

Association of the Pattern of Retinal Capillary Non-Perfusion and Vascular Leakage with Retinal Neovascularization in Proliferative Diabetic Retinopathy

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Abstract

Purpose: To explore the correlation between retinal capillary non-perfusion and the distribution of retinal neovascularization and vascular leakage (VL) in patients with proliferative diabetic retinopathy (PDR).

Methods: Ultra-widefield angiograms of 96 eyes of 69 patients with PDR were reviewed for the proportion of non-perfused area to total gradable area, and for the presence of neovascularization and VL.

Results: Retinal neovascularization was distributed as such: neovascularization elsewhere (NVE), 57.3%; neovascularization of the disc (NVD), 11.5%; both neovascularization of the disc and elsewhere (NVED), 31.3%. The proportion of non-perfused retina, so-called ischemic index, was greater in eyes with NVED compared to eyes with NVE only, but not when compared to NVD only. Overall, 83% of eyes had VL. The presence and the extent of VL correlated with the proportion of the ischemic index. While VL and ischemic index were more severe in the mid-periphery and far-periphery, the majority of NVE was located in the posterior pole.

Conclusions: The presence of both NVD and NVE is associated with a greater ischemic index than NVE alone. Although both VL and ischemic index is significantly higher in peripheral zones, the majority of neovascularization occurs at the posterior pole.

Keywords: Diabetic retinopathy, Neovascularization, Retinal ischemia, Retinal vasculitis, Ultra-widefield fluorescein angiography, Ultra-widefield imaging, Vascular leakage

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INTRODUCTION

Diabetes affected about 171 million people worldwide in the year 2000 and is projected to affect 366 million by 2030.¹ One of the most common complications and a leading cause of adult-onset blindness is diabetic retinopathy, which has been reported to affect up to 1 out of 3 people with diabetes.² Almost all patients with diabetes for longer than 15 years will have diabetic retinopathy at 15 or more years, and 67% will have proliferative diabetic retinopathy (PDR) at 35 years.³ The underlying pathogenesis of diabetic retinopathy is purported

to stem from a combination of mechanisms, including non-enzymatic glycation of retinal vasculature,⁴ increased phosphokinase C activation,^{5,6} and subclinical inflammation and capillary occlusion.^{7,8}

As ischemia progresses, retinal cells release vascular endothelial growth factor (VEGF) to encourage the growth of new blood vessels, a process known as neovascularization. This process marks the difference between non-PDR and PDR. In PDR, neovascularization is found on the optic disc and

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its surrounding area (neovascularization of the disc [NVD]) or on the remaining area of the retina (neovascularization elsewhere [NVE]). These two different locations have clinical implications, with some experts believing that NVD indicates a more advanced stage of PDR.⁹⁻¹¹ Without treatment, the neovascularization that occurs in PDR can lead to further complications that may result in permanent vision loss, such as tractional retinal detachment and vitreous hemorrhage.

Ultra-widefield fluorescein angiography (UWFA) is a novel imaging modality that allows for improved evaluation of the peripheral retinal vasculature and assessing capillary ischemic index, i.e., proportion of capillary non-perfusion. This technique uses confocal scanning laser ophthalmoscopy technology and a panoramic ellipsoid mirror to image up to 200° of the retina horizontally (representing approximately 82.5% of the total retina surface) in a single capture without the need for a special lens or pharmacologic dilation. UWFA is especially useful for the detection and management of diabetic retinopathy as it can reveal retinal non-perfusion and neovascularization in 10% of eyes missed by standard, conventional fluorescein angiography (FA).¹²

UWFA studies have revealed novel findings in PDR, particularly between retinal non-perfusion and neovascularization. Studies have shown an association between the more extensive peripheral ischemic index and diabetic retinopathy severity,¹³ with some evidence that there may be a certain threshold of retinal non-perfusion for the development of neovascularization.^{14,15} Recent evidence suggests that most ischemia is located in the periphery, while most neovascularization occurs at the posterior pole.¹⁶

Meanwhile, our ability to image greater areas of the retina have identified other important angiographic markers for diabetic retinopathy. Oliver and Schwartz described a new finding in active diabetic retinopathy termed late peripheral vascular leakage (VL), identified on UWFA by vasculitis-like hyper-fluorescence extending beyond the vessel wall into the surrounding capillary bed.¹⁷ They suggested that VL, like neovascularization, could be an equally relevant clinical marker for diabetic retinopathy.

