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# Automated Quantification of Nonperfusion Areas in 3 Vascular Plexuses With Optical Coherence Tomography Angiography in Eyes of Patients With Diabetes

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**IMPORTANCE** Diabetic retinopathy (DR) is a leading cause of vision loss that is managed primarily through qualitative clinical examination of the retina. Optical coherence tomography angiography (OCTA) may offer an objective and quantitative method of evaluating DR.

**OBJECTIVE** To quantify capillary nonperfusion in 3 vascular plexuses in the macula of eyes patients with diabetes of various retinopathy severity using projection-resolved OCTA (PR-OCTA).

**DESIGN, SETTING, AND PARTICIPANTS** Cross-sectional study at a tertiary academic center comprising 1 eye each from healthy control individuals and patients with diabetes at different severity stages of retinopathy. Data were acquired and analyzed between January 2015 and December 2017.

**MAIN OUTCOMES AND MEASURES** Foveal avascular zone area, extrafoveal avascular area (EAA), and the sensitivity of detecting levels of retinopathy.

**RESULTS** The study included 39 control individuals (20 women [51%]; mean [SD] age, 43.41 [19.37] years); 16 patients with diabetes without retinopathy (8 women [50%]; mean [SD] age, 56.50 [12.43] years); 23 patients with mild to moderate nonproliferative DR (18 women [78%] ; mean [SD] age, 62.48 [10.55] years); and 32 patients with severe nonproliferative DR or proliferative DR (12 women [38%]; mean age, 53.41 [14.05] years). Mean (SD) foveal avascular zone area was 0.203 (0.103) $mm^2$ for control individuals, 0.192 (0.084) $mm^2$ for patients with diabetes without retinopathy, 0.243 [0.079] $mm^2$ for mild to moderate nonproliferative DR, and 0.359 (0.275) $mm^2$ for severe nonproliferative DR or proliferative DR. Mean (SD) EAA in whole inner retinal slab in these groups, respectively, were 0.020 (0.031) $mm^2$, 0.034 (0.047) $mm^2$, 0.038 (0.040) $mm^2$, and 0.237 (0.235) $mm^2$. The mean (SD) sum of EAA from 3 segmented plexuses in each of the respective groups were 0.103 (0.169) $mm^2$, 0.213 (0.242) $mm^2$, 0.451 (0.243) $mm^2$, and 1.325 (1.140) $mm^2$. With specificity fixed at 95%, using EAA in inner retinal slab, the sensitivity of detecting patients with diabetes from healthy control individuals was 28% (95% CI, 18%-40%), 31% for patients with DR (95% CI, 19%-45%), and 47% for patients with severe DR (95% CI, 29%-65%) from whole inner retinal EAA. With the sum of EAA from 3 individual plexuses, the sensitivities were 69% (95% CI, 57%-80%), 82% (95% CI, 70%-91%), and 97% (95% CI, 85%-100%), respectively. Avascular areas were not associated with signal strength index. The commercial vessel density from the 2-plexus scheme distinguished the groups with lower sensitivity and were dependent on SSI.

**CONCLUSIONS AND RELEVANCE** Automatically quantified avascular areas from a 3-layer segmentation scheme using PR-OCTA distinguished levels of retinopathy with a greater sensitivity than avascular areas from unsegmented inner retinal slab or measurements from a commercially available vessel density in 2-layer scheme. Additional studies are needed to investigate the applicability of nonperfusion parameters in clinical settings.

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Supplemental content

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Diabetic retinopathy (DR) is a leading cause of vision loss in the United States.[1] Prevention of vision loss related to DR depends on identifying qualitative features from clinical examination, fundus photographs, or fluorescein angiography. Optical coherence tomography angiography (OCTA), a noninvasive modality capable of capillary resolution imaging of retinal vasculature,[2] is better suited for quantifying vascular changes related to diabetic retinopathy than conventional imaging and has opened the possibility of a quantitative biomarker to characterize DR.[3]

Unlike conventional modalities, OCTA is also able to image the retinal vasculature in 3 dimensions.[4] The retinal vasculature is composed of 3 distinct layers in the macula: superficial vascular complex (SVC), intermediate capillary plexus (ICP), and deep capillary plexus (DCP).[5] In DR, these layers can show incongruent areas of capillary nonperfusion that are not visible in whole inner retinal slabs,[6] suggesting that an examination of the individual plexuses may reveal more vascular abnormalities and possibly increase the diagnostic accuracy of the biomarkers based on the angiograms.

