Diabetic Neuroglial Changes in the Superficial and Deep Nonperfused Areas on Optical Coherence Tomography Angiography

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PURPOSE. To evaluate the relationship between lamellar capillary nonperfusion on optical coherence tomography angiography (OCTA) images and neuroglial changes on structural optical coherence tomography (OCT) images in diabetic retinopathy (DR).

METHODS. We retrospectively reviewed 101 consecutive eyes of 69 patients with DR. OCTA and OCT images within a 3 × 3-mm square centered on the fovea were acquired simultaneously. The nonperfused areas (NPAs) in the superficial capillary layer (sNPA) from the nerve fiber layer (NFL) to the inner plexiform layer (IPL), the deep capillary layer (dNPA) (corresponding mainly to the inner nuclear layer [INL]), or both layers (bNPA) were measured individually along 10 transverse lines. The corresponding lamellar neuroglial changes also were evaluated on OCT images.

RESULTS. The transverse lengths of the sNPA, dNPA, and bNPA were 2.54% (interquartile range, 0.81–5.55), 0.61% (0–1.99), and 5.96% (4.02–10.88), respectively. The length of the sNPA was correlated significantly with the lengths of no boundary between the NFL and ganglion cell layer (GCL)/IPL and the spots with inverted OCT reflectivity in the sNPA. The transverse length of the dNPA was associated with the length of cystoid spaces in the INL or Henle’s fiber layer (HFL) in the dNPA. There was a significant correlation between the transverse lengths of the bNPA and no boundary between the NFL and GCL/IPL within the bNPA.

CONCLUSIONS. Systematic evaluation of the OCTA–OCT association showed structural changes in the neuroglial tissues corresponding to the lamellar NPAs and suggested the feasibility of layer-by-layer evaluation of the capillary nonperfusion in DR.

Keywords: diabetic retinopathy, capillary nonperfusion, optical coherence tomography angiography

Diabetic retinopathy (DR) often leads to severe visual loss in working-age individuals, although it remains to be determined how the pathophysiologic changes in retinal vessels affect the neuroglial function and vice versa in DR.1–3 The neurovascular unit sustains the structural and functional health of retinas, whereas diabetes-induced damage in the retinal vessels promotes edematous changes and impairs the exchange of gases, nutrients, and waste in the retinal parenchyma, which can be recognized clinically as diabetic macular edema (DME) and nonperfused areas (NPAs).

Recent advances in optical coherence tomography angiography (OCTA) technology have enabled an appreciation of the three-dimensional vascular architecture.4–6 Histologic studies have reported that in healthy retinas the retinal vasculature resides mainly in the nerve fiber layer (NFL), ganglion cell layer (GCL), and the inner and outer borders of the inner nuclear layer (INL), although fluorescein angiography (FA) has shown a two-dimensional vascular picture.7,8 Most OCTA machines and their software for image analyses allow individual evaluations of the superficial and deep capillary plexuses, which contain vessels in the NFL and GCL, or those in the inner and outer borders of the INL in chorioretinal diseases.9–16 The capillaries in the FA images correspond approximately to those in the superficial layer on OCTA images, which suggests that a systematic evaluation of both the superficial and deep capillary layers would lead to a better understanding of the NPAs in DR.17 Despite the great advantage of OCTA imaging, we have to consider that the segmentation error or edematous changes did not allow correct evaluation on the en face images constructed according to the default setting, and capillaries should be evaluated three-dimensionally.

Optical coherence tomography (OCT) has visualized morphologic changes in the neuroglial components in diabetic retinas.18 Cystoid macular edema (CME) and serous retinal detachments (SRDs) were reported first as typical neuronal lesions in DME.19 A honeycomb pattern or petalloid pattern hyperfluorescence corresponds to cystoid spaces in the INL and Henle’s fiber layer (HFL) on OCT images, and foveal cystoid spaces are accompanied by an enlarged foveal avascular zone (FAZ) in DME.20–21 Other biomarkers of clinical relevance, the external limiting membrane and the ellipsoid zone of the photoreceptors (EZ), represent the integrity of the outer retinal layers, whereas disorganization of the retinal inner layers (DRIL) at the fovea also is related to visual function.22–25 The relationship between FA and OCT elucidated that the boundary between the NFL and GCL/INL plexiform layer...
(IPL) is often indistinct in the NPAs on FA images. En face OCT images delineate inner retinal spots with lower OCT reflectivity in the NFL and higher reflectivity in the GCL, referred to as inner retinal spots with inverted OCT reflectivity, most of which are seen in the hypofluorescent areas. However, it is largely unknown how the lamellar capillary nonperfusion contributes to the development or progression of these neuroglial lesions and concomitant visual impairment in DR.

The current study investigated the relationship between lamellar capillary nonperfusion and corresponding structural changes in the neuroglial components in DR using three-dimensional OCTA and OCT images.

