototype measurement software to quantify the size and location of individual areas of retinal nonperfusion associated with neovascularization of the disc (NVD) or neovascularization elsewhere (NVE) in eyes with untreated PDR. Because NVD and NVE are both forms of posterior segment neovascularization that can be imaged and quantified using UWFA, heretofore we will refer to them together as “NV.” We hypothesized that areas of retinal nonperfusion associated with NV would be larger in size than areas of nonperfusion without any adjacent NV. The goal of this investigation was to establish an approximate threshold size of nonperfusion above which adjacent NV would be likely to be present. This would provide a numerical metric that would indicate an elevated risk and potentially serve as a screening threshold. Defining such a threshold may facilitate earlier identification of patients at high risk of NV, which could lead to closer follow-up or earlier intervention that may improve the prognosis in advanced disease.

PATIENTS AND METHODS

Study Population and Data Collection

Prospective enrollment of adult patients with PDR presenting to the University of California San Diego (UCSD) Shiley Eye Institute was conducted during a 6-month period. The UCSD Institutional Review Board / Ethics Committee approval was obtained. The work was conducted in a manner compliant with the United States Health Insurance Portability and Accountability Act and was adherent to the tenets of the Declaration of Helsinki.

Potential patients presenting to the retina service at the UCSD Shiley Eye Institute for initial evaluation of possible diabetic eye disease underwent a complete ophthalmologic examination, including measurement of corrected visual acuity (VA), intraocular pressure, slit-lamp examination, and dilated fundus examination. Each patient underwent spectral-domain optical coherence tomography (SD-OCT) of the macula (Spectralis; Heidelberg Engineering, Heidelberg, Germany) and mydriatic UWFA (California A10650; Optos, Dunfermline, United Kingdom) using a standard image view of 200° in a single capture. Demographic information included age, gender, ethnicity, type of diabetes, years since diagnosis of diabetes, whether the patient was currently on insulin treatment at the time of evaluation, most recent hemoglobin A1c (%), and whether the patient had undergone any prior intraocular treatment for diabetic retinopathy.

The inclusion criteria for the study were adult diabetic patients (age 18 years and older) with PDR manifested by posterior segment neovascularization, which was defined by the presence of NVD or NVE with visible angiographic evidence on UWFA. Exclusion criteria included low-quality images (eg, significant vitreous hemorrhage, cataract, or other media opacity), any prior treatment for diabetic retinopathy (including anti-vascular endothelial growth factor [VEGF] therapy, intraocular or periocular steroids, and laser photocoagulation), and the presence of any other significant retinal pathology besides PDR. Patients with anterior segment neovascularization (neovascularization of the iris or of the angle) were excluded, as these forms of neovascularization cannot be visualized by UWFA. Eighteen eyes of 11 consecutive patients were identified that met these inclusion and exclusion criteria, and these study patients were subsequently included in the imaging analysis.

Imaging Analysis

Both SD-OCT of the macula and mydriatic Optos UWFA were reviewed. Diabetic macular edema was defined based on the presence of cystic fluid or thickening of greater than 250 μm within the central subfield on SD-OCT. For analysis of UWFA images, midphase angiograms with minimal eyelid or eyelash artifacts were chosen. For each UWFA image, manual tracings of the total image area, areas of retinal non-perfusion, and buds of NV were completed and verified by two separate unmasked graders (SLB and EN). The total image area was defined as the entire area of visible retina captured in the angiogram. Retinal non-perfusion was defined as any area(s) of the retinal image lacking fluorescein filled capillaries, with a size greater than the physiologic intercapillary distance. No maximum size was prespecified. A bud of NV was defined as a focal area of leakage on the angiogram with a characteristic appearance of fine loops or network of vessels. Each of these three categories of tracings was distinguished by a different color annotation. A representative image depicting these tracings is shown in Figure 1. If there was any disagreement in image tracings between the two graders, the senior retina specialist (EN) made the final decision for the tracing pattern.