However, projection artifacts in OCTA can confound the interpretation of the deeper plexus angiograms. The available commercial systems present the retinal vasculature in 2 plexuses: superficial and deep. They place the boundaries to include most of the ICP with the superficial layer, which makes the projection of the superficial vessels in the deep plexus less visible.[7,8] Our group has developed an algorithm to reduce projection artifacts without moving the segmentation boundaries, and present the 3 macular plexuses distinctly according to known histologic studies.[9] Using this algorithm and modern image processing techniques,[10] we have previously shown that extrafoveal avascular areas (EAAs) from projection-resolved, segmented retinal angiograms perform better than unsegmented angiograms in distinguishing mild nonproliferative diabetic retinopathy (NPDR) eyes from control eyes.[11] Avascular areas may have less age and signal strength dependence compared with vessel density (VD) measurements.[12] In this study, we test how these OCTA-derived biomarkers perform across a wider spectrum of disease in a larger number of patients with diabetes and compare them with commercial VD measurements.

### Key Points

**Question** How useful are automatically quantified nonperfusion parameters from projection-resolved optical coherence tomography angiography in patients with diabetes?

**Findings** In this cross-sectional study of 71 patients with diabetes and 39 healthy control individuals, avascular areas detected from projection-resolved optical coherence tomography angiography retinal plexuses distinguished between control individuals and various levels of diabetic retinopathy severity. These biomarkers were less dependent on age and signal strength and more sensitive than some commercially available vessel density measurements.

**Meaning** Automatically quantified avascular areas from projection-resolved optical coherence tomography angiography of individual plexuses in patients with diabetes may offer a more reliable, sensitive method to evaluate diabetic vascular abnormalities than previous methods.

## Methods

The study team recruited healthy participants and participants with diabetes from Oregon Health and Science University and obtained informed written consent under a protocol in accordance with the Declaration of Helsinki and compliant with the Health Insurance Portability and Accountability Act of 1996. The institutional review board of Oregon Health and Science University approved the protocol. Each participant underwent a full clinical examination including an Early Treatment of Diabetic Retinopathy Study (ETDRS) protocol visual acuity. The ETDRS 7-field color photographs were obtained from all participants with diabetes. Eyes with other significant ocular pathology (including hypertensive retinopathy, retinal vascular occlusions, and uveitis) were excluded from the study. Other exclusion criteria included history of major intraocular surgery (including vitrectomy, cataract extraction, scleral buckle, and glaucoma surgery) within prior 4 months and poor-quality scans (signal strength index [SSI] less than 55 or significant motion artifacts). Patients with history of laser photocoagulation or intravitreal injections were not excluded. The clinician retinopathy severity grading guided the recruitment goals, but a masked grading of the color photographs assigned eyes into no retinopathy, mild, moderate, and severe NPDR and proliferative DR groups for final analysis. A commercial 70-kHz spectral-domain OCT system (RTVue, Optovue) obtained two 3 × 3 mm scans, with a depth of 2 mm centered at the fovea. The optical resolution in retina is 15 µm and 5 µm on transverse and axial directions, respectively. The digital sampling interval is 15 × 15 × 3 $\mu m^3$/voxel. Two registered scans, 1 x-fast and 1 y-fast, were combined to form a single volume to reduce motion artifacts.[13] When 2 eyes were eligible for the study, the eye with better-quality scans was selected.

A previous publication details the image processing steps used in this study.[11] Briefly, a commercial version of split-spectrum amplitude decorrelation angiography algorithm detected blood flow.[14] Projection-resolved (PR) OCTA algorithm then suppressed the projection artifacts by comparing the normalized flow signal strength based on the reflectance signal with the more superficial signal in the same transverse location.[9] A semiautomated algorithm based on directional graph search segmented the volumes into SVC, ICP, and DCP. The SVC was defined as the inner 80% of ganglion cell complex, which includes the nerve fiber layer, the ganglion cell layer, and the inner plexiform layer with a minimum thickness of 61 µm. The ICP was defined as the outer 20% of the GCC plus the inner 50% of the inner nuclear layer. The DCP was defined as remaining slab internal to the OPL with the minimum thickness of 37 µm. The mix of proportional slab definition with a minimum thickness is to account for the thinning and merging of the layers at the foveal pit[8] and to avoid arbitrary separation of the vessels into plexuses that are merging.[5] Avascular areas were identified on the resulting PR-OCTA slabs

