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Running head: Clinically Significant NPAs on Widefield OCTA in Diabetic Eyes

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Key Words

diabetic retinopathy; nonperfusion areas; neovascularization; semi-automatic quantification; widefield optical coherence tomography angiography
ABSTRACT

Purpose

To investigate the distribution of clinically significant nonperfusion areas (NPAs) on widefield optical coherence tomography angiography (OCTA) images in diabetic patients.

Design

This was a prospective, cross-sectional, observational study.

Participants

One hundred and forty-four eyes of (114 diabetes patients).

Methods

Nominal 20 × 23 mm OCTA images were obtained using a swept source OCTA device (Xephilio OCT-S1), followed by the creation and selection of en face images with 20-mm (1614 pixels) in diameter centering on the fovea. The nonperfusion squares (NPSs) were defined as the 10 × 10 pixel squares without retinal vessels that were automatically detected and the ratio of eyes with the NPS to all eyes in each square was referred to as the NPS ratio. The areas with probabilistic differences (APD) for proliferative diabetic retinopathy (PDR) and nonproliferative diabetic retinopathy (NPDR) (APD[PDR] and APD[NPDR]) were defined as sets of squares with higher NPS ratios in eyes with PDR and NPDR, respectively. The P ratio (NPSs within APD[PDR] but not APD[NPDR]/all NPSs) was also calculated.

Main Outcome Measures

The probabilistic distribution of the NPSs and the association with diabetic retinopathy (DR) severity.

Results
The NPSs developed randomly in eyes with mild and moderate NPDR, and were more prevalent in the extramacular areas and the temporal quadrant in eyes with severe NPDR and PDR. The APD(PDR) was distributed mainly in the extramacular areas, sparing the areas around the vascular arcades and radially peripapillary capillaries. The APD(PDR) contained retinal neovascularization more frequently than the non-APD(PDR) ($P=0.023$).

The P ratio was higher in eyes with PDR than in those with NPDR ($P<0.001$). The multivariate analysis designated the P ratio (odds ratio=8.293 $\times$ 10$^3$; 95% confidence interval (CI): 6.529 $\times$ 10$^2$–1.053 $\times$ 10$^{13}$; $P=0.002$) and the total NPSs (odds ratio=1.002; 95% CI: 1.001–1.003; $P<0.001$) as independent risk factors of PDR. Most eyes with NPDR and 4-2-1 rule findings of DR severity had higher P ratios but not necessarily the greater NPSs numbers.

Conclusions

The APD(PDR) is uniquely distributed on widefield OCTA images and the NPAs location patterns are associated with DR severity, independent of the whole area of NPAs.
INTRODUCTION

Diabetic retinopathy (DR) is a leading cause of visual impairment in the working age in developed countries.\textsuperscript{1} DR is characterized by structural and functional alterations of the retinal circulation.\textsuperscript{2} Retinal ischemia is associated with DR progression, and the concomitant expression of vascular endothelial growth factor (VEGF) promotes vision-threatening DR, i.e., proliferative diabetic retinopathy (PDR) and diabetic macular edema.\textsuperscript{1,3} Therefore, automatic and objective quantification methods for nonperfusion areas (NPAs) in patients with DR may help further identify referable disease.

Each DR case may have a unique distribution of NPAs, which can appear to develop and progress at random. Several fundus findings are associated with specific capillary nonperfusion. Cotton-wool spots correspond to focal NPAs, and most intraretinal microvascular abnormalities (IRMAs) are accompanied with NPAs in their peripheral areas.\textsuperscript{4,5} Lamellar NPAs appear to exacerbate neurodegeneration in the inner retina and edematous changes in the middle layers.\textsuperscript{6} Several perfusion and nonperfusion indices are associated with visual acuity (VA) and have been proposed to characterize diabetic macular ischemia.\textsuperscript{7} These previous publications implicate the importance of nonperfusion indices but the mechanisms and patterns of the distribution of NPAs during the progression of DR remains unknown.