To date, the geographic characteristics of VL in diabetic retinopathy, and their association with non-perfusion and neovascularization, remain unclear. We, therefore, sought to characterize the extent and distribution of retinal ischemic index, VL, and neovascularization, as well as their possible association in PDR.

METHODS

A single-center retrospective cross-sectional study was conducted on patients seen at the Los Angeles County + University of Southern California (USC) Medical Center from August 2014 to July 2016. The database of angiograms consisted of 836 patients with the diagnosis of diabetic retinopathy noted in their medical record charts. Angiograms that met the grading

for PDR were included in the study. Patients with any prior treatment with pan-retinal photocoagulation or intravitreal anti-VEGF treatment were excluded. Images of poor quality or images significantly occluded by artifacts (i.e., eyelids, eyelashes) and vitreous hemorrhage were also excluded. Ninety-six eyes from 69 patients met our inclusion and exclusion criteria and were included in the analysis. Eyes were divided into three groups: those with NVE, those with NVD, and those with evidence of simultaneous neovascularization of disc and elsewhere (NVED). Representative examples of each are shown in Figure 1.

Retinal photographers acquired ultra-widefield (UWF) angiogram images using the Optos Tx200 device (Optos Plc, Dunfermline, UK) after standard intravenous infusion of 5 mL of sodium fluorescein 10%. Acquired images were stored in the Synergy ophthalmic data management system (Topcon, Oakland, NJ). Mid-phase images taken at around 60 seconds were chosen for the analysis. Imaging was exported as unaltered .tiff files and were reviewed by two trained, masked graders. Analysis of non-perfusion was performed with ImageJ software (National Institutes of Health, Bethesda, MD). Areas of capillary dropout were manually traced and used to determine the ischemic index, i.e., the proportion of non-perfused retina to the total viewable area. The proportion of non-perfused area to the total gradable area was reported as a percentage and did not include portions of the image that were obscured by artifact. Three concentric perfusion zones were established based on distance from the center of the fovea as in previously published literature:¹³ posterior pole (<10 mm from the fovea, zone 1), mid-periphery (10–15 mm from fovea, zone 2), and far-periphery (>15 mm from fovea, zone 3).

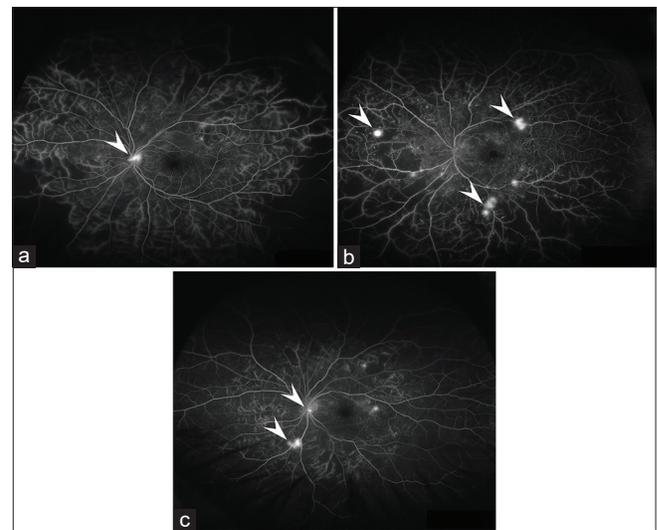


Figure 1: Examples of varying distributions of neovascularization based on disc, retina elsewhere, or both. (a) neovascularization of the disc eye with vascular leakage pattern. (b) neovascularization elsewhere eye (c) neovascularization of disc and elsewhere eye. Select areas of neovascularization indicated by arrowheads