Table 1. Participant Characteristics

| Characteristic | Mean (SD) | | | |
|---|---|---|---|---|
| | Control (n = 39) | DM Without DR (n = 16) | Mild to Moderate NPDR (n = 23) | Severe DR (n = 32) |
| Participant characteristics | | | | |
| Age, y | 43.41 (19.37) | 56.50 (12.43) | 62.48 (10.55) | 53.41 (14.05) |
| Female, No. (%) | 20 (51) | 8 (50) | 18 (78) | 12 (38) |
| Type 1 diabetes, No. (%) | NA | 4 (25) | 3 (13) | 9 (28) |
| $HbA_{1c}$ level, % | NA | 6.5 (0.9) | 8.1 (1.9) | 8.4 (1.6) |
| Diabetes duration, y | NA | 15.7 (12.2) | 21.0 (11.1) | 19.3 (12.5) |
| BP, mm Hg | | | | |
| Systolic | 121.5 (18.7) | 126.8 (19.2) | 123.0 (18.4) | 145.6 (22.3) |
| Diastolic | 75.6 (11.9) | 60.7 (12.4) | 72.5 (13.2) | 83.8 ± 14.7) |
| Ocular characteristics | | | | |
| Presence of DME, No. (%) | NA | NA | 11 (48) | 19 (83) |
| ETDRS letter score | 87.17 (4.46) | 82.69 (4.77) | 81.61 (5.79) | 78.00 (8.87) |
| IOP, mm Hg | 14.75 (2.63) | 17.25 (3.75) | 14.22 (2.89) | 14.84 (3.87) |
| Axial length, mm | 24.35 (0.98) | 24.48 (1.10) | 23.92 (0.97) | 23.55 (1.17) |
| SSI | 76.69 (6.93) | 71.23 (3.94) | 68.50 (6.38) | 66.84 (5.75) |

Abbreviations: BP, blood pressure; DCP, deep capillary plexus; DM, diabetes mellitus; DME, diabetic macular edema; DR, diabetic retinopathy; ETDRS, Early Treatment Diabetic Retinopathy Study; FAZ, foveal avascular zone; $HbA_{1c}$, glycated hemoglobin; IOP, intraocular pressure; NA, not applicable; NPDR, nonproliferative diabetic retinopathy; SSI, signal strength index.

SI conversion factor: To convert glycated hemoglobin to proportion of total hemoglobin, multiply by 0.01.

and the whole inner retinal slab, which included all layers internal to OPL based on their vessel-distance map generated by Euclidean distance transform algorithm.[11]

We calculated the foveal avascular zone (FAZ) area from the whole inner retinal slab and EAA, which excludes the central 1-mm diameter circle, in the inner retinal slab and each of the individual 3 plexus angiograms. We excluded the central area a priori to reduce the effect of normal variation in the FAZ size that may regrade the diagnostic accuracy, especially in a 3 × 3-mm angiogram.[11] The sum of AAs from individual plexuses were also calculated.

The eyes were then assigned into 4 predetermined groups: healthy control participants, patients with diabetes without retinopathy, patients with mild to moderate NPDR, and patients with severe NPDR and PDR based on fundus photograph grading. The Mann-Whitney *U* test evaluated the difference of the AAs between the groups. Spearman rank-order correlation analysis tested the correlation between the AAs and the increasing level of retinopathy severity and hemoglobin $A_{1c}$ level. *P* values were adjusted for multiple comparisons using the Holm-Bonferroni method.[15] The *P* value level of significance was .05, and all *P* values were 2-sided. With the specificity fixed at 95%, the sensitivity of detecting patients with diabetes from healthy control individuals, patients with mild to moderate NPDR from those without retinopathy, and patients with severe NPDR or PDR from patients with less severe retinopathy were calculated for EAA of the inner retinal slab, each of the 3 individual plexuses, and the sum of the 3 plexuses. The diagnostic accuracy was further evaluated with area under the receiver operating characteristic curve between each of the severity groups. Pooled standard deviation verified the repeatability of these measurements by testing values from images obtained in the same visit but different sitting at the machine.

Finally, we tested the correlation between the AAs and the SSI and the patient's age using Spearman correlation analysis. These parameters were compared with the VD measurements produced by the default commercial software on the machine. The commercial software used a 2-layer scheme to divide the retinal vasculature into superficial and deep plexuses. The superficial plexus is defined as 15 µm below the ILM to 15 µm below inner plexiform layer, which includes the superficial portion of the inner nuclear layer and thus the ICP. The deep plexus is defined as 15 to 70 µm below the inner plexiform layer, which includes the deep capillary plexus and dips into the Henle fiber layer and outer nuclear layer centrally in the foveal pit. The commercial system did not have an algorithm to reduce the projection artifacts.