Fluorescein angiography (FA) is the gold standard for the assessment of DR,\textsuperscript{8} though it is an invasive and time-consuming method. The automatic detection of NPAs via FA is difficult due to background choroidal fluorescence and dye leakage from neovascularization (NV), damaged retinal vessels, and changes in signal levels in the retinal vessels over time. The clinical application of optical coherence tomography angiography (OCTA), a technique that isolates the microvascular circulation in OCT
image data by detecting the motion contrast of blood flow, allows for noninvasive evaluations of both the retinal and choroidal vasculature. As high resolution OCTA images delineate retinal vessels selectively with less background noise, OCTA may be more feasible to detect NPAs than FA. However, few studies have objectively evaluated the geometric distribution of NPAs using widefield OCTA in patients with DR. In this study, we investigated the distribution of clinically significant NPAs that were stochastically determined using widefield OCTA images and evaluated their associations with DR severity.

**METHODS**

**Participants**

This prospective, observational, cross-sectional case series was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee, and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from each participant prior to the study. We enrolled patients with diabetes mellitus who were examined at the Department of Ophthalmology in Kyoto University Hospital between January 2021 and January 2022. Individuals with diabetes for whom widefield OCTA images of sufficient quality were obtained were included in the study if they provided written informed consent. The exclusion criteria were the presence of media opacities interfering with VA or image acquisition, other chorioretinal diseases, cataract surgery within three months, a history of vitrectomy, prior anti-VEGF treatment, and prior ocular steroid treatment; and an axial length < 22 mm or > 26 mm. Eyes with weak signal strength (< 5) or severe image artifacts
on en face OCTA images were also excluded. Resultantly, several eyes received prior panretinal photocoagulation (PRP).

**OCTA**

All patients underwent comprehensive ophthalmologic examinations and the best-corrected decimal VA was measured and converted into a logarithm of the minimum angle of resolution (logMAR). The axial length was measured using partial coherence interferometry (IOL Master, Carl Zeiss Meditec, Inc., Dublin, CA). Ultra-widefield color fundus photographs using Optos 200Tx (Optos PLC, Dunfermline, UK) were obtained and graded for DR severity according to the International Clinical Disease Severity Scale for Diabetic Retinopathy. Two retinal specialists evaluated fundus findings and the severity grades using the photographs, and any disagreements were discussed until the specialists reached an agreement.

Two en face, swept source OCTA images centered on the upper and lower quadrants were obtained by moving the internal target upward and downward with a scanning area of nominal 20 (height) × 23 (width) mm (1614 × 1856 pixels) using a Xephilio OCT-S1 device (Canon, Tokyo, Japan). En face combined images of superficial and deep layers (from the internal limiting membrane to the outer plexiform layer) were generated using the default setting and, after the application of artificial intelligence (AI)-based denoising, a montage image was created from two images using the built-in manufacturer’s software. (Fig 1A and B).

**Assessment of the NPA distribution and its relationship with clinical findings**
The 1614-pixel (nominal 20 mm)-diameter circle centered on the fovea in the images were evaluated. The images of the left eye were inverted horizontally to position the nasal subfield on the right-hand side. The 100-pixel diameter circle centered on the optic disc was excluded for the evaluation of NPAs. Vessel edges were automatically detected using the Canny Edge Detector plugin (gaussian kernel radius=1.5, low threshold=2.5, high threshold=7.5) of imageJ software (NIH, Bethesda, MD; http://imagej.nih.gov/ij/) (Fig 1C and D), after the optimization of each parameter. The image was divided into squares of 10 × 10 pixels (Fig 1E-H). The pixels of vessel edges were automatically counted in each 10 × 10 pixels square using ImageJ. The squares with no pixels of vessel edges were defined as nonperfusion squares (NPSs).

The differences of NPSs between nonproliferative diabetic retinopathy (NPDR) and PDR were investigated. For each square, the NPS ratio for each DR severity grade was defined as follows:

$$\text{NPS ratio} = \frac{\text{The number of eyes with NPSs}}{\text{The number of all eyes}}.$$  

The squares in which the differences of the NPS ratios between the groups were large (greater than the median) were categorized as areas with probabilistic differences (APD). The APDs between the no apparent retinopathy group and the DR group; between the no apparent retinopathy group and the NPDR group; and between the NPDR group and the PDR group were termed APD(DR), APD(NPDR), and APD(PDR), respectively. Furthermore, Clinically significant NPAs was defined as the NPAs in APD(PDR) but not APD(NPDR).