Images were also qualitatively graded for the presence of late VL. VL was defined as late leakage of fluorescein from retinal vessels and capillaries identified on UWFA by vasculitis-like hyper-fluorescence extending beyond the vessel wall. The location of both neovascularization and VL was recorded. The presence of VL and neovascularization was verified by a retina specialist. Location was categorized by four quadrants surrounding the optic disc as center (superonasal [SN]; inferonasal [IN]; superotemporal [ST]; inferotemporal [IT]). Paired *t*-tests were used to compare the mean ischemic index for all eyes by zone. Clustered Wilcoxon rank-sum tests were used to determine statistical significance, with a $P < 0.05$ considered significant.

This research was conducted in accordance with the Declaration of Helsinki. The collection and evaluation of all protected patient health information were performed in a Health Insurance Portability and Accountability Act-compliant manner. Ethical approval for this study was obtained from the USC Institutional Review Board.

RESULTS

Statistical analysis focused on comparisons between three groups of eyes: (1) NVE, (2) NVD, and (3) NVED. Out of the 96 eyes (from 69 patients) with PDR, retinal neovascularization was distributed as such: 55 with NVE (57.3%); 11 with NVD (11.5%); and 30 with NVED (31.3%). Of the 69 patients, 35% (24/69) were female and 65% (45/69) were male. There was no significant difference in systemic factors such as age, gender, ethnicity, or between the three groups. There was a slightly higher hemoglobin A1c in the NVD group, which may have been influenced by the smaller sample size [Table 1]. The intraclass correlation coefficient quantifying inter-grader reliability for the measurement of retinal non-perfusion was 0.87 (95% confidence interval: 0.82–0.92).

The retinal ischemic index in each zone, as well as in all 3 zones combined, was calculated to be as such: for the NVE group, posterior pole: $15.6\% \pm 10.7\%$, mid-periphery: $21.7\% \pm 17.9\%$, far-periphery: $24.4\% \pm 21.2\%$, all zones combined: $20.0\% \pm 13.7\%$; NVD, posterior pole: $20.2\% \pm 11.3\%$, mid-periphery: $30.4\% \pm 15.9\%$, far-periphery: $30.3\% \pm 22.6\%$, all three zones combined: $26.4\% \pm 13.0\%$; and NVED, posterior pole: $22.4\% \pm 12.0\%$, mid-periphery: $34.8\% \pm 17.8\%$, far-periphery: $39.7\% \pm 23.5\%$; all zones combined: $31.1\% \pm 14.1\%$. This data are represented in Figure 2. In general, the proportion of non-perfused retina increased from posterior pole to the far-periphery in all three groups. For all eyes combined, there was an increase in mean ischemic index when moving from the posterior pole to the periphery (that is, mid-periphery has a significantly greater ischemic index than the posterior pole ($P < 0.0001$), and far-periphery has a significantly greater ischemic index than mid-periphery ($P = 0.0396$) and the posterior pole ($P < 0.0001$)).

Eyes with NVED exhibited a greater retinal ischemic index compared to eyes with NVE only in all three zones, separately

Table 1: Demographics of eyes in study (neovascularization elsewhere vs. neovascularization of the disc vs. neovascularization of disc and elsewhere)

	NVE (n=55)	NVD (n=11)	NVED (n=30)
Average age	50.8	52.1	47.6
Average hemoglobin A1c level	8.63	9.76	8.11
Gender (# female)	21	4	11

Average age, average hemoglobin A1c level, and gender count of each group. There were no significant age or gender differences. Average hemoglobin A1c levels were higher in the NVD group. NVE: Neovascularization elsewhere (of area of the retina besides disc), NVD: Neovascularization of the disc, NVED: Neovascularization of disc and elsewhere