## Results

The study included 1 eye each from 39 healthy control individuals, 16 patients with diabetes without retinopathy, 23 with mild to moderate NPDR, and 32 with more severe retinopathy. **Table 1** summarizes the patient characteristics. **Table 2** presents the automatically quantified AAs and commercial VD measurements, as well as the statistical differences between groups. **Tables 3** and **4** present the correlation analyses and diagnostic accuracy of the nonperfusion metrics.

Mean (SD) foveal avascular zone area was 0.203 (0.103) $mm^2$ for control individuals, 0.192 (0.084) $mm^2$ for patients with diabetes without retinopathy, 0.243 [0.079] $mm^2$ for mild to moderate NPDR, and 0.359 (0.275) $mm^2$ for severe NPDR or PDR. Mean (SD) EAA in the whole inner retinal slab in these groups, respectively, were 0.020 (0.031) $mm^2$, 0.034 (0.047) $mm^2$, 0.038 (0.040) $mm^2$, and 0.237 (0.235) $mm^2$. The mean (SD) sum of EAA from 3 segmented plexuses in each of the respective groups were 0.103 (0.169) $mm^2$, 0.213 (0.242) $mm^2$, 0.451 (0.243) $mm^2$, and 1.325 (1.140) $mm^2$. With specificity fixed at 95%, using EAA in inner retinal slab, the sensitivity of detecting patients with diabetes from healthy control individuals

Table 2. Avascular Areas and Commercial Vessel Density Measurements

| Comparison | FAZ | EAA | | | | | Commercial VD | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Inner Retina | SVC | ICP | DCP | Sum of Plexuses[a] | SVC | | DVC | |
| | | | | | | | Whole | Parafovea | Whole | Parafovea |
| **Measurements, Mean (SD)** | | | | | | | | | | |
| Control | 0.20 (0.103) | 0.020 (0.031) | 0.042 (0.075) | 0.026 (0.046) | 0.034 (0.053) | 0.103 (0.169) | 53.84 (2.92) | 55.94 (2.78) | 59.33 (2.37) | 61.85 (3.03) |
| DM no DR | 0.192 (0.084) | 0.034 (0.047) | 0.107 (0.110) | 0.025 (0.035) | 0.081 (0.127) | 0.213 (0.242) | 47.91 (3.50) | 49.74 (3.77) | 53.28 (3.78) | 55.55 (3.69) |
| Mild to moderate NPDR | 0.243 (0.079) | 0.038 (0.040) | 0.221 (0.134) | 0.058 (0.053) | 0.171 (0.139) | 0.451 (0.243) | 49.91 (4.01) | 51.33 (3.74) | 57.09 (1.97) | 57.09 (2.09) |
| SNPDR and PDR | 0.359 (0.275) | 0.237 (0.235) | 0.594 (0.421) | 0.249 (0.372) | 0.482 (0.439) | 1.325 (1.140) | 44.48 (3.13) | 45.76 (4.43) | 50.42 (2.59) | 52.26 (0.49) |
| **Mann-Whitney *U* Test of Comparisons Between Study Groups, *P* Value[b]** | | | | | | | | | | |
| Control | | | | | | | | | | |
| DM no DR | .67 | .28 | .02 | .41 | .22 | .02 | .01 | .01 | .009 | .01 |
| Mild to moderate NPDR | .10 | .002 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| SNPDR and PDR | .002 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| DM no DR | | | | | | | | | | |
| Mild to moderate NPDR | .079 | .18 | .01 | .01 | .02 | .002 | .24 | .13 | .07 | .18 |
| SNPDR and PDR | .003 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| Mild to moderate NPDR | | | | | | | | | | |
| SNPDR and PDR | .09 | <.001 | <.001 | .002 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |

Abbreviations: DCP, deep capillary plexus; DM, diabetes mellitus; DR, diabetic retinopathy; DVC, deep vascular complex; EAA, extrafoveal avascular area; FAZ, foveal avascular zone; ICP, intermediate capillary plexus; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; SNPDR, severe NPDR; SVC, superficial vascular complex.

[a] Sum of plexuses was defined as sum of EAA from 3 plexuses.

[b] Significant *P* values were adjusted for multiple comparisons using Holm-Bonferroni method.

was 28% (95% CI, 18%-40%), 31% for patients with DR (95% CI, 19%-45%), and 47% for patients with severe DR (95% CI, 29%-65%) from whole inner retinal EAA. With the sum of EAA from 3 individual plexuses, the sensitivities were 69% (95% CI, 57%-80%), 82% (95% CI, 70%-91%), and 97% (95% CI, 85%-100%), respectively.