We planned to validate the clinical relevance of the APD. The leave-one-out cross validation method was selected in order to avoid overfitting. Briefly, one eye was first left out, and the NPS ratio of each square was calculated in the remaining eyes to
determine the APD(PDR) and APD(NPDR). The NPSs in the APD(PDR) or
APD(NPDR) were counted and evaluated in the left-out eye. These procedures were
repeated for all eyes.
In each eye, the number of NPSs within the APD was examined. When we investigated
the distribution and the extents of NPSs in each DR stage, we found several PDR cases
with a smaller number of NPSs. In addition, the NPSs were distributed uniquely in eyes
with PDR, compared to eyes with NPDR. It suggests that the progression to PDR or the
NPA progression after NV development depends on not only the extent but also the
location of NPAs. In order to test this hypothesis, we defined the ratios of NPSs specific
to PDR as the P ratio. The P ratio and N ratio were calculated as follows:
\[
P \text{ ratio } = \frac{\text{The number of NPSs in APD(PDR) but not in APD(NPDR)}}{\text{The total number of NPSs}}
\]
\[
N \text{ ratio } = \frac{\text{The number of NPSs in APD(NPDR) but not in APD(PDR)}}{\text{The total number of NPSs}}
\]
The location of the NV was determined in three-dimensional OCTA images, as described
previously. NV within one disc-radius value from the disc was defined as
neovascularization of the disc (NVD). Other NV was defined as retinal
neovascularization (NVE).

**Statistical analyses**
All values were expressed as median and interquartile range (IQR). Statistical
significance was set at $P<0.05$. The agreement of qualitative parameters was assessed
using Kappa coefficient. Mann–Whitney U tests and Kruskal-Wallis test with Bonferroni
correction were performed to compare continuous variables. Fisher’s exact test or chi-
square test was used to compare categorical variables. Spearman’s correlation coefficient
was used to analyze the association between two continuous variables. Multivariable logistic regression analysis (independent variables=number of NPSs in whole areas and P ratio; dependent variable=PDR) was conducted to determine the risk factors discriminating eyes with PDR from those with NPDR. The NPSs were analyzed using R software (version 3.6.1; R Foundation for Statistical Computing, Vienna, Austria) and all statistical analyses, except for hierarchical clustering, were performed using EZR software. Hierarchical agglomerative clustering was conducted using SPSS (version 24; IBM, Armonk, NY).

RESULTS

The unique distribution of NPAs in severe NPDR and PDR

We acquired OCTA images of 178 eyes from 139 participants with diabetes. After excluding 34 eyes due to the weak signal strength or severe image artifacts, we investigated 144 eyes from 114 participants in the study (Table 1). The agreement of qualitative findings and DR grades were shown in Table S1 (available at www.aaojournal.org). The percentages of NPSs in each eye were 0.45% (IQR: 0.31–0.77) in 27 eyes with no apparent retinopathy, 0.92% (IQR: 0.48–1.58) in 15 eyes with mild NPDR, 1.00% (IQR: 0.52–1.89) in 40 eyes with moderate NPDR, 1.90% (IQR: 1.16–3.32) in 18 eyes with severe NPDR, and 5.42% (IQR: 2.55–10.12) in 44 eyes with PDR. The percentages in the extramacular sectors (nominal 10-20 mm) were higher in eyes with severe NPDR and PDR (Fig S2).

We evaluated the perfusion status in each square of 10x10 pixels and the NPSs ratios were shown on pseudo-colored rate map images in each DR severity grade (Fig 3). The NPSs developed at random locations in eyes with mild and moderate NPDR (Fig 3),
whereas the ratios increased particularly in the extramacular areas and temporal quadrant in eyes with severe NPDR and PDR (Fig S2). The maps of the differences in the NPS ratios between individual DR severity grades confirmed the high frequency of NPAs in these areas in the later stages (Fig S4). Pseudo-colored rate map images demonstrated that 17 PDR eyes with NVD had high NPS ratios in the nasal subfield of the extramacular areas, compared to 27 PDR eyes without NVD (Fig S5).