The areas of these tracings were calculated using a prototype software provided by Optos, which uses a proprietary algorithm that accounts for size distortion from spherical projection. The sizes of the tracings of nonperfused areas were normalized to the total angiographic image size by calculating the size as a percentage of the total image. The linear distances between areas of nonperfusion and adjacent NV (if present) were measured, as well as the linear distances between all areas of nonperfusion and the optic disc. A bud of NV was considered “isolated” if its location was greater than approximately 1 disc diameter (defined as 1.5 mm) from the border of the nearest area of nonperfusion. Location of the areas of nonperfusion was defined using criteria from the Early Treatment of Diabetic Retinopathy Study (ETDRS)² into posterior (< 10 mm from the disc), mid-peripheral (10 mm to 15 mm), and peripheral (> 15 mm) locations. If an area of nonperfusion traversed more than one location category, it was categorized in the most posterior location.

Statistical Analysis

The sizes of nonperfused areas associated with NV were compared to the sizes of nonperfused areas without associated NV using unpaired two-sided t-tests. The distribution of the locations of areas of nonperfusion associated with NV was compared to the distribution of locations for areas of nonperfusion without NV using a chi-squared analysis. A *P* value of less than .05 was considered statistically significant in both instances. A linear regression analysis was performed to describe the relationship between sizes of nonperfusion and the number of buds of NV.

To define a “threshold” size, the lower limit of the 95% confidence interval for the mean size of retinal nonperfusion associated with NV was used. Areas above this threshold were compared with those below the threshold with respect to the number of associated buds of NV, the distance of associated NV from the optic nerve, and the distance of associated NV from the border of the nonperfused area using unpaired two-sided t-tests. The distribution of associated buds of NV by retinal quadrant location was compared between areas of nonperfusion above and below the threshold size using a chi-squared analysis. A *P* value of less than .05 was considered statistically significant. Statistical analyses were performed using STATA 12 (StataCorp, College Station, TX).

RESULTS

UWFA images of 18 eyes from 11 patients with treatment-naïve PDR were included in the analysis. The patients ranged in age from 25 years to 72 years, with an average age of 45.7 years (Table 1). Most (73%) of the participants had type 2 diabetes, and the same percentage of patients were insulin-dependent. The mean hemoglobin A1c was $8.5\% \pm 0.7\%$. The mean number of years from diagnosis of diabetes to time of presentation was 16.1 (range: 1 year to 32 years). More than half of the eyes (61%) had corrected visual acuity of 20/40 or better. OCT demonstrated macular edema, defined as central thickening of greater than 250 μm or the presence of intraretinal fluid, in 11% of eyes. Two eyes ultimately required vitrectomy for treatment of PDR.

The mean total area of retinal nonperfusion in each image was $297.7 \pm 34.4 \text{ mm}^2$. The total area of retinal nonperfusion was divided by the total angiographic image size to determine the percent of retinal nonperfusion in each eye. The mean percentage of retinal nonperfusion in this cohort was 39.7%. The mean size of areas of retinal nonperfusion associated with NV was $32.0\% \pm 5.24\%$ of the total retinal area, which was approximately 10-times the mean size of areas of retinal nonperfusion without any associated NV, at $3.3\% \pm 0.92\%$ ($P < .001$; Table 2). Areas of nonperfusion were most likely to be located in the mid-peripheral retina, regardless of association with NV. However, almost one-third of areas of nonperfusion with associated NV were posteriorly located, whereas none of the nonperfused areas without associated NV were located in the posterior retina ($P < .001$) (Table 2).

Comparing the sizes of nonperfused areas with the number of associated buds of NV revealed a moderately strong linear relationship ($R^2 = 0.64$; Figure 2), suggesting that as areas of nonperfusion become larger in size, there is a possible association with the development of more numerous buds of NV.

The 95% confidence interval (CI) for the mean size of nonperfusion associated with NV was 23.44% to 40.54% of the total angiographic image size. Based on the lower limit of this 95% CI, the threshold size for nonperfused areas associated with NV was defined as 23% of the total angiographic image. Areas of nonperfusion that were larger than this threshold size were associated with 9.64 ± 2.16 buds of NV, compared with 0.86 ± 0.29 for areas smaller than threshold size ($P < .001$) (Table 3). Areas of nonperfusion above threshold size were also associated with NV that was more posteriorly located (mean distance of $7.53 \text{ mm} \pm 0.27 \text{ mm}$ from the optic nerve) compared with nonperfused areas below the threshold size (mean distance of $9.24 \text{ mm} \pm 0.64 \text{ mm}$ from the optic nerve; $P = .01$) (Table 3). Using the threshold size distinction did not reveal any statistically significant differences in the distances of associated NV from the border of the nonperfused areas or in the distribution of retinal quadrant location of associated NV.