In general, AAs were greater and VDs were less in eyes with more severe DR (**Figure**; eFigure in the Supplement). All parameters distinguished the severe NPDR to PDR group from control group with statistical significance. The FAZ size correlated significantly with increasing retinopathy severity (Tables 2 and 3) but did not distinguish between control, patients with diabetes without retinopathy, or the mild to moderate NPDR group. The EAA from SVC angiogram and the sum of EAA from the 3 plexuses distinguished with statistical significance between all groups. The commercial VD distinguished between all groups with statistical significance except between patients with diabetes without retinopathy and mild to moderate NPDR groups.

With the specificity fixed at 95%, EAA from the SVC was the most sensitive biomarker to detect all levels of diabetic retinopathy (70%, 84%, and 100%), with the sum of EAA from all 3 plexuses performing similarly (69%, 82%, and 97%). They were more sensitive than the commercial VD measurements for either superficial (36%, 43%, 66%) or deep (61%, 70%, 94%) plexuses, especially for the lower level of retinopathy (Table 4).

All vessel parameters were significantly correlated with ETDRS vision scores and retinopathy severity groups (Table 3). In the control group, the projection-resolved AA measurements had minimal association with age and no significant association with SSI, whereas the commercial VD measurements were strongly associated with age and SSI (Table 3). There was a strong association between SSI and retinopathy severity group with Spearman ρ of −0.625 ($P < .001$).

The area under the receiving operating curve evaluating the diagnostic accuracy between each of the groups was best for SVC EAA and the sum of EAA of all 3 plexuses (Table 4). The commercial VD measurements were nearly as good as these EAA measurements and performed better than ICP or DCP EAA measurements. The repeatability of AA, as measured by pooled SD, were 0.018 $mm^2$ for FAZ and 0.026 $mm^2$, 0.057 $mm^2$, 0.021 $mm^2$, and 0.048 $mm^2$ for EAA of the inner retina, SVC, ICP, and DCP, respectively.

Table 3. Spearman Rank-order Correlation Coefficient

| Spearman Rank-Order Correlation Coefficient | FAZ | EAA | | | | | Commmercial VD | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | SVC | | DVC | |
| | | Inner Retina | SVC | ICP | DCP | Sum of Plexuses[a] | Whole | Parafovea | Whole | Parafovea |
| Correlation with ETDRS vision score | −0.184 | −0.336 | −0.371 | −0.298 | −0.433 | −0.429 | 0.409 | 0.407 | 0.427 | 0.399 |
| *P* value[b] | .06 | .001 | <.001 | .003 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| Correlation with severity group | 0.328 | 0.675 | 0.815 | 0.609 | 0.713 | 0.819 | −0.781 | −0.795 | −0.833 | −0.809 |
| *P* value | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| Correlation with $HbA_{1c}$ | 0.334 | 0.428 | 0.482 | 0.343 | 0.292 | 0.428 | −0.342 | −0.304 | −0.298 | −0.298 |
| *P* value | .02 | .002 | .003 | .03 | .04 | .003 | .09 | .14 | .10 | .14 |
| Correlation with age (control) | 0.097 | 0.192 | 0.299 | 0.075 | 0.3 | 0.36 | −0.57 | −0.615 | −0.684 | −0.678 |
| *P* value | .56 | .24 | .06 | .65 | .06 | .02 | <.001 | <.001 | <.001 | <.001 |
| Correlation with SSI (control) | 0.068 | −0.038 | −0.188 | 0.107 | −0.181 | −0.216 | 0.678 | 0.663 | 0.573 | 0.587 |
| *P* value | .68 | .82 | .25 | .52 | .27 | .19 | <.001 | <.001 | <.001 | <.001 |

Abbreviations: DCP, deep capillary plexus; DM, diabetes mellitus; DR, diabetic retinopathy; DVC, deep vascular complex; ETDRS, early treatment diabetic retinopathy study; EAA, extrafoveal avascular area; FAZ, foveal avascular zone; $HbA_{1c}$, glycated hemoglobin; ICP, intermediate capillary plexus; SSI, signal strength index; SVC, superficial vascular complex.

[a] Sum of plexuses was defined as sum of EAA from 3 plexuses.

[b] Significant *P* values were adjusted for multiple comparisons using Holm-Bonferroni method.

## Discussion

We have demonstrated that projection-resolved OCTA derived AA with a 3-layer segmentation scheme that correspond to the known anatomic boundaries of retinal vasculature can distinguish between control individuals, patients with diabetes without retinopathy, patients with mild to moderate NPDR, and severe NPDR to PDR groups. The sensitivity of detecting retinopathy groups was greater when the vascular plexuses were examined individually, and the AA from the superficial vascular complex was the most sensitive. All AA measurements had good repeatability as measured by pooled standard deviation. This demonstrates the potential of OCTA to objectively and reliably evaluate DR.