**Stochastic determination of clinically significant NPAs**

Fig 6A-C showed the differences of the NPS ratios for each square between no apparent retinopathy and DR; between no apparent retinopathy and NPDR; and between NPDR and PDR. The APDs in eyes with DR, NPDR, and PDR were defined after thresholding (Fig 6D-F and Fig S7). The APD(PDR) was absent around the vascular arcades, around the radial peripapillary capillaries (RPC), and in the macula except for the temporal quadrant, whereas APD(NPDR) was randomly present throughout the retina.

The retinas were divided into four categories: both APD(PDR) and APD(NPDR); APD(PDR) but not APD(NPDR); APD(NPDR) but not APD(PDR); and neither APD(PDR) nor APD(NPDR) (Fig 6G and Supplemental Figs S8 and S9). In order to consider the likelihood of PDR, we investigated the association of PDR with the P ratios or N ratios (Fig 10). Interestingly, the P ratios ranged from 0.08–0.30 in eyes with NPDR, whereas most eyes with PDR had higher ratios ($P<0.001$; Fig 10A, Fig S11). The number of NPSs in the whole areas ranged from 129–6555 in eyes with PDR, although most eyes with NPDR had less than 1200 NPSs ($P<0.001$; Fig 10A). There was a significant association between P ratio and the total number of NPSs ($\rho=0.539$, $P<0.001$). The P ratio (odds ratio=$8.293 \times 10^7$; 95% confidence interval (CI): $6.529 \times 10^7$–$1.053 \times 10^{13}$;
Hierarchical clustering divided the eyes with NPDR into two major groups: a high-P-ratio group of 45 eyes and a low-P-ratio group of 28 eyes (Fig 12A). The P ratios in eyes with PDR were similar to those of eyes with NPDR of a high-P-ratio group. The high-P-ratio group had poorer logMAR (P=0.039), more pseudophakia (P=0.014), more severe DR (P=0.004), and more NPSs (P=0.008) than the low-P-ratio group (Table 3).

Relationship between clinically significant NPAs and DR severity

We evaluated the association between clinically significant NPAs and the international DR severity grades. NVEs were delineated in the outer areas of the nasal quadrant, though they were also observed within the posterior pole in the superotemporal and inferotemporal quadrants (Fig 13). The APD(PDR) contained NVEs more frequently than the non-APD(PDR) (89 [62.2%] vs. 54 [37.8%]; P=0.023) (Fig 13A and B). Most eyes with 4-2-1 rule findings had higher P ratios, although the number of NPSs did not necessarily increase in these eyes (Fig 12C-E, Table 3, and Fig S14).

DISCUSSION

In this study, we investigated the distribution of NPAs stochastically and demonstrated that the NPAs developed at random in eyes with mild and moderate NPDR, whereas the NPAs were distributed mainly in the extramacular areas and the temporal subfield in eyes with severe NPDR and PDR. Interestingly, the APD(PDR) were not present around the vascular arcade or RPCs and contained 62.2% of NVEs. A higher P ratio is a risk of PDR, independent of the NPA areas. Additionally, eyes with severe NPDR also had higher P...
ratios. These data might designate the NPAs in APD(PDR) but not APD(NPDR) as 
clinically significant NPAs for the progression of DR.

Previous studies that have subjectively analyzed and manually defined the NPAs using 
ultra-widefield FA found that the ischemic index is correlated with DR severity.\textsuperscript{18-20} 
However, OCTA images with higher signal/noise ratios provide an advantage for the 
automatic detection of NPAs. The clinical relevance of widefield OCTA images for the 
discrimination of PDR and NPDR has been reported by some studies, though how 
widefield imaging delineates the pathogenesis of the NPAs and concomitant NV 
development remain unclear.\textsuperscript{13,21} In this study, the objective and semi-automatic 
determination of areas and the distribution of NPAs allowed us to propose the APD and 
quantify the clinically significant NPAs. This image processing method can be used to 
determine the risks of PDR and to predict PDR development in a clinical setting.