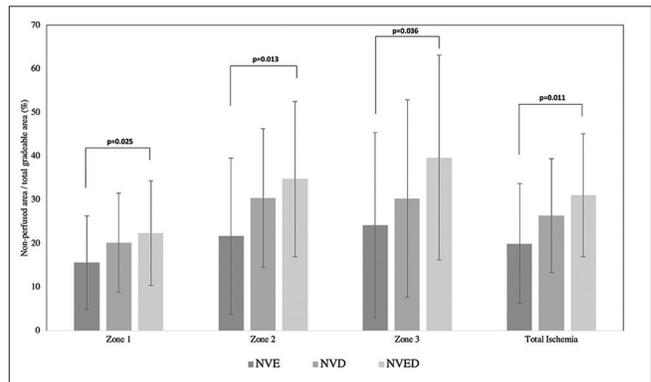


Figure 2: Proportion of non-perfused retina in eyes with proliferative diabetic retinopathy (PDR) across all zones. Proportion of ischemic retina increased from zone 1, to zone 2, to zone 3, in all three groups of eyes with PDR. The mean ischemic index increased from neovascularization elsewhere (NVE) to neovascularization of the disc (NVD) and was highest in the neovascularization of disc and elsewhere (NVED) group. Although the ischemic index difference reached statistical significance between NVED and NVE, it did not reach statistical significance between NVE and NVD, nor between NVED and NVD

and combined, (posterior pole: $P = 0.025$, mid-periphery: $P = 0.013$, far-periphery: $P = 0.036$, all zones combined: $P = 0.011$). Although eyes with NVED also had a greater proportion of ischemic index compared to NVD alone across all zones, this relationship was not significant (posterior pole: $P = 0.629$, mid-periphery: $P = 0.456$, far-periphery: $P = 0.196$, all zones combined: $P = 0.401$). Eyes with NVD demonstrated increased proportion of ischemic index compared to NVE, but this relationship was also non-significant (posterior pole: $P = 0.217$, mid-periphery: $P = 0.145$, far-periphery: $P = 0.409$, all zones combined: $P = 0.136$).

Next, we analyzed the relationship between the proportion of ischemia (as measured by ischemic index) and the number of quadrants with VL. Eighty-three percent of eyes (80/96) were noted to have the VL pattern. All NVD and NVED eyes had VL. In the posterior pole and in the mid-periphery, there was no statistically significant difference in mean ischemic index between eyes with VL and eyes without VL when stratifying by zone, while in the far-periphery eyes with VL had a significantly greater mean ischemic index than eyes without

VL ($P = 0.033$). Overall, eyes with VL had a significantly greater mean ischemic index ($P = 0.011$) than eyes without VL when considering all zones.

Our findings are represented in Figure 3. Subjects with VL in all four quadrants had 8.61% more ischemia in the posterior pole than those without VL ($P = 0.0035$), after controlling for age and inter-eye correlation. Similar results were seen between subjects with VL in all four quadrants compared to those without any quadrants for mid-periphery (15.1% more ischemia, $P = 0.0015$), far-periphery (18.5% more ischemia, $P = 0.0017$), and all zones combined (12.0% more ischemia, $P = 0.0003$). For zones 2, 3, and all zones combined, subjects with 3 quadrants of VL compared to no VL were also found to have an increased amount of ischemia (mid-periphery: 11.91% more ischemia $P = 0.019$; far-periphery: 16.8% more ischemia, $P = 0.0103$; all zones combined: 9.1% more ischemia, $P = 0.016$). There was no evidence for a correlation between localization of vasculitis and NVE in any quadrant, in any zone, as evidenced by non-significant point-biserial Pearson correlation coefficients—no Bonferroni correction was applied because all P values were already non-significant at the standard 0.05 level.

We also characterized the location of neovascularization outside of the optic disc in NVE and NVED, by zone and by quadrant. A retinal chart depicting the location of all neovascular lesions is shown in Figure 4. Neovascularization occurred more frequently in the nasal hemisphere in both NVE (57.39%, $P = 0.057$) and NVED eyes (64.06%, $P = 0.0122$). There was also significant predilection of these lesions for posterior pole for NVE (85.48%, $P < 0.001$) and NVED (93.1%, $P < 0.0001$) eyes. A summary of data representing the percentages of eyes having neovascularization and VL in different locations is shown in Table 2.