Our automated algorithm for detecting AA was designed to mimic the human grader's detection of nonperfusion areas, identifying only the contiguous and pathologic areas of capillary dropout while ignoring increased intercapillary spaces. By using the mean reflectance in the ganglion cell layer and inner plexiform layer to compensate for variation in signal strength within an image, we improved the accuracy of classifying vessel vs static tissue on en face OCTA. The use of vessel-distance map on vascular binary image avoided exaggerated contribution of large vessels in determining VD abnormality and eliminates the false-positive detection along large arterioles. We have previously demonstrated high repeatability and high detection accuracy with this approach.[10] Based on the observation that the VD measurements are dependent on SSI and age, we hypothesized that our approach would detect capillary nonperfusion caused by disease but would be less likely to detect artifactitious changes in perfusion index caused by image quality or normal changes in capillary density with aging. We have demonstrated that the AA measurements are independent from SSI and correlate minimally with age, potentially improving their value in detecting diabetic retinopathy.

Another unique feature of this study is the projection-resolved algorithm and segmentation of the macular vasculature into 3 plexuses respecting the true known boundaries. We hypothesized that (1) projection artifacts would make detection of nonperfusion more difficult in the deeper plexuses, (2) the inclusion of the ICP into the superficial plexus as done in many commercial segmentation schemes would decrease the sensitivity of nonperfusion detection, and (3) even if the deep plexus included the ICP and DCP together without projection artifacts, an en face analysis of this layer would only reveal congruous AA between the 2 layers. We expected that our approach would lead to more sensitive and specific detection of microvasculopathy, and in turn, better correlation with clinical grading of diabetic retinopathy.

The results demonstrate that 3-layer segmentation with projection resolution indeed detects more nonperfusion than unsegmented inner retinal angiogram and is better able to distinguish between retinopathy severity groups. However, the diagnostic accuracy as measured by area under the receiving operator characteristic curve did not show a clearly superior performance compared with the 2-layer commercial VD measurements in this study. This may be owing to the association between the SSI and the retinopathy groups in this study (Spearman ρ, −0.568; $P < .001$). The SSI may be associated with retinopathy severity for various factors that are not associated with microangiopathy of diabetes. Because these factors are likely not be consistently present in different

Table 4. Diagnostic Accuracy of Nonperfusion Biomarkers and Commercial Vessel Density Measurements

| | % (95% CI) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | EAA | | | | | Commercial VD | | | | |
| | | | | | | | SVC | | DVC | | |
| DR Severity | FAZ | Inner Retina | SVC | ICP | DCP | Sum of Plexuses[a] | Whole | Parafovea | Whole | Parafovea |
| Sensitivity to detect retinopathy from controls | | | | | | | | | | | |
| DM | 19 (10-30) | 28 (18-40) | 70 (58-81) | 30 (19-42) | 58 (46-70) | 69 (57-80) | 36 (24-48) | 45 (33-57) | 61 (48-73) | 45 (33-57) |
| DR | 23 (12-36) | 31 (19-45) | 84 (71-92) | 36 (24-50) | 70 (56-81) | 82 (70-91) | 43 (29-57) | 50 (36-64) | 70 (56-82) | 52 (38-66) |
| Severe DR | 29 (15-49) | 47 (29-65) | 100 (89-100) | 50 (32-68) | 82 (65-94) | 97 (85-100) | 66 (47-81) | 72 (53-86) | 94 (79-99) | 78 (60-91) |
| Area under receiver operating characteristic curve between levels | | | | | | | | | | |
| DM | 0.63 (0.53-0.72) | 0.80 (0.71-0.87) | 0.93 (0.84-0.96) | 0.77 (0.68-0.84) | 0.85 (0.76-0.91) | 0.90 (0.83-0.95) | 0.89 (0.82-0.95) | 0.90 (0.83-0.95) | 0.93 (0.86-0.97) | 0.91 (0.84-0.96) |
| DR | 0.69 (0.59-0.77) | 0.83 (0.74-0.89) | 0.94 (0.87-0.97) | 0.82 (0.74-0.89) | 0.88 (0.81-0.94) | 0.94 (0.87-0.97) | 0.89 (0.82-0.94) | 0.90 (0.83-0.95) | 0.93 (0.86-0.97) | 0.92 (0.85-0.96) |
| Severe DR | 0.70 (0.61-0.79) | 0.92 (0.85-0.96) | 0.93 (0.87-0.97) | 0.84 (0.75-0.90) | 0.90 (0.84-0.95) | 0.95 (0.89-0.98) | 0.94 (0.87-0.98) | 0.94 (0.88-0.98) | 0.95 (0.89-0.98) | 0.94 (0.88-0.98) |

Abbreviations: DCP, deep capillary plexus; DM, diabetes mellitus; DR, diabetic retinopathy; DVC, deep vascular complex; EAA, extrafoveal avascular area; FAZ, foveal avascular zone; ICP, intermediate capillary plexus; SVC, superficial vascular complex.

