Association Between Retinal Layer Thickness and Perfusion Status in Extramacular Areas in Diabetic Retinopathy

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• PURPOSE: **This study was performed to investigate the association between changes in retinal layer thickness and perfusion status in the extramacular areas of eyes with diabetic retinopathy.**

• DESIGN: **Retrospective cross-sectional study.**

• METHODS: **The medical records of 70 eyes from 55 patients with diabetes were reviewed. The status of retinal perfusion in extramacular areas was evaluated using swept-source optical coherence tomography angiography. Retinal layer thickness was measured in nonperfused areas (NPA) larger than 2 optic disc areas, areas of sparse capillaries (SC), and perfused areas (PA-DR) in eyes with diabetic retinopathy. Retinal layer thickness was also measured in perfused areas in eyes without diabetic retinopathy (PA-NDR), and the thicknesses were then compared. In addition, swept-source optical coherence tomography angiography images and retinal thickness maps were compared to investigate the distribution of retinal thickness changes and spatial relationships to areas of retinal perfusion.**

• RESULTS: **The inner retinal thickness in NPA was significantly thinner than the inner retinal thicknesses in SC, PA-DR, and PA-NDR (all $P < .001$), and the inner retinal thickness in PA-NDR and SC was significantly thinner than that in PA-DR ($P = .006$ and .031, respectively). In a distribution analysis of the extramacular areas, NPA spatially overlapped with areas of severe retinal thinning in all locations. Local thickening with smooth shapes and gentle borders overlapped with areas of capillary abnormalities. Neovascularization was present at sites of local thickening with irregular shapes and unnatural clear borders.**

• CONCLUSIONS: **Changes in retinal layer thickness were associated with perfusion status, suggesting that retinal thickness maps can reflect perfusion status.** **(Am J Ophthalmol 2020;215:25–36. © 2020 Elsevier Inc. All rights reserved.)**

Accepted for publication Mar 17, 2020.

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THE RETINAL VASCULATURE IS IN THE INNER RETINA and is responsible for nourishing the inner retina. Impairment of retinal circulation causes ischemia of the inner retina. In eyes with the central retinal artery occlusion, transient inner retinal edema and subsequent inner retinal thinning has been observed.[1–4] In eyes with diabetic retinopathy, disorganization of the retinal inner layers (DRIL) in the macular areas, which was first reported by Sun and associates, is associated with capillary nonperfusion.[5,6] DRIL in the macular areas is also associated with alterations in visual function and future changes in visual acuity.[7–9] Inner retinal layers in the macular area were selectively thinned in minimal diabetic retinopathy compared with healthy control subjects.[10] The thickness of the inner retinal layer in the macular area diminishes over time in patients with type 1 diabetes, even before the onset of diabetic retinopathy.[11] A lower tolerance to glucose is significantly associated with reductions in the thickness of the circumpapillary retinal nerve fiber layer.[12] The impairment of retinal circulation can cause structural changes to the inner retina in the macular area.

However, previous studies of structural changes in eyes associated with diabetic retinopathy were performed on the macular areas, whereas the extramacular areas have been less well studied. Recently, the development of swept-source optical coherence tomography (SS-OCT) has made wide-area scanning possible.[13–15] The scan area of the latest commercially available SS-OCT is much wider than that of the spectral-domain OCT. By using SS-OCT angiography, changes in retinal structure and circulation in the extramacular retina can be easily evaluated. Thus, the current study was performed using SS-OCT angiography to determine any associations between changes in retinal perfusion and retinal layer thickness in the extramacular areas in eyes with diabetic retinopathy.

METHODS

• ETHICS APPROVAL: This was a retrospective cross-sectional study. The protocol for this retrospective study was approved by the Institutional Review Board of Nagoya University School of Medicine, and the

0002-9394/$36.00
https://doi.org/10.1016/j.ajo.2020.03.019

procedures used were in accordance with the tenets of the Declaration of Helsinki.

• **PATIENTS:** A retrospective review was performed using the medical records of consecutive diabetic patients who were examined using SS-OCT (PlexElite 9000; Carl Zeiss Meditec, Dublin, California, USA) for the evaluation of diabetic retinopathy between October 2016 and September 2019 at Nagoya University Hospital, Japan. The severity of diabetic retinopathy was graded based on the International Clinical Diabetic Retinopathy Disease Severity Scale.[16,17]

Eyes with other retinal diseases, including retinal vascular occlusion, glaucoma, and age-related macular degeneration, were excluded. Eyes that had received an intravitreal injection of anti–vascular endothelial growth factor (anti-VEGF) and laser treatment within the previous 6 months and those with a history of vitreoretinal surgery were excluded. Signal intensity of SS-OCT images <7 was also excluded.

• **SS-OCT ANGIOGRAPHY:** Perfusion status was evaluated by SS-OCT angiography (PlexElite 9000). The scan program used was 12 × 12 mm, 15 × 9 mm centered on the fovea, or a montage of two 15- × 9-mm scans. The SS-OCT angiography segmentation used was full-thickness retinal slab. In SS-OCT angiography images, capillary nonperfused areas (NPA) larger than 2 optic disc areas, areas with sparse capillaries (SC), and perfused areas (PA-DR) in eyes with diabetic retinopathy and perfused areas in eyes without diabetic retinopathy (PA-NDR) were identified in the extramacular areas.

SC was defined as follows: 1) most of fine capillaries were lost; 2) residual capillaries were dilated; 3) the area adjacent to NPA; 4) the largest gap between capillaries was >100 μm; and 5) vessel length density in the circle with diameter of 500 μm at the location of measurement was between 2.0-7.0 mm/mm^2. PA was defined as follows: 1) no visible capillary dropout was seen, 2) the largest gap between capillaries was <50 μm, and 3) vessel length density in the 500-μm circle at the location of measurement was >7 mm/mm^2. NPA was defined as follows: 1) all fine capillaries were lost and 2) vessel length density in the 500-μm circle at the location of measurement was <2 mm/mm^2, including signal noise. Vessel length density in the 500-μm circle was calculated after applying Niblack auto local threshold and the skeletonize function of ImageJ software (National Institutes of Health, Bethesda, Maryland, USA).

At each location, the retinal layer thickness of the entire retina, the inner retina (the inner limiting membrane to the outer plexiform layer), and the outer retina (the outer nuclear layer to the retinal pigment epithelium) was measured manually using the caliper function of the built-in software of the SS-OCT device. The measurement at the NPA was performed ≥1 mm away from the perfused area. Areas with neovascularization, photocoagulation scars were excluded. Fluorescein angiography was also used to evaluate the capillary status and leakage.

Retinal layer thicknesses were compared between NPA, SC, PA-DR, and PA-NDR. As for the locations of thickness measurement, NPA was first identified, then matched SC, PA-DR, and PA-NDR areas were selected at a similar distance from the optic disc in different eyes.