In addition, we observed an association between different areas of neovascularization and VL location by quadrant. Subjects with NVE were 30.0% less likely to have VL in the

SN quadrant ($P = 0.0003$) and 22.5% less likely to have VL in the IN quadrant ($P = 0.0092$) compared to subjects with NVD or NVED. Conversely, subjects with NVED were 49.2% more likely to have VL in the SN quadrant ($P < 0.0001$) and 27.1% more likely to have VL in the IN quadrant ($P = 0.0253$) compared to subjects with NVE or NVD alone. A significant relationship was not found regarding the ST and IT quadrants in either group. Furthermore, a significant correlation was not found for the location of VL in NVD subjects compared to the other groups.

When examining the mean best corrected visual acuity represented in the logMAR, NVD was associated with poorer visual acuity at the time of imaging than NVE (0.459 ± 0.283 vs. 0.256 ± 0.240 , $P = 0.027$). There was no significant difference in visual acuity when eyes with NVED were compared to NVE ($P = 0.084$) or NVD ($P = 0.190$).

DISCUSSION

The additional pathology that is revealed on ultra-widefield imaging may have some predictive value in the progression of diabetic retinopathy. Untreated peripheral non-perfusion and late VL as detected on ultra-widefield FA have been found to be associated with neovascularization in diabetic retinopathy,¹⁷ and the presence of predominantly peripheral lesions, such as microaneurysms, hemorrhages, and venous beading, have been found to be associated with an increased risk of disease progression over 4 years, independent of baseline diabetic retinopathy severity and hemoglobin A1c levels.¹⁸

Using UWFA, we were able to more completely image the vasculature and associated features in PDR. A robust explanation for why certain patients develop neovascularization in different areas remains out of reach. Our study does, however, indicate that the location and extent of retinal non-perfusion is a factor associated with neovascularization of varying forms.

Earlier wide-angle angiography studies established the concept that vasculopathy and non-perfusion in diabetic retinopathy

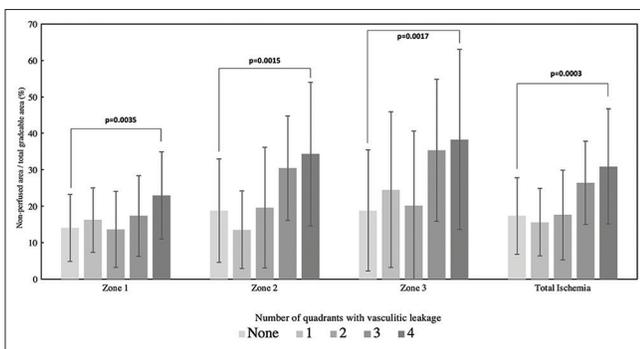


Figure 3: Proportion of non-perfused retina and the presence of late vascular leakage (VL) in fundus quadrants. The presence of VL in all four quadrants was significantly associated with an increased proportion of ischemic retina in zone 1. The presence of VL in all four quadrants, as well as in three quadrants, was significantly associated with an increased proportion of ischemic retina in zones 2 and 3. Zone 2: Mid-periphery, Zone 3: Far-periphery

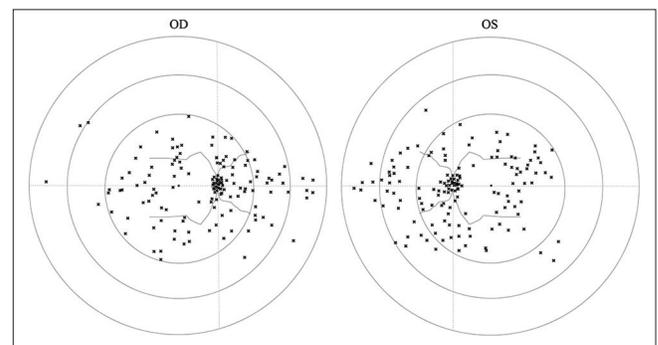


Figure 4: Scatter fundus diagrams showing the location of neovascularization in all eyes. Retinal chart is shown with macula as center and three circles denoting previously mentioned zones. Note predominance of neovascularization elsewhere in the posterior pole and higher density in nasal retina. OD: Right eye, OS: Left eye