[a] Sum of plexuses was defined as sum of EAA from 3 plexuses.

populations, an SSI-dependent nonperfusion parameter may give less reproducible study results. Furthermore, all scans included in the study had high SSI (mean [SD], 71.35 [7.45]; range, 59-88) and it is possible that in the situations with more variable scan qualities, VD may be less useful in distinguishing between the groups.

Other groups have reported on the differences in nonperfusion parameters from OCTA and patients with diabetes. Simonett et al,[16] in comparing patients with type 1 diabetes without retinopathy with healthy control eyes, reported a significant difference in the deep plexus parafoveal VD but not in the superficial plexus.[16] Carnevali et al[17] also compared VD in patients with type 1 diabetes without retinopathy with healthy control individuals and similarly found a significant difference in the deep but not in the superficial plexus.[17] In contrast, Dimitrova et al,[18] using similar methods, reported a significantly reduced VDs in both superficial and deep plexuses in diabetic patients without retinopathy compared to controls.

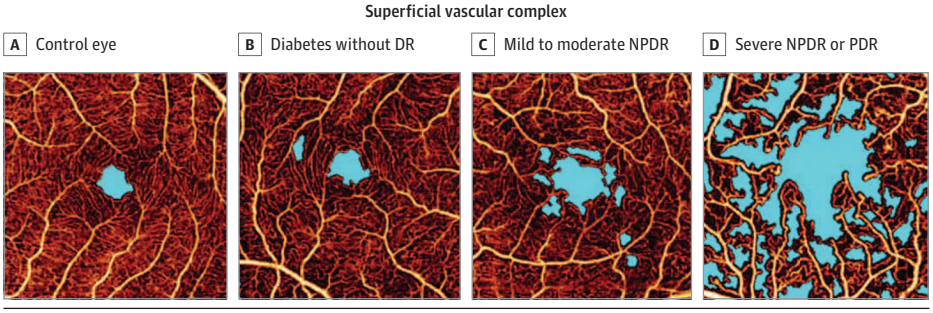
Samara et al[19] examined control, mild NPDR, moderate to severe NPDR, and PDR eyes with VD measurements and found that deep plexus VD measurements distinguished between the diabetic retinopathy groups better than superficial plexus. Both plexuses showed significant difference between the control group and the retinopathy groups of all levels, and none of the measurements had significant difference between PDR and moderate-to-severe NPDR groups.[19] In contrast, Pedinielli et al[20] did not show a statistically significant difference between patients with diabetes without retinopathy, patients with mild NPDR, patients with moderate NPDR, or patients with severe NPDR vs the control eyes in deep or superficial plexus VD.

These studies had different patient populations and different analysis methods. Their divergent conclusions and the lack of reproducibility of some of the findings with respect to the differences in superficial and deep plexus VD highlight the challenges of quantification using OCTA. Many steps are required to process the images, and there are numerous potential confounders. Image acquisition and quality, segmentation of the layers, projection artifacts, strategies for motion artifact reduction, and image processing can all influence the results. In particular, the role of image quality as measured by SSI may be particularly important. The disagreements in the conclusions from various studies may be because the SSI and retinopathy severity are variably related quantities. The exclusion of scans with low SSI does not appear to eliminate the influence of SSI on VD measurements.

## Limitations and Strengths

Limitations of this study include the cross-sectional design and a modest number of participants. Furthermore, hypertension, laser therapy, and anti-vascular endothelial growth factor are confounders that might influence retinal vasculature. Participants with any ocular pathology that might interfere with diabetic vasculopathy, including hypertensive retinopathy, were excluded from the the study. In addition, 2017 and 2018 studies by Ghasemi Falavarjani et al[21] and Karst et al[22] reported that no significant changes were

Figure. Optical Coherence Tomography Angiograms of the Superficial Vascular Complex From Healthy Participants and Participants With Diabetes




Projection-resolved optical coherence tomography angiograms with automatically detected nonperfusion areas in blue. DR indicates diabetic retinopathy; NPDR, nonproliferative DR; PDR, proliferative DR.

observed in retinal perfusion after anti-vascular endothelial growth factor treatment in patients with DR. Another limitation of this study is that we did not fully explore other methods of vascular quantification methods, such as vessel length density, vascular perfusion density mapping, automated perfusion density, and fractal dimension analysis, to compare with our PR-OCTA AA.[23,24] The strengths of this study were the consistently high-quality images, advanced image processing algorithms, rigorous grading of the retinopathy levels by masked graders from 7 field ETDRS photographs, and the visual acuity assessment using ETDRS protocol vision.