• **DISTRIBUTION OF CHANGES IN RETINAL THICKNESS:** Retinal thickness maps and SS-OCT angiography images overlaid on retinal thickness maps were created for all eyes using the same SS-OCT angiography scans through the built-in software of the SS-OCT device. Retinal thickness maps, SS-OCT angiography images, and fluorescein angiography images were compared to investigate the distribution of retinal thickness changes and spatial relationships to retinal perfusion. The accuracy of segmentations of all scans was verified using the built-in software of the SS-OCT device. When identifying a capillary lesion, capillary dropout was defined as the area of capillary loss and gap between capillaries >50 μm. Capillary abnormality is a generic term that defines any capillary dropout, including SC and microaneurysms.

In 6 eyes, SS-OCT angiography was conducted twice during >6 months, and the changes in the retinal thickness and perfusion status of NPA during this period were evaluated.

• **REPRODUCIBILITY OF THE MANUAL RETINAL LAYER THICKNESS MEASUREMENTS:** We evaluated the reproducibility of the manual measurement of retinal layer thickness. Retinal layer thickness was measured twice by a single grader for 10 randomly selected SS-OCT angiography images of diabetic retinopathy and normal eyes for intragrader intraclass correlation coefficients (ICCs). For the intergrader ICCS, each retinal layer thickness was independently measured by 2 graders. For both measurements, the graders were masked to the clinical characteristics of the subjects, such as age, sex, normal, or with diabetic retinopathy.

• **STATISTICAL ANALYSIS:** Repeated analysis of variance tests, followed by the Tukey post hoc test, were used to determine if there were any significant differences between eyes in terms of retinal layer thickness, spherical equivalent refractive error, and distance between the location of measurement and optic disc.

For this study, we adopted the usual P value of $< .05$ to indicate statistical significance. SPSS statistical software (v 26.0; IBM Corp, Chicago, Illinois, USA) was used to perform all statistical analyses.

TABLE 1. Patient Characteristics

Eyes/patients, n	70/55
Age, years, mean ± SD (range)	58.4 ± 7.7 (36-69)
Sex, men/women, n	38/17
Hemoglobin A1c, %, mean ± SD	7.9 ± 2.2
Logarithm of minimal angle of resolution, mean ± SD	−0.10 ± 0.20
Spherical equivalent refractive error, diopters, mean ± SD (range)	−1.1 ± 2.1 (−5.75 to +3.75)
Crystalline lens status, phakia/pseudophakia, n	64/6
Panretinal photocoagulation, eyes, n	17
Diabetic retinopathy classification, n	
No retinopathy	27
Moderate nonproliferative retinopathy	7
Severe nonproliferative retinopathy	4
Proliferative retinopathy	32

SD = standard deviation.

RESULTS

THIS STUDY INCLUDED 70 EYES OF 55 PATIENTS. THERE WERE 38 men and 17 women with a mean age ± standard deviation (SD) of 58.4 ± 7.7 years (range 36-69 years). Among the 70 eyes, 11 had nonproliferative diabetic retinopathy and 32 showed proliferative diabetic retinopathy. No diabetic retinopathy was found in 27 eyes. The mean spherical equivalent refractive error was −1.1 ± 2.1 diopters (D) (range −5.75 to +3.75 D). Fluorescein angiography was performed in 33 of 70 eyes using an ultra–wide-field scanning laser ophthalmoscope (Optos PLC, Dunfermline, Scotland, United Kingdom) (Table 1).

Among 70 eyes, PA-NDR, PA-DR, SC, and NPA were identified in 27, 30, 27, and 27 eyes, respectively. Representative cases are shown in Figure 1 and Supplemental Figures 1 and 2.

The total retinal thickness in PA-NDR, PA-DR, SC, and NPA was 211.3 ± 13.6, 233.4 ± 23.3, 223.3 ± 24.6, and 196.3 ± 23.5 μm, respectively. The total retinal thickness in NPA was significantly thinner than that in PA-NDR, PA-DR and SC ($P = .019$, $P < .001$ and $P < .001$, respectively). The total retinal thickness in PA-NDR was significantly thinner than that in PA-DR ($P < .001$). There was no significant difference in the total retinal thickness between PA-NDR and SC ($P = .09$) (Figure 2A, Table 2).

The inner retinal thickness in PA-NDR, PA-DR, SC, and NPA was 113.3 ± 12.6, 125.6 ± 16.6, 115.2 ± 17.3, and 82.7 ± 13.5 μm, respectively. The inner retinal thickness in NPA was significantly thinner than that in PA-NDR, PA-DR, and SC (all $P < .001$). The inner retinal thickness in PA-NDR and SC was significantly thinner than that in PA-DR ($P = .006$ and $P = .031$, respectively). There was no significant difference in the inner retinal thickness between PA-NDR and SC ($P = 0.95$) (Figure 2B, Table 2).

The outer retinal thickness in PA-NDR, PA-DR, SC, and NPA was 98.0 ± 10.4, 107.8 ± 11.8, 108.1 ± 13.3, and 113.5 ± 16.9 μm, respectively. The outer retinal thickness in PA-NDR was significantly thinner than that in PA-DR, SC, and NPA ($P = .019$, $P = .010$, and $P < .001$, respectively). The outer retinal thickness in PA-DR, SC, and NPA was not significantly different (PA-DR vs SC, $P = 1.0$; SC vs NPA, $P = .32$; PA-DR vs NPA, $P = .31$) (Figure 2C, Table 2).

The spherical equivalent refractive error in PA-NDR, PA-DR, SC, and NPA was −0.8 ± 1.9, −1.1 ± 1.9, −1.2 ± 2.5, and −1.5 ± 2.5 D, respectively. There were no significant differences between groups ($P = .69$). The distances between the optic disc and the measured location in PA-NDR, PA-DR, SC, and NPA were 8.2 ± 2.2, 8.0 ± 1.9, 8.3 ± 1.9, and 8.2 ± 2.3 mm, respectively. There were no significant differences between groups ($P = .95$).

• **DISTRIBUTION OF CHANGES IN RETINAL THICKNESS:** In SS-OCT angiography images, an NPA larger than a 2-disc area was observed at 52 locations in the extramacular areas of 43 eyes; these locations overlapped with areas of aberrant retinal thinning in all eyes (52/52, 100%) in retina thickness maps (Figures 3).

In retinal thickness maps, local retinal thickening with smooth shapes and gentle borders was observed in 104 locations in 31 eyes; these locations overlapped with areas of capillary abnormalities in all locations (100%), capillary dropout in 92 locations (88%), and microaneurysms in 68 locations (65%) based on SS-OCT angiography. In those areas, fluorescein leakage was observed at all locations in fluorescein angiography (100%). Absence of neovascularization at these areas was confirmed by both the fluorescein angiography images and observation of all cross-sectional SS-OCT images at these areas (Figure 4).