Table 2: Locations of neovascularization and vasculitic lesions by quadrant and zone

	SN (%)	IN (%)	ST (%)	IT (%)	Zone 1 (%)	Zone 2 (%)	Zone 3 (%)
Location of neovascularization							
NVE (<i>n</i> =56)	58.90	58.90	39.20	48.20	94.60	46.40	16.10
NVED (<i>n</i> =28)	67.90	78.60	46.40	35.70	96.40	53.60	7.10
Location of VL							
NVE (<i>n</i> =42)	64.20	71.40	83.30	78.60	54.80	81.00	76.20
NVD (<i>n</i> =8)	100	87.50	75.00	75.00	50.00	100	100
NVED (<i>n</i> =25)	96.00	84.00	88.00	84.00	52.00	100	100

Data is represented as percentage of eyes that had lesions in the various quadrants and zones. Neovascularization most commonly occurred in the nasal hemisphere zone 1 for both NVE and NVED. SN: Superonasal, IN: Inferonasal, ST: Superotemporal, IT: Inferotemporal, Zone 1: Posterior pole, Zone 2: Mid-periphery, Zone 3: Far-periphery, NVE: Neovascularization elsewhere (of area of the retina besides disc), NVD: Neovascularization of the disc, NVED: Neovascularization of disc and elsewhere, VL: Vascular leakage

occurred more commonly in the mid-periphery than the posterior pole.¹⁹⁻²¹ While it was true that the posterior pole showed the least amount of ischemic retina in our study's own PDR groups, we found that zone 3 (far-periphery) contained the highest proportion of non-perfused retina. Our ability to image the far-periphery with this ultra-wide field modality may indicate that the furthest areas of the retina were previously not accounted for in some earlier studies – these earlier studies captured images that ranged from 60°¹⁹ to 130° field of view,²¹ and not necessarily simultaneously. This highlights the importance of visualizing the far-periphery in understanding the nuanced pathophysiology and clinical course of ocular disease like diabetic retinopathy.

Our study found a strong predilection for neovascularization in the posterior pole despite the abundance of non-perfusion in outer retinal zones (mid- and far-periphery). These results are supported by similar findings in recently published studies.^{15,16} Fan *et al.* used a different steering method for image acquisition and stereographic correction techniques but similarly found that ischemia was most severe in the far-periphery while neovascularization was most likely to occur in the posterior pole than the mid periphery or far-periphery.¹⁶ In addition, Nicholson *et al.*¹⁴ found that eyes with NVD generally had increased non-perfusion when compared to eyes with only NVE. The current study suggests a more nuanced interpretation, given the fact that it was only the NVED group, and not NVD, that demonstrated increased ischemic index compared to NVE in a statistically significant manner.

Based on our results, it is quite possible that VEGF's actions extend beyond simple local effects. The posterior pole's increased susceptibility to neovascularization may indicate a more robust release of VEGF in response to hypoxia in the posterior pole. Alternatively, the posterior pole may have an increased density of VEGF tyrosine kinase receptors compared to the more peripheral regions. A similar phenomenon of differential VEGF release or receptor density may explain the predilection of neovascularization in the nasal hemisphere as well. Another hypothesis is that the retinal areas of greatest non-perfusion – the far-periphery – may be too severely injured to produce or secrete VEGF. Understanding this complex relationship has important clinical implications and may help

guide decision-making, such as the geographical distribution of retinal photocoagulation administered.