Piccolino et al. proposed three patterns of NPA distribution in eyes with PDR and reported 
the association between early NVE and the midperipheral capillary nonperfusion which 
was observed in more than 80% eyes.\textsuperscript{22} It may be consistent to the distribution of 
APD(PDR) to some extent. The objective and quantitative analyses in the current study 
demonstrated more precise distribution and probability of NPAs in eyes with PDR, 
comparing to those in eyes with NPDR. It remains to be investigated in the future how 
the number and the distribution of NPSs change after treatment with PRP or anti-VEGF 
 injection.

Despite the clinical significance of the uniquely distributed APD(PDR), what factors 
determine the locations of NPAs in eyes with PDR remains unclear. The stochastic 
distribution of the NPSs were different between eyes with venous beading and those with 
IRMA s, suggesting multiple pathways to NVE development. In the posterior pole, vessels
in the temporal quadrants were accompanied by fewer RPCs and had a lower perfusion pressure than those in other quadrants. This may explain the imbalance of the NPAs between the nasal and temporal subfields. The macula showed a lower frequency of NPAs than the extramacular area. In the macula, where the retina is thicker and has few arterioles with a large diameter, deep capillaries bridge under the arterioles and may function as collateral vessels. On the other hand, in the extramacular area, large arterioles residing in both the superficial and deep layers could be the perfusion boundaries. This may explain the difference in the frequency of NPAs between the macula and extramacular area. The NPA analysis of each layer would provide more detailed data, although the current wide-field OCTA device does not have the sufficient accuracy in auto-segmentation, especially in the periphery, and en face images of each layer with good quality were not available. Future research using improved image acquisition and processing is desired. IRMAs can serve as shunt vessels. NVEs were more frequently observed within the APD(PDR) in the current study; the severity of ischemia in the posterior pole and midperiphery reportedly correlates with NVEs. Whether peripheral NPA cause these vascular lesions or the lesions cause peripheral NPA by stealing blood vessels requires further investigation.

The P ratio was used in this study to investigate the clinical significance of NPAs, and we observe that it was significant in the APD(PDR) but not in the APD(NPDR). The parenchyma and vasculature do not have a uniform structure throughout the retina. Some eyes with PDR had small NPA and a higher P ratio, suggesting that the NPA in the APD(PDR) but not in APD(NPDR) contribute to the development of nearby NVEs. NVEs develop mainly in the extramacular areas, and their development is dependent on several factors, such as the adherent vitreous cortex, local VEGF expression, and loss of
retinal ganglion cells which exert anti-angiogenic effects.\textsuperscript{30,31} We therefore speculate that clinically significant NPAs as well as the total areas of NPAs throughout the retina should be carefully evaluated during screening for PDR. NVD is generally a marker of advanced PDR. In this study, PDR eyes with NVD had higher NPS ratios in the nasal subfield, whereas the NPS ratios were higher in the temporal subfield in PDR eyes without NVD. It suggests that the extramacular NPAs in the nasal side contribute to the development of NVD. Future longitudinal studies with a large number of participants should confirmed the association between NVD and the locations of NPAs.

A method for identifying NPDR eyes with a high risk of progressing to PDR would be of great clinical significance. Cluster analysis revealed that NPDR eyes with higher P ratios were often accompanied with multiple retinal hemorrhages, venous beading, or IRMAs, which predict PDR development in the international DR severity grades. This suggests that the P ratios also have clinical relevance in eyes with NPDR. However, several NPDR eyes with higher P ratios did not have these fundus findings. Future longitudinal studies should elucidate whether higher P ratios are a novel predictor of progression to PDR.