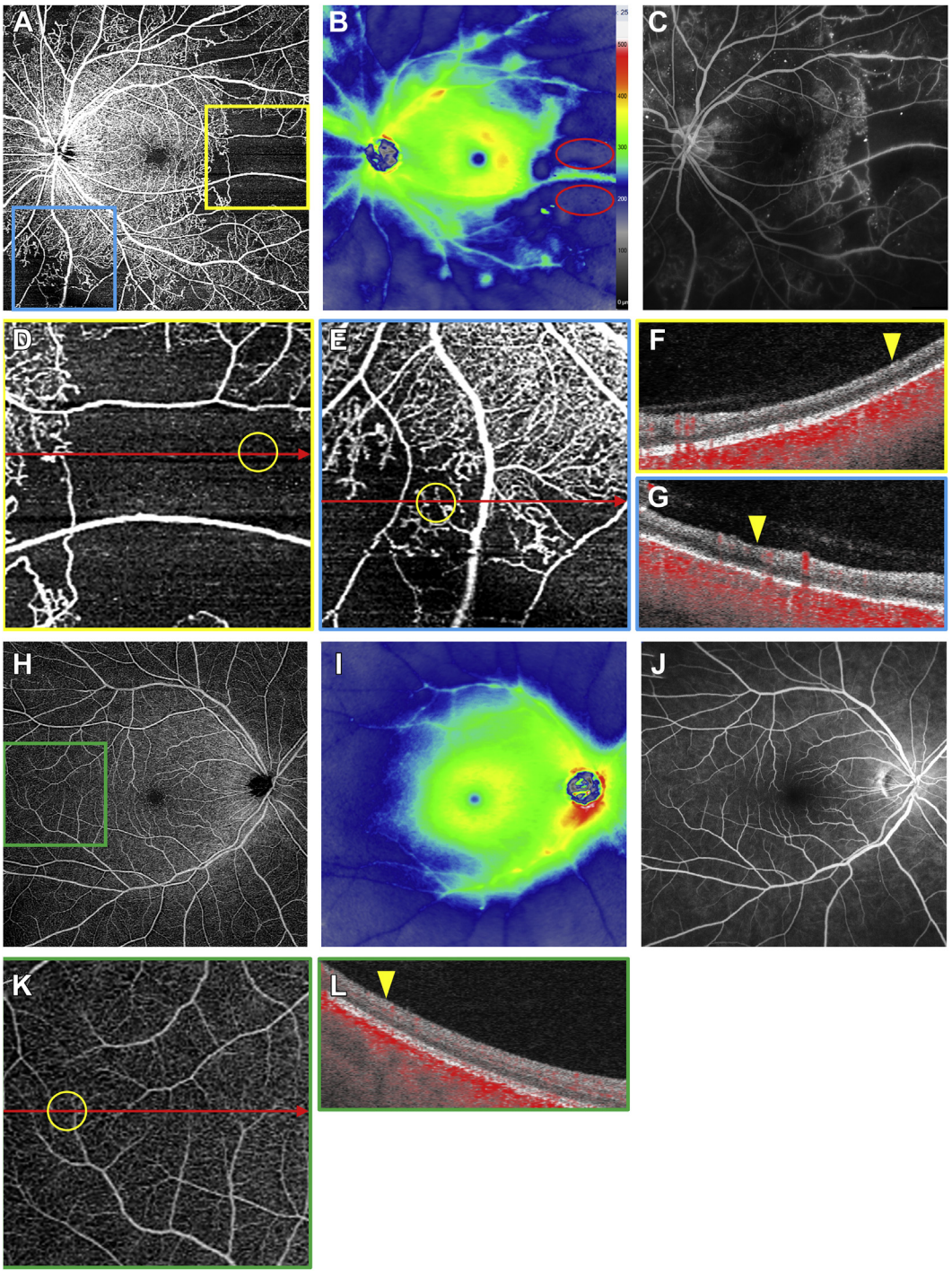

FIGURE 1. (A-G) Representative case 1 with diabetic retinopathy. (H-L) Representative case 2 without diabetic retinopathy. (A) Swept-source optical coherence tomography (SS-OCT) angiography image of the eye with diabetic retinopathy (12 × 12 mm, retinal slab). Note the large nonperfused area (NPA; yellow rectangle) and area of sparse capillaries (SCs; blue rectangle). (B) Retinal thickness map. Note that the NPA in (A) corresponds with retinal thinning (red circles). (C) Fluorescein angiography image. The NPA is shown, similar to the SS-OCT angiography image (A). (D) Magnified image of the yellow rectangle in (A). The red arrow indicates the location of the SS-OCT scan at the NPA (F). The thickness of the retinal layer was measured at the center of the yellow circle. (E) Magnified image of the blue rectangle in (A). The red arrow indicates the location of the SS-OCT scan at the SC (G). At the site of thickness measurement, a circle with diameter of 500 μm was delineated (yellow circle). If the largest gaps between capillaries was > 100 μm and vessel length density in the 500-μm circle was between 2.0 and 7.0 mm/mm^2, retinal thickness at SC was measured. (F) Cross-sectional SS-OCT image of the NPA with flow signal (red dots) at the red arrow in (D). The thickness of the retinal layer was measured at the yellow arrowhead. Note that 1) the inner retina at the NPA is thin, 2) there is no flow signal in the inner retina at

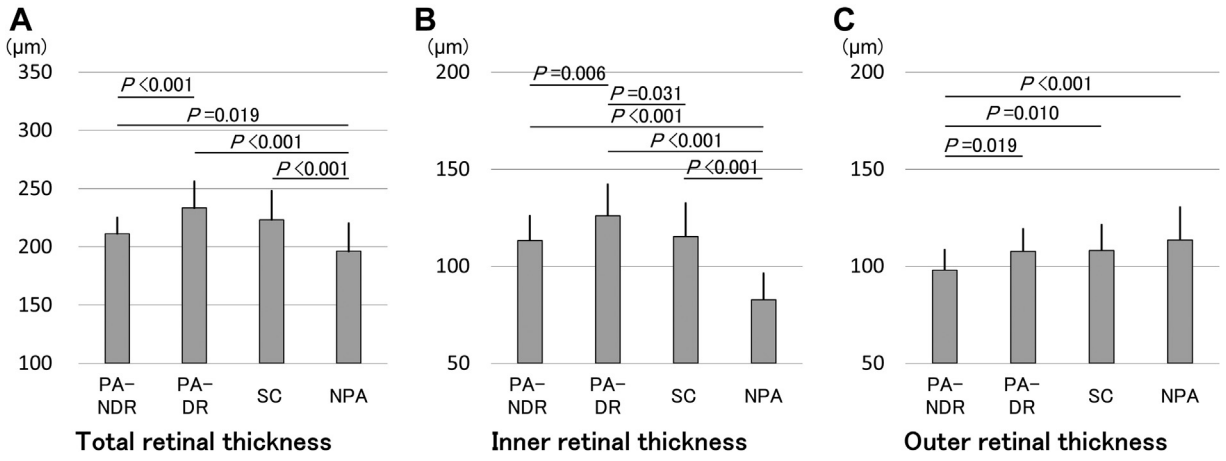

FIGURE 2. Comparison of retinal layer thickness between groups. (A) Comparison of total retinal thickness. Retinal thickness at the nonperfused area (NPA) is significantly thinner than the thickness in other groups. (B) Comparison of inner retinal thickness. Retinal thickness at the NPA is significantly thinner than the thickness in other groups. (C) Comparison of outer retinal thickness. Retinal thickness at the perfused area in eyes without diabetic retinopathy is significantly thinner than the thickness in other groups.