It is currently unclear whether other markers of diabetic retinopathy relate to retinal non-perfusion in a similar way. The study is the first to use UWF imaging to include late VL in this discussion. Oliver and Schwartz first described the finding of late VL in diabetic retinopathy, and found that the presence of peripheral VL was strongly associated with both peripheral retinal ischemia and posterior pole neovascularization.¹⁷ We similarly observed that increased ischemia was associated with an increased presence of VL, as measured by the number of quadrants involved. In addition, while VL showed a predilection for peripheral regions, as did non-perfusion, about 50% of VL involved the posterior pole in our study. Neovascularization of the nasal disc (NVED and NVD) seemed to be associated with the increased presence of nasal VL. This additionally suggests that the vasculitic pattern observed may be due to a robust VEGF drive, though with a potentially stronger and more relevant geographical correlation with non-perfusion than neovascularization. VEGF's action to increase capillary permeability and degrade vascular basement membrane could lead to the development of VL before neovascularization is seen. As we learn more about the differences in patients that lead to neovascularization of the disc versus elsewhere, we might consider the presence of VL lesions to be a prognostic indication of the risk of NVED/NVD development and worse visual outcome.

Of note, when looking at the entire viewable retina, there was a higher proportion of ischemic retina in eyes without any signs of VL, compared to eyes with VL in 1 quadrant. This effect seemed to be primarily driven by the mid-periphery [Figure 3]. VL may thus be additionally influenced by a combination of factors independent of VEGF; for example, the capillary hyperpermeability leading to observed VL might be caused by microvascular damage directly from hyperglycemia, hypoxia, and other pathways. However, further studies are needed to determine the true significance of VL and why certain patients do not exhibit this feature. VEGF has been historically detected throughout the vitreous,²² but the varied distribution of VL suggests that VEGF's effects are not uniform.

A limitation of this study is that the study population was collected from a single healthcare institution, namely a county hospital, where the demographics may not be generalizable to other populations. In addition, the current study did not assess potential confounding factors such as macular edema, macular ischemia, and media opacity, which could have affected our results, especially visual acuity. Other sources of limitation come from the image analysis itself. In many cases, the peripheral retina is poorly visible or obscured by eyelids and eyelashes, especially in the inferior and superior fields. These areas were excluded from analysis; however, if these regions did not have the same pattern of non-perfusion as the posterior pole and the nasal and temporal areas, the data may be skewed. In addition, another potential source of error is that UWFA systems convert a three-dimensional image into a flat, two-dimensional image and inherently introduce some peripheral distortion that worsens with an increased field of view. Certain stereographic projection techniques have been developed to minimize this phenomenon.²³ However, Tan *et al.* demonstrated a strong correlation between these techniques and the commonly used ischemic index technique that we used in our study.