Eyes of the high-P-ratio group had pseudophakia more frequently. In this study, high P ratio was associated with DR severity and greater NPAs, that suggests that higher levels of VEGF expression and concomitant vascular hyperpermeability may promote cataract progression, resulting in frequent cataract surgery. Alternatively, the common regulators, e.g., biochemical pathways stimulated by hyperglycemia, might contribute to cataract progression and NPA development in the APD(PDR). Intraocular inflammation after cataract surgery might promote the NPA progression there. Further studies with a larger number of patients are warranted.
This study had several limitations. First, all patients were Asians from a single center, with relatively few eyes with mild NPDR and severe NPDR, which may result in selection bias. A few parameters have been shown to be associated with the retinal vascular metrics. In particular, we did not find the association of number of NPSs with age and axial length (data not shown), although we did not completely exclude the effects of these confounders on the statistical analyses. As patients with prior PRP were not excluded, the natural course of DR was not necessarily analyzed. Although no reperfusion of vessels or capillary network was detected in the short to medium term after PRP, PRP has been reported to cause retinal oxygenation, intraocular VEGF reduction, and vasoconstriction, which may affect NPA distribution. Second, the widefield OCTA images do not completely correspond to the anatomical images of retinal vasculature after AI-based denoise processing. Distortions from the spherical surface to the plane could not be corrected; therefore, the approximate values were analyzed. In addition, the length of one pixel on the retina was not strictly consistent among different eyes and the areas of a 10 × 10 pixel square were varied after the correction for the axial length according to the Littman-Bennett formula (14817 ± 1213 μm²). Further, refraction including astigmatism also should be taken into account. Third, the areas of NPA might have been underestimated or overestimated due to the evaluation of the NPSs. Fourth, the thresholds for the APD may have affected the calculations of the P ratios. Future multi-center studies should confirm the generalizability in the image acquisition, processing, and analytic methods.

In summary, this is the first study to report clinically significant NPA in DR on widefield OCTA images. Stochastic methods revealed the unique distribution of the APD(PDR) and concomitant NVEs, which may depend on the non-uniform vascular architecture.
REFERENCES


FIGURE LEGENDS

Figure 1. Semi-automatic assessment of nonperfusion areas on optical coherence tomography angiography in two representative diabetic eyes.

The left eye of a 32-year-old patient with no apparent retinopathy (A, C, E, G), and the left eye of a 48-year-old patient with proliferative diabetic retinopathy (B, D, F, H). (A, B) The montage image of two en face optical coherence tomography angiography (OCTA) images. (C, D) The binary image using the edge detection function of the ImageJ software plugin. (E, F) The binary image is divided into squares of 10 × 10 pixels. Magnified images of the green rectangles in panels E and F are shown in panels G and H, respectively.

Figure 3. Ratios of the nonperfusion squares in each square based on diabetic retinopathy (DR) severity.

The nonperfusion square (NPS) ratios in each square in eyes with each DR severity grade in pseudo-colored maps. The nasal quadrant is on the right-hand side. NPDR=nonproliferative diabetic retinopathy; PDR=proliferative diabetic retinopathy.

Figure 6. Probabilistic definition of clinically significant nonperfusion areas in diabetic retinopathy (DR).

(A, B, C) The differences in the ratios of nonperfusion squares (NPSs) in each square between eyes with no apparent retinopathy and those with DR (A), between eyes with no apparent retinopathy and those with nonproliferative diabetic retinopathy (NPDR) (B), and between eyes with NPDR and those with proliferative diabetic retinopathy (PDR) (C). The values are obtained by subtracting the NPS ratios of a milder group from those
of a more severe group. (D, E, F) After thresholding with the median, the areas with probabilistic differences in panels A, B, and C were defined as the APD(DR), APD(NPDR), and APD(PDR), respectively. (G) The merged image of PA(NPDR) and APD(PDR) shows unique distributions of areas with probabilistic differences between the groups. The nasal quadrant is shown on the right-hand side.

**Figure 10. Relationship between number of nonperfusion squares and clinically significant nonperfusion areas.**

A scatter plot of the number of nonperfusion squares (NPSs) and the P ratios (A) and N ratios (B). Eyes with proliferative diabetic retinopathy (PDR) have higher P ratios and lower N ratios, though the number of NPSs is not necessarily high in eyes with PDR.