In retinal thickness maps, retinal thickening with irregular shapes and unnatural clear borders was observed in 32 locations in 20 eyes; all thickening corresponded with neovascularization (32/32, 100%). The presence of neovascularization at these areas was confirmed by both the fluorescein angiography images and observation of all cross-sectional SS-OCT images at these areas (Figure 5).

In 6 eyes in which SS-OCT angiography was performed twice in >6 months, there were no changes in retinal thinning or perfusion in the NPA (6/6, 100%) (Figure 6). No treatment was applied to these eyes during this period. In 2 eyes, there were large NPA in the macular and extramacular areas. Large NPA overlapped with areas of aberrant retinal thinning in both eyes (Figure 7).

• **REPRODUCIBILITY OF THE MANUAL RETINAL LAYER THICKNESS MEASUREMENTS:** The intragrader ICC for inner, outer, and total thickness measurement was 0.96, 0.87, and 0.94, respectively, whereas the intergrader ICC was 0.83, 0.75, and 0.87, respectively. Therefore, the reproducibility of the retinal layer thickness measurements in the SS-OCT angiography images was confirmed.

DISCUSSION

THIS STUDY SHOWED THAT THERE WAS AN ASSOCIATION between changes in retinal thickness and perfusion. After the onset of diabetic retinopathy, inner retinal thickness increased and then inner retinal thickness decreased as retinopathy progressed. Thickening after the onset of diabetic retinopathy may be caused by the breakdown of the blood–retina barrier, whereas thinning after retinopathy progression may be caused by the loss of inner retinal tissue related to ischemia. At sites of local thickening with smooth shapes and gentle borders, there were capillary abnormalities, including capillary dropout and microaneurysms. At sites of local thickening with irregular shapes and unnatural clear borders, neovascularization was present. These results suggest that retinal thickness maps can reflect perfusion status and may be used to provide clues as to the status of perfusion and leakage (Table 3).

In proliferative diabetic retinopathy, the formation of large NPA in extramacular areas is common. In contrast, large NPA in macular areas is rare, even in eyes with proliferative diabetic retinopathy. This may be because of the high density of capillaries in macular areas. This may

the NPA, and 3) the area of inner retinal thinning corresponds with the NPA in (D). (G) Cross-sectional SS-OCT image of the SC with flow signal at the red arrow in (E). Note that the inner retinal structure at the SC is disorganized, but its thickness is relatively preserved. (H) SS-OCT angiography image in the eye without diabetic retinopathy. There is no abnormality of the retinal capillary network. (I) Retinal thickness map. There is no irregularly thickened or thinned area. (J) Fluorescein angiography image. There is no abnormality of retinal vasculature and no leakage. (K) Magnified image of the green rectangle in (H). The red arrow indicates the location of the SS-OCT scan at the perfused area in eyes without diabetic retinopathy (L). Retinal layer thickness was measured at the center of the yellow circle. (L) Cross-sectional SS-OCT image of the perfused area in eyes without diabetic retinopathy with flow signal (red dots) at the red arrow in (K). All of the retinal layer structure is intact, and there is blood flow in the inner retina. The thickness of the retinal layer was measured at the yellow arrowhead.

TABLE 2. Retinal Layer Thickness

	PA-NDR	PA-DR	SC	NPA	*P* Value: PA-NDR vs PA-DR	PA-NDR vs SC	PA-NDR vs NPA	PA-DR vs SC	PA-DR vs NPA	SC vs NPA
Total retinal thickness, μm (±SD)	211.3 ± 13.6	233.4 ± 23.3	223.3 ± 24.6	196.3 ± 23.5	<.001	.09	.019	.24	<.001	<.001
Inner retinal thickness, μm (±SD)	113.3 ± 12.6	125.6 ± 16.6	115.2 ± 17.3	82.7 ± 13.5	.006	.95	<.001	.031	<.001	<.001
Outer retinal thickness, μm (±SD)	98.0 ± 10.4	107.8 ± 11.8	108.1 ± 13.3	113.5 ± 16.9	.019	.010	<.001	1.00	.31	.32

NPA = nonperfused areas; PA-DR = perfused areas in eyes with diabetic retinopathy; PA-NDR = perfused areas in eyes without diabetic retinopathy; SC = sparse capillaries; SD = standard deviation.