Ultra-widefield angiography allows us to image greater areas of the retina and glean useful information regarding the characteristics of PDR. Neovascularization of both the disc and retina is associated with greater areas of retinal ischemic index than neovascularization of the retina alone. In addition, the development of NVED and the presence of VL may be caused by massive amounts of retinal ischemic index and high VEGF drive. In this study, ischemic index and late VL was distributed more in the mid-periphery and far-periphery of the retina, while neovascularization more commonly occurred at the posterior pole. These findings add new insight into the processes underlying PDR.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004;27:1047-53.
2. Wong TY, Klein R, Islam FM, Cotch MF, Folsom AR, Klein BE, *et al.* Diabetic retinopathy in a multi-ethnic cohort in the United States. *Am J Ophthalmol* 2006;141:446-55.
3. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. *Arch Ophthalmol* 1984;102:520-6.
4. Chibber R, Molinatti PA, Kohner EM. Intracellular protein glycation in cultured retinal capillary pericytes and endothelial cells exposed to high-glucose concentration. *Cell Mol Biol (Noisy-le-grand)* 1999;45:47-57.
5. Kim JH, Kim JH, Jun HO, Yu YS, Kim KW. Inhibition of protein kinase C delta attenuates blood-retinal barrier breakdown in diabetic retinopathy. *Am J Pathol* 2010;176:1517-24.
6. Koya D, King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes* 1998;47:859-66.
7. Luttly GA, Cao J, McLeod DS. Relationship of polymorphonuclear leukocytes to capillary dropout in the human diabetic choroid. *Am J Pathol* 1997;151:707-14.
8. Schröder S, Palinski W, Schmid-Schönbein GW. Activated monocytes and granulocytes, capillary nonperfusion, and neovascularization in diabetic retinopathy. *Am J Pathol* 1991;139:81-100.
9. Deckert T, Simonsen SE, Poulsen JE. Prognosis of proliferative retinopathy in juvenile diabetics. *Diabetes* 1967;16:728-33.
10. Jansson RW, Frøystein T, Krohn J. Topographical distribution of retinal and optic disc neovascularization in early stages of proliferative diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2012;53:8246-52.
11. Valsania P, Warram JH, Rand LI, Krolewski AS. Different determinants of neovascularization on the optic disc and on the retina in patients with severe nonproliferative diabetic retinopathy. *Arch Ophthalmol* 1993;111:202-6.
12. Soliman AZ, Silva PS, Aiello LP, Sun JK. Ultra-wide field retinal imaging in detection, classification, and management of diabetic retinopathy. *Semin Ophthalmol* 2012;27:221-7.
13. Silva PS, Dela Cruz AJ, Ledesma MG, van Hemert J, Radwan A, Cavallerano JD, *et al.* Diabetic retinopathy severity and peripheral lesions are associated with nonperfusion on ultrawide field angiography. *Ophthalmology* 2015;122:2465-72.
14. Nicholson L, Ramu J, Chan EW, Bainbridge JW, Hykin PG, Talks SJ, *et al.* Retinal nonperfusion characteristics on ultra-widefield angiography in eyes with severe nonproliferative diabetic retinopathy and proliferative diabetic retinopathy. *JAMA Ophthalmol* 2019;137:626-31.
15. Baxter SL, Ashir A, Nguyen BJ, Nudleman E. Quantification of retinal nonperfusion associated with posterior segment neovascularization in diabetic retinopathy using ultra-widefield fluorescein angiography. *Ophthalmic Surg Lasers Imaging Retina* 2019;50:86-92.
16. Fan W, Nittala MG, Velaga SB, Hirano T, Wykoff CC, Ip M, *et al.* Distribution of Nonperfusion and Neovascularization on Ultrawide-Field Fluorescein Angiography in Proliferative Diabetic Retinopathy (RECOVERY Study): Report 1. *Am J Ophthalmol* 2019;206:154-60.
17. Oliver SC, Schwartz SD. Peripheral vessel leakage (PVL): A new angiographic finding in diabetic retinopathy identified with ultra wide-field fluorescein angiography. *Semin Ophthalmol* 2010;25:27-33.
18. Silva PS, Cavallerano JD, Haddad NM, Kwak H, Dyer KH, Omar AF, *et al.* Peripheral lesions identified on ultrawide field imaging predict increased risk of diabetic retinopathy progression over 4 years. *Ophthalmology* 2015;122:949-56.
19. Bek T, Helgesen A. The regional distribution of diabetic retinopathy lesions may reflect risk factors for progression of the disease. *Acta Ophthalmol Scand* 2001;79:501-5.
20. Kern TS, Engerman RL. Vascular lesions in diabetes are distributed non-uniformly within the retina. *Exp Eye Res* 1995;60:545-9.
21. Shimizu K, Kobayashi Y, Muraoka K. Midperipheral fundus involvement in diabetic retinopathy. *Ophthalmology* 1981;88:601-12.
22. Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, *et al.* Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med* 1994;331:1480-7.
23. Tan CS, Chew MC, van Hemert J, Singer MA, Bell D, Sadda SR. Measuring the precise area of peripheral retinal non-perfusion using ultra-widefield imaging and its correlation with the ischaemic index. *Br J Ophthalmol* 2016;100:235-9.