## Conclusions

Automatically quantified AA detected in 3-plexus segmentation using PR-OCTA can detect differences between control individuals, patients with diabetes without retinopathy, patients with mild to moderate NPDR, and patients with severe NPDR to PDR. With PR-OCTA, 3 macular plexuses were examined independently according to the known anatomic boundaries. These biomarkers were not dependent on SSI and may be less likely to be confounded by other covariate factors that are not the result of microvasculopathy of diabetes.


### ARTICLE INFORMATION

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*Concept and design:* Hwang, Hagag, Wilson, David, Jia.
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Invited Commentary

# Optical Coherence Tomography Angiography to Evaluate Ischemia in Diabetic Eyes

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**The retinal vasculature** consists of a superficial vascular plexus (SVP), an intermediate capillary plexus (ICP), and a deep capillary plexus (DCP). There is also a fourth regional vascular network, the radial peripapillary capillary plexus (RPCP). The RPCP runs in parallel with the nerve fiber layer, the SVP is located within the ganglion cell layer and the deeper plexuses, and the ICP and DCP are above and below the inner nuclear layer, respectively. The SVP receives a blood supply from the central retinal artery and the deeper vascular layers are supplied by vertical anastomoses from the SVP.[1]

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Fluorescein angiography (FA) has been used during the last 50 years as the preferred method of retinal vasculature imaging. However, because of various factors, including light scattering in the retina, FA is unable to image the ICP and DCP. Optical coherence tomography angiography (OCTA) is a powerful noninvasive method with which to study blood flow in the retina and choroid. It provides high-resolution depth-resolved images of blood flow in the retina and choroid. The contrast that is generated between nonstatic and static tissue results in a vascular signal that is processed by a variety of algorithms. These algorithms help to calculate the decorrelation of the signal amplitude from repeated consecutive B scans at the same location. This technology has opened new avenues as we can now acquire information for all the vascular plexuses of the retina.

Optical coherence tomography angiography may be particularly important in patients with diabetic retinopathy as changes in the deep plexus can occur early in the pathophysiologic process of the disease.[2] In addition, areas of capillary nonperfusion that are adjacent to the foveal avascular zone can increase as the diabetic retinopathy worsens,[3] and changes in the DCP have been associated with the disorganization of the retinal inner layers.[4]

In this issue of *JAMA Ophthalmology*, Hwang et al[5] quantified the capillary nonperfusion in 3 vascular plexuses in the macula of diabetic and healthy control eyes using projection-resolved OCTA. Their study included 39 healthy control eyes, 16 eyes from patients with diabetes without retinopathy, 23 eyes with mild to moderate nonproliferative diabetic retinopathy, and 32 eyes with severe nonproliferative diabetic retinopathy or proliferative diabetic retinopathy. A semiautomated algorithm segmented the volumes in the superficial vascular complex (RPCP and SVP), ICP, and DCP. The authors show that automatically quantified avascular areas that use their algorithm from a 3-layer segmentation scheme that uses projection resolved OCTA distinguishes levels of retinopathy with a greater sensitivity compared with unsegmented slabs or commercial vascular density measurements in 2 layers (superficial and deep).

This study[5] adds to the growing literature that studies all the vascular plexuses of the retina in diabetic retinopathy as well as other retinal vascular diseases. Projection artifacts and segmentation errors can problematize interpretations of OCTA images and lead to erroneous conclusions. The authors have made substantial efforts to overcome these difficulties, first by using an algorithm to resolve the projection artifacts[6] and second by creating a reproducible automated system to segment the 3 layers, namely the superficial vascular complex, the ICP, and DCP. Another important aspect of the study[5] is that the avascular areas that were quantified were independent of the signal strength index, which was not the case for the commercial vascular density measurements that depended on the signal strength index. Their work shows that diabetic retinopathy can reliably and objectively be evaluated by OCTA.

The limitations of the study include the small number of patients in each group, as stated by the authors in their discussion, and perhaps the confounding association of prior light amplification by stimulated emission of radiation or anti-