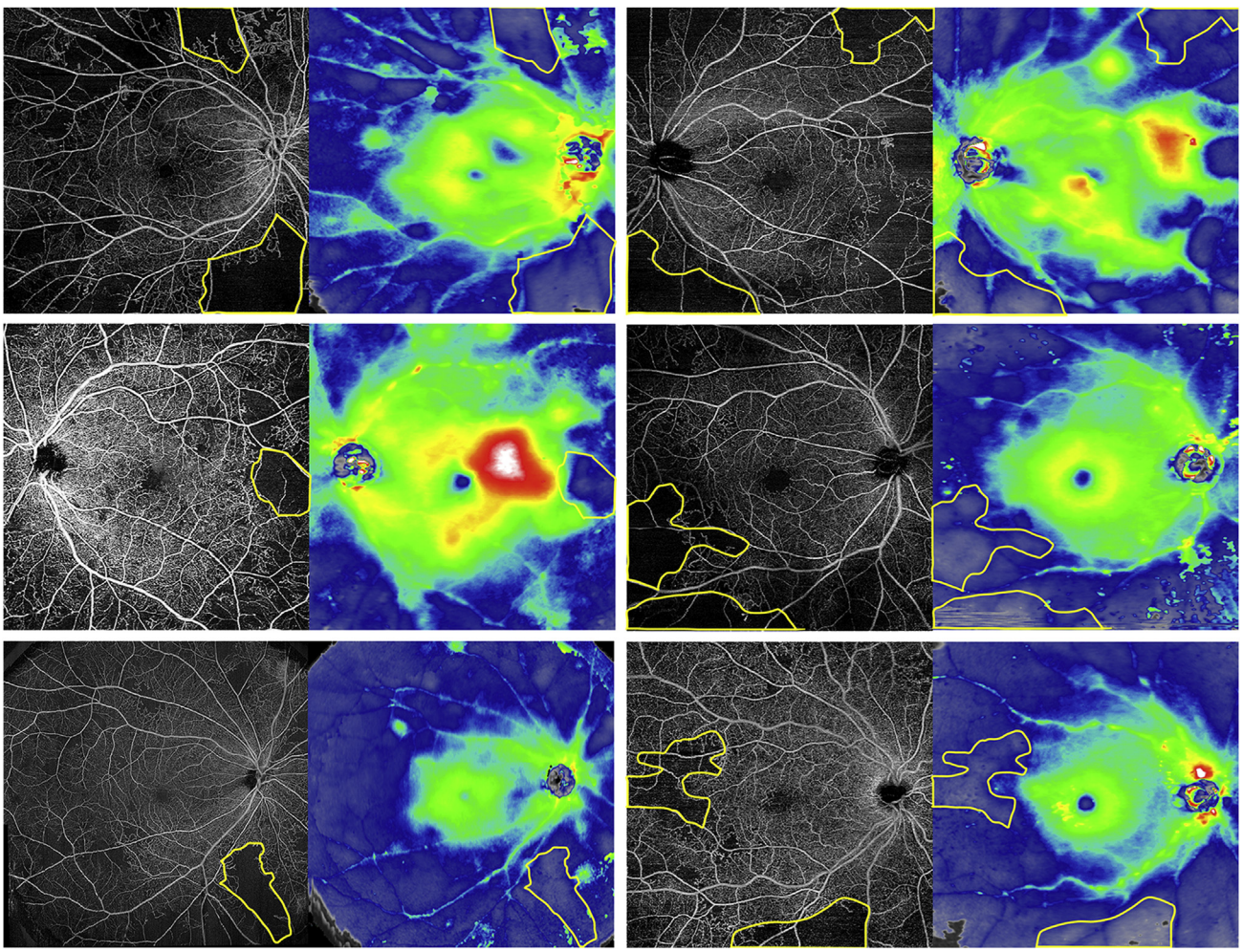

FIGURE 3. Six representative cases of diabetic retinopathy with nonperfused areas (NPAs). Left. Swept-source optical coherence tomography angiography images. Right. Retinal thickness maps. Areas of severe retinal thinning correspond with nonperfused areas (yellow lines).

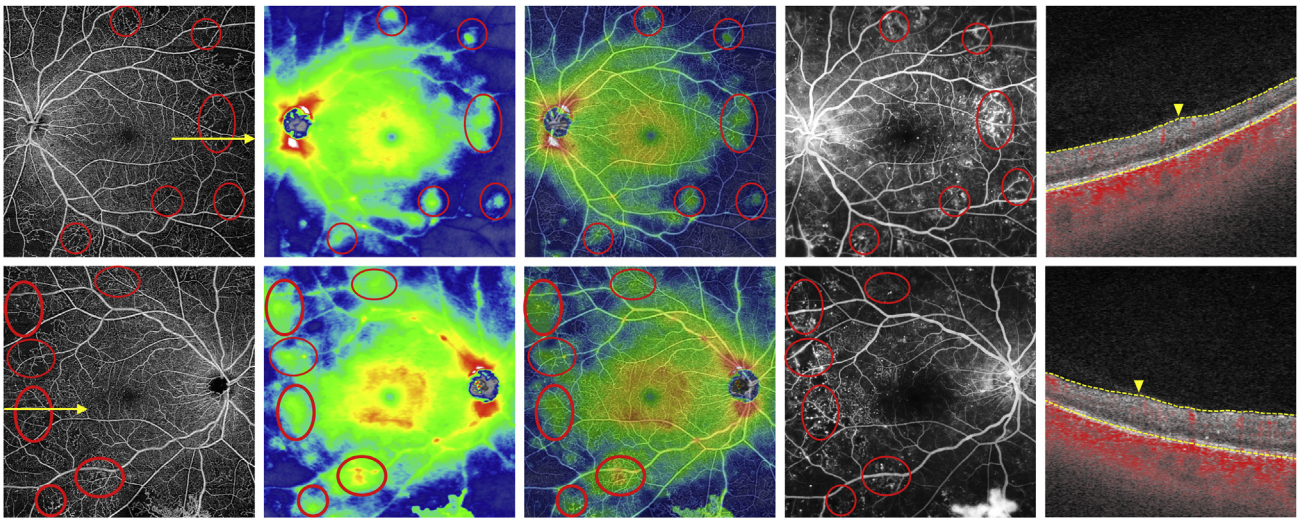

FIGURE 4. Two representative cases of local retinal thickening with smooth shapes and gentle borders in the extramacular areas. Column 1 shows 12 × 12 mm swept-source optical coherence tomography (SS-OCT) angiography images. Column 2 shows retinal thickness maps. Column 3 shows SS-OCT angiography images overlaid on retinal thickness maps. Column 4 shows fluorescein angiography images. Column 5 shows cross-sectional SS-OCT angiography images with flow signals (red dots) at the site with retinal thickening [yellow arrows in SS-OCT angiography images (column 1)]. Local retinal thickening with smooth shapes and gentle borders shown as red circles in the retinal thickness maps (columns 2 and 3) appears as capillary dropout with leakage in the SS-OCT angiography images (column 1) and fluorescein angiography images (column 4). At the site with retinal thickening (yellow arrowheads), inner retinal thickening was observed, and no neovascularization was observed (column 5).

prevent the formation of large NPA. However, the retina became severely thinned at the large NPA, even in macular areas (Figure 7).

There are reports that studied the association between retinal layer thickness changes and retinal perfusion status in the macula using OCT angiography. Kim and associates[18] reported that macular ganglion cell/inner plexiform layer thickness significantly correlated with vessel density and perfusion index in eyes without diabetic retinopathy and nonproliferative diabetic retinopathy. Moreover, they reported the progressive macular ganglion cell/inner plexiform layer thinning during 24 months of observation in early stage diabetic retinopathy.[19] The inner retina in the macula without macular edema becomes mildly thinned, correlating with a decrease in vessel density during the early stage of diabetic retinopathy. Lavia and associates[20] reported that different retinal structural changes associated with reduced vascular density and patchy polygonal nonperfusion areas in the superficial vascular plexus corresponded well to areas of inner retinal thinning in eyes without macular edema of patients with type 1 diabetes. Inner retinal thinning occurs at the location of impaired perfusion in the macular and extramacular area.

Because inner retinal atrophy occurs as a result of inner retinal tissue loss, our study suggests that the reperfusion of large NPA is difficult. In our study, 6 cases underwent SS-OCT angiography twice. Reperfusion was not observed during this period and there was no intervention (Figure 6). Couturier and associates[21] reported that no reperfusion of vessels or capillary networks was detected in NPA using 2 imaging techniques, ultra–wide-field fluorescein angiography, and SS-OCTA, in eyes with diabetic retinopathy after 3 anti-VEGF injections. Bonnin and associates[22] reported that after anti-VEGF injections the diabetic retinopathy severity score based on color fundus photographs had improved with no retinal reperfusion, based on ultra–wide-field fluorescein angiography. Further studies are needed to determine whether NPA in diabetic retinopathy is irreversible.