NPDR = nonproliferative diabetic retinopathy;

\[
P \text{ ratio } = \frac{\text{The number of NPSs in APD(PDR) but not in APD(NPDR)}}{\text{The total number of NPSs}}
\]

\[
N \text{ ratio } = \frac{\text{The number of NPSs in APD(NPDR) but not in APD(PDR)}}{\text{The total number of NPSs}}
\]

**Figure 12. Two major patterns of location of nonperfusion areas in eyes with nonproliferative diabetic retinopathy (NPDR).**

(A) A dendrogram of hierarchical clustering by P ratios in eyes with NPDR is shown. Unsupervised clustering divides 73 eyes with NPDR into two major groups: a high-P-ratio group (n=45 eyes) and a low-P-ratio group (n=28 eyes). The threshold of the P ratios is 0.18. (B) The P ratios in eyes with proliferative diabetic retinopathy (PDR) were similar to those of eyes with NPDR in the high-P-ratio group. (C, D, E) Scatter plots of eyes with or without each 4-2-1 fundus finding. Hem = more than 20
intraretinal hemorrhages in each of the four quadrants; VB = venous beading in two or more quadrants; intraretinal microvascular abnormalities in one or more quadrants;

\[
P \text{ ratio} = \frac{\text{The number of NPSs in APD(PDR) but not in APD(NPDR)}}{\text{The total number of NPSs}}
\]

\[
N \text{ ratio} = \frac{\text{The number of NPSs in APD(NPDR) but not in APD(PDR)}}{\text{The total number of NPSs}}
\]

**Figure 13.** Association between retinal neovascularization (NVE) and areas with probabilistic differences between eyes with nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) (APD[PDR]).

(A) The locations of APD(PDR) and neovascularization (NV) are shown. The nasal quadrant is shown on the right-hand side. (B) The directions of NVs and their distances to the nearest square in the APD(PDR) are shown. Most NVEs are within or near the APD(PDR). (C) The distances between the foveal center and NVs. The NVEs in the inferotemporal and superotemporal quadrants are nearest to the foveal center and those in the nasal quadrant are furthest. The white circle represents neovascularization of the disc. The black circle represents NVEs. T=temporal; I=inferior; N=nasal; S=superior.
Table 1. Participant characteristics

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<td>17 (7–22)</td>
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<td>14/4</td>
<td>28/33</td>
<td>20/15</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>13/101</td>
<td>2/16</td>
<td>6/55</td>
<td>5/30</td>
</tr>
<tr>
<td>Renal disease (present/absent)</td>
<td>38/76</td>
<td>7/11</td>
<td>21/40</td>
<td>10/25</td>
</tr>
<tr>
<td>Prior myocardial infarction</td>
<td>5/109</td>
<td>0/18</td>
<td>4/57</td>
<td>1/34</td>
</tr>
<tr>
<td>Prior stroke (present/absent)</td>
<td>8/106</td>
<td>0/18</td>
<td>6/55</td>
<td>2/33</td>
</tr>
<tr>
<td>logMAR VA</td>
<td>0.000</td>
<td>-0.079</td>
<td>-0.079</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>(-0.079–0.046)</td>
<td>(-0.176–0.023)</td>
<td>(-0.079–0.000)</td>
<td>(0.000–0.097)</td>
</tr>
<tr>
<td>Phakia / pseudophakia</td>
<td>96/48</td>
<td>19/8</td>
<td>47/26</td>
<td>30/14</td>
</tr>
<tr>
<td>International DR severity grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(eyes/patients)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild NPDR</td>
<td>15/13</td>
<td>-</td>
<td>15/13</td>
<td>-</td>
</tr>
<tr>
<td>Moderate NPDR</td>
<td>40/33</td>
<td>-</td>
<td>40/33</td>
<td>-</td>
</tr>
<tr>
<td>Severe NPDR</td>
<td>18/15</td>
<td>-</td>
<td>18/15</td>
<td>-</td>
</tr>
<tr>
<td>PDR</td>
<td>44/35</td>
<td>-</td>
<td>-</td>
<td>44/35</td>
</tr>
<tr>
<td>More than 20 intraretinal hemorrhages in each of four quadrants (eyes)</td>
<td>10</td>
<td>-</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Definite venous beading in two or more quadrants (eyes)</td>
<td>19</td>
<td>-</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>Prominent intraretinal microvascular abnormalities in one or more quadrants (eyes)</td>
<td>43</td>
<td>-</td>
<td>13</td>
<td>30</td>
</tr>
<tr>
<td>Retinal neovascularization (eyes)</td>
<td>42</td>
<td>-</td>
<td>0</td>
<td>42</td>
</tr>
<tr>
<td>Neovascularization of the disc (eyes)</td>
<td>17</td>
<td>-</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Prior PRP (eyes)</td>
<td>45</td>
<td>-</td>
<td>7</td>
<td>38</td>
</tr>
</tbody>
</table>
Data are shown as numbers or median (interquartile range).