DISCUSSION

PDR is a potentially blinding eye condition with increasing prevalence worldwide.⁹ A key goal in the management of PDR is to identify the disease early, so that appropriate treatment can be delivered, and progression to end-stage disease can be prevented. UWFA has become a powerful tool toward achieving this goal, given its superior ability to image the mid peripheral and peripheral retina in comparison with conventional imaging techniques. In this pilot study, we employed a prototype software to analyze Optos UWFA images of patients with treatment-naïve PDR in order to quantify individual areas of retinal non-perfusion with increased precision and to characterize features, such as size and location, which were associated with the presence of adjacent posterior segment NV. Areas of retinal nonperfusion associated with posterior segment NV tended to be significantly larger in size and more posteriorly located compared with areas that had no associated NV. A threshold size of nonperfusion of greater than 23% of the total angiographic image was associated with a significantly elevated risk of that nonperfused area to be associated with NV.

The majority of the patients with PDR whose images were analyzed in this study had good vision; more than 60% of eyes had corrected VA of 20/40 or better. Furthermore, only about one-tenth of eyes had diabetic macular edema detected on SD-OCT of the macula. In some patients, the areas of NV were small and peripheral. These lesions are often difficult to identify by either ophthalmoscopy or angiography within the ETDRS 7SF. This suggests that UWFA facilitated earlier detection of areas of nonperfusion and NV in these patients.

Areas of nonperfusion were most likely to be located in the mid-peripheral retina, consistent with prior studies.^{4,5,7,8} In our analysis, about one-third of the areas of nonperfusion associated with NV were posteriorly located. In contrast, none of the nonperfused areas without adjacent NV were in the posterior retina. First, this implies that nonperfusion in the posterior pole is a particularly high risk for development of posterior segment NV. In addition, these data are consistent with larger areas of nonperfusion being associated with NV, likely due to disease progression resulting in nonperfusion expanding toward the posterior pole. Frequent monitoring with widefield imaging, therefore, may be useful to detect pathology before it reaches a posterior location.

The purpose of defining a threshold size of non-perfusion was to provide a quantitative metric to be applied to UWFA images to indicate elevated risk of posterior segment NV. This would serve as a screening threshold to alert the provider that the patient may be at elevated risk of complications from proliferative disease. Further specific risk stratification for any given patient would require integration of additional variables, including the location of the nonperfusion, the duration of disease, and level of disease control. With ongoing progress in automated imaging analysis using artificial intelligence, increased interoperability between imaging platforms and the electronic health record, and improved integration of clinical decision support algorithms, this type of screening threshold metric may be able to initiate a point-of-care alert about the patient's potentially elevated risk to the physician at the time of the initial encounter, prompting them to consider a shorter duration between visits, or to initiate earlier treatment. However, larger studies are needed to refine the estimation of the threshold size.

Limitations to this study include the small sample size and the cross-sectional nature of the analysis that only captured a single point in time for each eye. Due to the small sample size, subgroup analyses such as examining the relationship between retinal nonperfusion and diabetic macular edema or analyzing NVD and NVE separately could not be performed. Additionally, because this study analyzed UWFA images at a single point in time, the incidence of NV and longitudinal risk over time could not be ascertained. In addition, the study was limited by the presence of eyelid and eyelash artifacts in the UWFA images, which could have affected the exact measurements of the total angiographic image area and of peripheral areas of non-perfusion.

In summary, UWFA may be an informative tool to establish more precise quantification and characterization of nonperfused areas in PDR. These results suggest that nonperfused areas greater than about a quarter of the total angiographic image are likely to have associated posterior segment NV that is more numerous and more posteriorly located and may prompt closer follow-up or earlier intervention to help avoid vision-threatening complications and improve outcomes.