In the extramacular area analysis in this study, inner retinal structures could not be observed in the NPA because of severe inner retinal atrophy. The retinal thickness at the extramacular area is physiologically thinner than that at the macular area but thinning of the inner retina at NPA was much severe than physiologic thinning. In SC areas, inner retina was not atrophied, but DRIL was seen in the SC in some eyes (Figure 1). DRIL may begin to occur at the SC stage, and inner retinal thickness then decreases after the complete loss of capillaries. Unfortunately, the resolution of the scan used in this study was not sufficient to precisely visualize fine structural changes within the inner layer of the extramacular areas. Additional studies using scans with higher resolution are needed to investigate layer-by-layer changes in the extramacular areas.

Segmentation errors always occur at the site of vitreoretinal adhesion because the algorithms used to detect the inner limiting membrane cannot usually differentiate

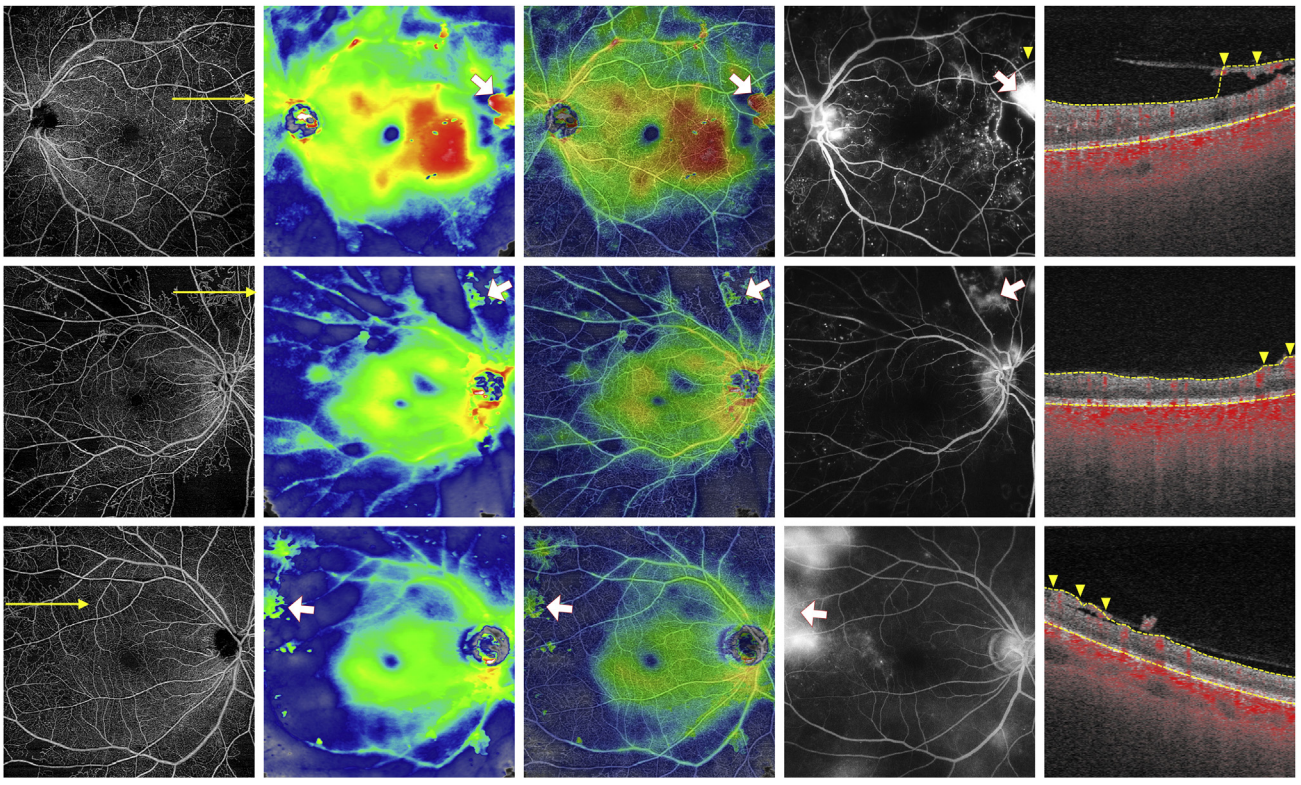

FIGURE 5. Three representative cases of neovascularization in the extramacular areas. Column 1 shows 12 × 12 mm swept-source optical coherence tomography (SS-OCT) angiography images. Column 2 shows retinal thickness maps. Column 3 shows SS-OCT angiography images overlaid on retinal thickness maps. Column 4 shows fluorescein angiography images. Column 5 shows cross-sectional SS-OCT angiography images with flow signals (red dots) at the site with retinal thickening (yellow arrows in SS-OCT angiography images [column 1]). Irregularly shaped retinal thickening with clear unnatural margins shown as white arrows in the retinal thickness maps (columns 2 and 3) corresponded with neovascularization of the retina in the fluorescein angiography images (arrows in column 4). At the neovascularization site (yellow arrowheads), segmentation (yellow dotted line) errors occurred in the cross-sectional SS-OCT images with flow signal (red dots; column 5).

between the inner limiting membrane and attached vitreous. These segmentation errors cause artifacts in the form of irregular thick areas to appear in retinal thickness maps. In diabetic retinopathy, neovascularization arises from the retina and grows along the posterior vitreous; therefore, peripheral, irregularly thick areas of retina in a retinal thickness map can sometimes be a sign of neovascularization. Retinal thickness maps can be used as a clue to help identify the location of neovascularization, although the retinal thickness map needs to be carefully interpreted along with B-scans (Figure 5).

The outer retinal thickness in PA-DR, SC, and NPA was significantly thicker than that in PA-NDR. This may have been caused by leakage from the retinal vasculature of the inner retina reaching the outer retina or because outer retinal thickness does not become thinner at NPA where there is no leakage; inner retinal perfusion impairment may cause structural changes to the outer retina. There may be another possibility—that the method used in this study likely underestimated the deep capillary plexus and that plexus was already not perfused in the eyes with NDR, resulting in outer retinal thinning that precedes the onset of retinopathy with secondary breakdown of the blood–retinal barrier manifesting as inner retinal thickening in these eyes.

In the macular areas, severe outer retinal edema can result in outer retinal atrophy, but in our study, we did not observe this in the extramacular areas. This may be because of differences in retinal structure, including capillary density.