Abbreviations: logMAR VA=logarithm of the minimum angle of resolution visual acuity; DR=diabetic retinopathy; NPDR=nonproliferative diabetic retinopathy; PDR=proliferative diabetic retinopathy; PRP=panretinal photocoagulation
Table 3. Comparisons of each parameter between low and high P ratio groups in eyes with nonproliferative diabetic retinopathy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low P ratio group (n=28)</th>
<th>High P ratio group (n=45)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67 (60—73)</td>
<td>68 (58—76)</td>
<td>0.443</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>21/7</td>
<td>30/15</td>
<td>0.601</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
<td>7.1 (6.7—8.0)</td>
<td>7.3 (6.8—8.6)</td>
<td>0.305</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>19 (13—23)</td>
<td>18 (12—22)</td>
<td>0.579</td>
</tr>
<tr>
<td>Systemic hypertension (present/absent)</td>
<td>22/6</td>
<td>15/30</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Dyslipidemia (present/absent)</td>
<td>14/14</td>
<td>16/29</td>
<td>0.235</td>
</tr>
<tr>
<td>Cardiovascular disease (present/absent)</td>
<td>3/25</td>
<td>3/42</td>
<td>0.669</td>
</tr>
<tr>
<td>Renal disease (present/absent)</td>
<td>9/19</td>
<td>13/32</td>
<td>0.798</td>
</tr>
<tr>
<td>Prior myocardial infarction (present/absent)</td>
<td>3/25</td>
<td>2/43</td>
<td>0.365</td>
</tr>
<tr>
<td>Prior stroke (present/absent)</td>
<td>3/25</td>
<td>6/39</td>
<td>1.000</td>
</tr>
<tr>
<td>LogMAR VA (-0.103—0.059)</td>
<td>-0.079</td>
<td>0.000</td>
<td>0.039</td>
</tr>
<tr>
<td>Phakia/pseudophakia</td>
<td>23/5</td>
<td>24/21</td>
<td>0.014</td>
</tr>
<tr>
<td>International DR severity grade (eyes)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild NPDR</td>
<td>10</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Moderate NPDR</td>
<td>16</td>
<td>24</td>
<td>0.004</td>
</tr>
<tr>
<td>Severe NPDR</td>
<td>2</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>More than 20 intraretinal hemorrhages in each of four quadrants (present/absent)</td>
<td>1/27</td>
<td>5/40</td>
<td>0.396</td>
</tr>
<tr>
<td>Definite venous beading in two or more quadrants (present/absent)</td>
<td>0/28</td>
<td>6/39</td>
<td>0.076</td>
</tr>
<tr>
<td>Prominent intraretinal microvascular abnormalities in one or more quadrants (present/absent)</td>
<td>1/27</td>
<td>12/33</td>
<td>0.012</td>
</tr>
<tr>
<td>NPSs number of NPAs in whole areas</td>
<td>178 (117—239)</td>
<td>316 (179—461)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Data are shown as numbers or median (interquartile range).
Abbreviations: logMAR VA = logarithm of the minimum angle of resolution visual acuity; DR = diabetic retinopathy; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy; NPSs = nonperfusion squares; NPAs = nonperfusion areas
**Precis**
Stochastic screening on widefield optical coherence tomography angiography images reveals nonperfusion areas developing uniquely in extramacular areas in eyes with proliferative retinopathy, and validation study confirms their associations with fundus findings of severe nonproliferative diabetic retinopathy.