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Figure 1.

Representative tracings of an ultra-widefield fluorescein angiogram (UWFA) in a patient with diabetes with retinal nonperfusion. Color fundus photo (A) and UWFA (B) of a patient with proliferative diabetic retinopathy. The total visible retinal image is traced in green (C), areas of nonperfusion are delineated in red (D), and buds of retinal neovascularization are noted in fuchsia (D). The distance from the retinal neovascularization to the optic nerve and to the associated nonperfusion is noted in yellow (E).

Figure 2.

Linear regression analysis of the size of nonperfusion with the number of buds of associated posterior segment neovascularization. Almost two-thirds of the variation in the number of neovascular buds was explained by variation in the sizes of areas of retinal nonperfusion, implying that larger areas of nonperfusion are related to the number of associated neovascular buds in a linear fashion.

TABLE 1

Characteristics of Patients With Treatment-Naïve Proliferative Diabetic Retinopathy With Retinal Nonperfusion and Posterior Segment Neovascularization on Ultra-Widefield Fluorescein Angiography

Number of Patients	11
Average Age (Years)	45.7 (Range: 25–72)
Gender	
Male	5 (45%)
Female	6 (55%)
Ethnicity	
Hispanic	4 (36%)
Non-Hispanic White	5 (45%)
Other	2 (18%)
Type of Diabetes	
Type 1	3 (27%)
Type 2	8 (73%)
Mean Number of Years Since Diagnosis of Diabetes	16.1 (Range: 1–32)
Insulin-Dependent	8 (73%)
Mean Hemoglobin A1c (%)	8.5 (Range: 4.4–10.8)
Number of Eyes	18
Corrected Visual Acuity	
20/40 or better	11 (61%)
< 20/40 and 20/80	3 (17%)
< 20/80 and 20/200	3 (17%)
< 20/200	1 (6%)
Lens Status	
Phakic	12 (67%)
Pseudophakic	6 (33%)
Presence of Diabetic Macular Edema	2 (11%)

TABLE 2

Characteristics of Nonperfused Areas Associated With Posterior Segment Neovascularization in Patients With Treatment-Naive Proliferative Diabetic Retinopathy

	Areas of Nonperfusion Associated With NV (n = 21)	Areas of Nonperfusion Not Associated With Any NV (n = 12)	P Value
Mean Size of Area of Nonperfusion (% of Retinal Image)	32.0 ± 5.24	3.3 ± 0.92	<i>P</i> < .001
Location of Nonperfusion			<i>P</i> < .001
Posterior (< 10 mm from the disc)	6 (29%)	0 (0%)	
Mid-periphery (10 mm to 15 mm from the disc)	12 (57%)	8 (67%)	
Periphery (> 15 mm from the disc)	3 (14%)	4 (33%)	

NV = neovascularization

TABLE 3

Characteristics of Neovascularization in Patients With Proliferative Diabetic Retinopathy Based on Threshold Size of Retinal Nonperfusion^a

	All Areas of Nonperfusion (n = 122)	Areas of Nonperfusion < Threshold Size (n = 19)	Areas of Nonperfusion Threshold Size (n = 103)	P Value
Mean Number of Associated Buds of NV	4.56	0.86 ± 0.29	9.64 ± 2.16	<i>P</i> < .001
Mean Distance of Associated NV From the Optic Nerve (mm)	7.79	9.24 ± 0.64	7.53 ± 0.27	<i>P</i> = .01
Mean Distance of Associated NV From the Border of Nonperfused Area (mm)	0.20	0.08 ± 0.03	0.22 ± 0.05	<i>P</i> = .21
Location (Quadrant) of Associated Buds of NV				<i>P</i> = .63
Supernasal	38 (31%)	7 (37%)	31 (30%)	
Inferonasal	27 (22%)	3 (16%)	24 (23%)	
Inferotemporal	29 (23%)	7 (37%)	22 (21%)	
Superotemporal	28 (23%)	2 (11%)	26 (25%)	

^aThe threshold size of nonperfusion was defined as 23% of the total retinal image, which represented the lower limit of the 95% confidence interval for the mean size of retinal nonperfusion associated with neovascularization.

NV = neovascularization