Recent advances in SS-OCT angiography have increased the width of the scan area, making it possible to evaluate retinal perfusion in the extramacular areas. Yasukura and associates[23] reported an association between large arterioles and NPA in the extramacular areas. Morino and associates[24] reported that most extramacular white spots could be distinguished from macular spots with respect to diabetic NPA using SS-OCT angiography images. When analyzing retinal thickness in the extramacular areas, the current SS-OCT system (PlexElite 9000) can

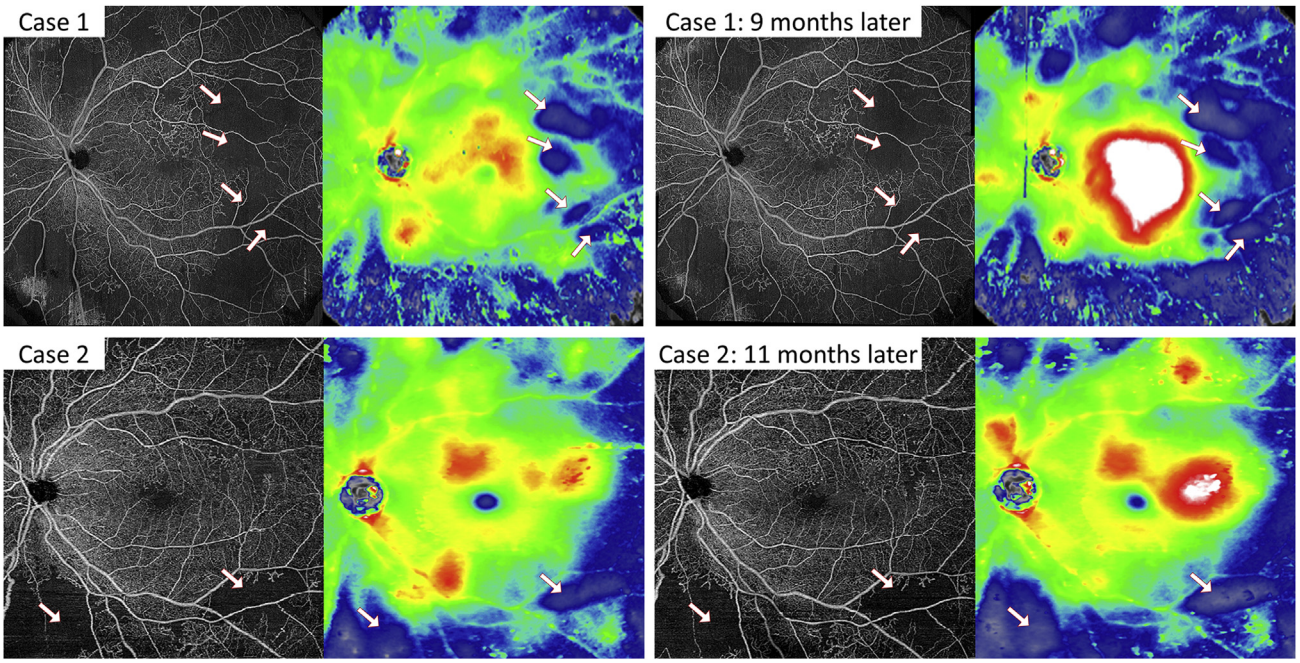

FIGURE 6. Two representative cases observed for > 6 months. Note that retinal thinning corresponded with the nonperfused areas (white arrows), and neither retinal thinning nor nonperfused areas changed during the period of observation (case 1, 9 months; case 2, 11 months) in either case. In contrast, retinal thickness in the macular perfused area increased in both eyes; however, there were no visible changes in the perfusion status in those areas in swept-source optical coherence tomography angiography.

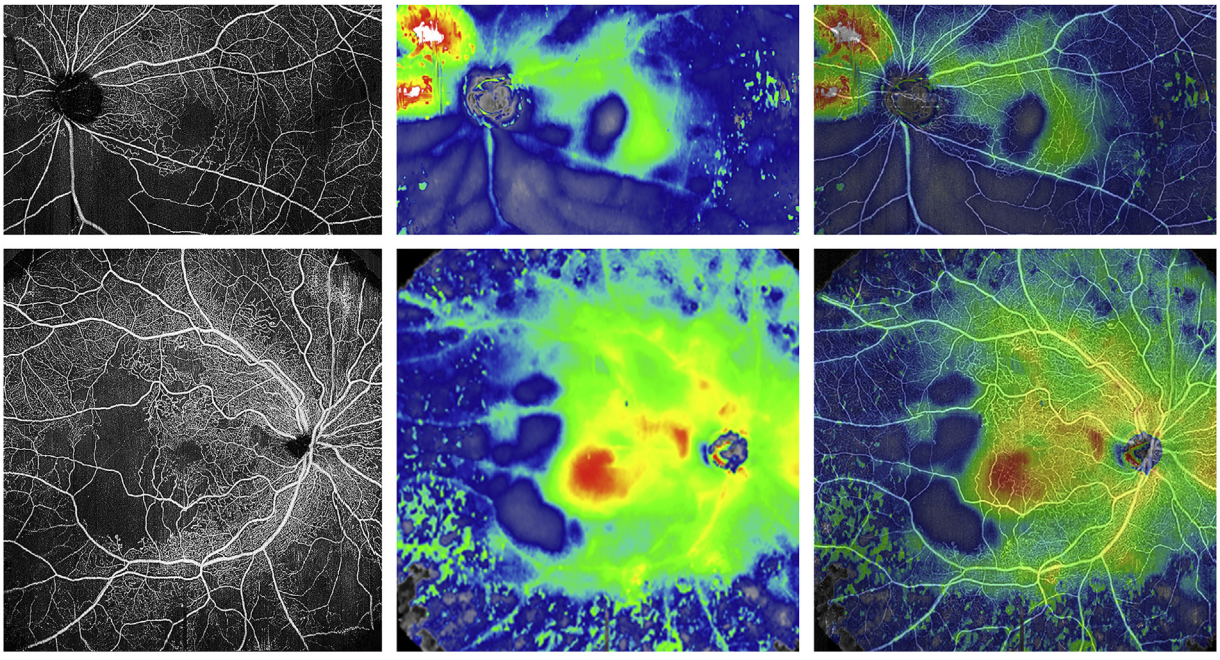

FIGURE 7. Two representative cases of large nonperfused areas and retinal thinning in the macular areas. Column 1 shows swept-source optical coherence tomography (SS-OCT) angiography images. Column 2 shows retinal thickness maps. Column 3 shows SS-OCT angiography images overlaid on retinal thickness maps. Large nonperfused areas in the macular areas also corresponded with retinal thinning, similarly to nonperfused areas in the extramacular areas.

TABLE 3. Perfusion Status and Retinal Thickness Status

Perfusion Status	Retinal Thickness Status
Perfused area	Slightly thicker than no retinopathy
Sparse capillaries	Retinal thickness similar to that of perfused area, sometimes with disorganization of the retinal inner layers
Nonperfused area	Extremely thin, especially in the inner retina

only create retinal thickness maps for the total retina. As our study has shown, in diabetic retinopathy, changes to the retinal layer thickness occur in the inner retina; therefore, inner retinal thickness maps are more sensitive to detect retinal layer thickness changes in diabetic retinopathy. Advances in SS-OCT technology continue to progress, so the analysis of retinal layer thickness in the periphery should soon be possible.

OCT angiography can precisely show retinal perfusion status. However, OCT devices that have OCT angiography function are more expensive than OCT devices without angiography function. In addition, OCT angiography images are obtained by repeating OCT scans, determining the change in backscattering between consecutive B-scans, and attributing the differences entirely to the flow of erythrocytes through retinal blood vessels.[25–27] Therefore, there are situations that make obtaining OCT angiography images difficult but obtaining a retinal thickness map possible, such as unstable fixation and cataracts. In circumstances where both OCT angiography and fluorescein angiography are difficult, analysis of retinal thickness map can provide a clue to speculate the functioning of retinal circulation, although the retinal thickness map needs to be carefully interpreted along with B-scans.

This study has some limitations. The resolution of the SS-OCT angiography scan program used in this study was not very high and layer structures were not clearly visible. However, when SS-OCT angiography and fluorescein angiography images were compared, the distribution of NPA was similar in both, suggesting the SS-OCT scan density used in this study can be used to evaluate capillary perfusion status. Another limitation may be that the slab used in this study was a full-thickness retinal slab. Changes in smaller capillaries in the deep capillary plexus may not be well reflected in the OCT angiography images with a full-thickness retinal slab. There were areas where retinal thinning was wider than area of NPA. This may result from undetected abnormalities in the middle and deep capillaries, especially in the posterior pole that cannot be detected in the OCT angiography with full-thickness retinal slab. Future studies regarding the association between thickness and capillary density in each layer are necessary. Another limitation is the potential for patient selection bias because of the retrospective nature of the study. The final limitation is that there were eyes with panretinal photocoagulation, which may have induced some changes in retinal thickness in the distant measured area.

In conclusion, this study showed that the inner retina was thinned at large NPA, that there were capillary abnormalities, including capillary dropout and microaneurysms at sites of local thickening with smooth shapes and gentle borders, and that neovascularization was present at sites of local thickening with irregular shapes and unnatural clear borders in the extramacular area. Changes in retinal thickness reflected the retinal perfusion status. Although the retinal thickness maps need to be carefully interpreted along with B-scans, retinal thickness maps can be used to provide clues about the perfusion status and leakage.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

HIROTAKA ITO: CONCEPTUALIZATION, METHODOLOGY, Investigation, Data curation, Writing - review & editing. **Yasuki Ito:** Conceptualization, Methodology, Validation, Investigation, Data curation, Formal analysis, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Keiko Kataoka:** Methodology, Writing - review & editing, Project administration. **Shinji Ueno:** Methodology, Writing - review & editing, Project administration. **Jun Takeuchi:** Investigation, Data curation, Writing - review & editing. **Yuyako Nakano:** Investigation, Data curation, Writing - review & editing. **Ai Fujita:** Investigation, Data curation, Writing - review & editing. **Etsuyo Horiguchi:** Methodology, Investigation, Data curation, Writing - review & editing. **Hiroki Kaneko:** Methodology, Writing - review & editing, Project administration. **Takeshi Iwase:** Methodology, Writing - review & editing, Project administration. **Hiroko Terasaki:** Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition.

ALL AUTHORS HAVE COMPLETED AND SUBMITTED THE ICMJE FORM FOR DISCLOSURE OF POTENTIAL CONFLICTS OF INterest. Funding/Support: This work was supported in part by the Japan Society for the Promotion of Science KAKENHI Grant Numbers 16K11320 (S.U.) and 16K11265 (Y.I.). The swept-source optical coherence tomography device used in this study was on loan from Carl Zeiss Meditec. Financial Disclosures: Dr Ito has received honoraria from Alcon Japan Ltd, Bayer Health Care Japan, Canon Life Care Solutions Inc, Carl Zeiss

Meditec Co, Ltd, Japan, Kowa Pharmaceutical Company, Novartis Pharma KK Japan, Pfizer Japan, and Santen Pharmaceutical Co, Ltd, Japan. Dr Terasaki has received honoraria from Astellas Pharma Inc, Otsuka Pharmaceutical Co, Ltd, Sanwa Kagaku Kenkyusho Co, Nidek Co, Kowa Pharmaceutical Company Ltd, Santen Pharmaceutical Co, Ltd, Senju Pharmaceutical Co, Ltd, Alcon Japan Ltd, Novartis Pharma KK, Bayer Health Care Japan, Pfizer Japan Inc, Wakamoto Co, Ltd, Aichi Ophthalmologists Association, Carl Zeiss Meditec Co, Ltd, Nitten Pharmaceutical Co, Ltd, Takeda Pharmaceutical Company Limited, Chiba Ophthalmologist Association, Japan Medical Association, and the Fukushima Ophthalmologist Association; grants from Otsuka Pharmaceutical Co, Ltd, Kowa Pharmaceutical Company Ltd, Santen Pharmaceutical Co, Ltd, Senju Pharmaceutical Co, Ltd, Alcon Japan Ltd, Novartis Pharma KK, Pfizer Japan Inc, Hoya Corporation, and Wakamoto Co, Ltd; been a consultant for Ono Pharmaceutical Co, Ltd, Bayer Health Care Japan, and Carl Zeiss Meditec Co, Ltd; received travel expenses from Bayer Health Care Japan, Chiba Ophthalmologist Association, Japan Medical Association, and the Fukushima Ophthalmologist Association; appeared on the Rohto Award Selection committee for Rohto Pharmaceutical Co, Ltd; and provided writing assistance to Nitten Pharmaceutical Co, Ltd. Dr Iwase has received honoraria from Santen Pharmaceutical Co, Ltd, Bayer Health Care Japan, and Alcon Japan Ltd. Dr Kataoka has received honoraria from Santen Pharmaceutical Co, Ltd, Novartis Pharma KK, Senju Pharmaceutical Co, Ltd, Pfizer Japan Inc, and Wakamoto Co, Ltd; has received grants from Novartis Pharma KK and Senju Pharmaceutical Co, Ltd; and has been a consultant for Novartis Pharma KK. Dr Ueno has received honoraria from Nidek Company Ltd, Canon Life Care Solutions Inc, Novartis Pharma KK, and Hoya Company Ltd and received grants from Novartis Pharma KK and the Tomey Corporation. The following authors have no financial disclosures: Jun Takeuchi, Yuyako Nakano, Etsuyo Horiguchi, and Hiroki Kaneko. All authors attest that they meet the current ICMJE criteria for authorship.

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