**METHODS**

**Patients**

We retrospectively reviewed 101 consecutive eyes of 69 patients with DR who visited the Department of Ophthalmology of Kyoto University Hospital from March 2015 to November 2016. The inclusion criterion was the availability of OCTA images of sufficient quality obtained using Optovue RTVue XR Avanti (Optovue, Inc., Freemont, CA, USA). The exclusion criteria were the presence of other chorioretinal diseases; glaucoma or ocular hypertension; optic nerve diseases including ischemic optic neuropathy; a history of any intervention for macular lesions; intraocular surgery other than cataract surgery; and cataract surgery within 6 months of study enrollment. We further excluded eyes with a displacement artifact, which corresponds to the discontinuity along the B-scan direction caused by eye motion during the raster scan, or a signal strength index (SSI) score of 50 or less to guarantee image quality. Thirty eyes had center-involved DME, which was determined by two-dimensional mapping using the Optovue RTVue XR Avanti as described recently.

All research and measurements adhered to the tenets of the Declaration of Helsinki. The ethics committee of Kyoto University Graduate School and Faculty of Medicine approved the study protocol. All participants provided written informed consent before study entry.

**Optical Coherence Tomography Angiography**

After a comprehensive ophthalmic examination, retinal specialists evaluated fundus findings, and we reviewed the international grades of DR severity from medical records. OCTA and OCT images of the 3×3-mm square (corresponding to 304×304 pixels) centered on the fovea were obtained simultaneously and coregistered using the Optovue RTVue XR Avanti (Fig. 1). This instrument enables acquisition of reflectivity signals at a high A-scan rate of 70,000 scans/second using a light source of approximately 840 nm; the split-spectrum amplitude decorrelation angiography algorithm demonstrates motion-dependent angiography as described previously. Two consecutive B-scans were acquired at a fixed position before proceeding to the next position. After processing the volume scans, the calculation of the signal decorrelation between the sequential images leads to detection of erythrocyte motion and resultant construction of a motion contrast “angiow” image.

We then evaluated the NPAs in the superficial or deep layers (sNPA and dNPA, respectively) on the OCTA images. The perfusion status along 10 of 304 transverse lines (5th, 35th, 65th, 95th, 125th, 155th, 185th, 215th, 245th, and 275th lines from the most superior line) was estimated three-dimensionally using both en face and sectional images of the decorrelation signals (Fig. 1). We first screened the sNPA or dNPA using the OCTA images along 10 white arrows were evaluated. (d) The bNPA (yellow lines) and dNPA (green line) were shown in the retinal sectional image along the fourth line in (c). (e) The sNPA (red lines) on the B-scan image along the eighth line in (c).
to the default setting of the manufacturer’s software (Optovue, Inc.). We then confirmed the absence of decorrelation signals in the corresponding layers on the B-scan images. Retinal capillaries are mainly in the NFL, GCL, and the inner and outer borders of the INL.24,25 The superficial layer contains most of the capillary plexuses in the NFL and GCL/IPL, whereas two capillary layers in the INL are in the deep layer. We determined the sNPAs as the areas without decorrelation signals in the NFL or GCL/IPL alone on B-scan images. Similarly, the dNPAs were defined as the areas without decorrelation signals in the INL alone. The NPAs in both the superficial and deep capillary layers were referred to as the bNPA in this study. Two masked retina specialists measured the transverse length of the sNPA, dNPA, or bNPA along 10 horizontal lines, and the averages of the measurements were used for further investigation. Briefly, the B-scan images with the decorrelation signals were exported into ImageJ software (National Institutes of Health, Bethesda, MD, USA). The straight line tool and the following measure function were applied to count the horizontal pixel number, which was converted to the transverse length.

**Optical Coherence Tomography**

We evaluated the structural changes in the neuroglial tissues corresponding to the NPA in individual layers, that is, areas with no boundary between the NFL and GCL/IPL, inner retinal spots with inverted OCT reflectivity, and cystoid spaces in the INL and HFL.20,26,27 The transverse lengths of these lesions were individually quantified using the ImageJ software as described above. The OCT reflectivity of the NFL is much higher than that of the GCL/IPL on B-scan images in healthy eyes, which enabled identification of the boundary between these layers. In contrast, the boundary is often indistinct in the areas with a NPA on FA images in diabetic eyes, whose transverse length was measured.26

Another OCT finding in the inner retinal layers, the spot with inverted reflectivity on OCT images using the Optovue RTVue XR Avanti, also was evaluated according to modified methods as described previously.27 We sometimes found well-defined spots with lower reflectivity in the NFL and higher reflectivity in the GCL/IPL in the sNPA, after the en face OCT images in the NFL or GCL were constructed using the manual setting of the inner and outer borders of these layers in the localized areas. When the boundary between the GCL and IPL was indefinite, we constructed en face images from the inner border of the GCL to the center of the GCL/IPL to evaluate the patchy lesions in the GCL. We determined such lesions using en face and B-scan images and measured the transverse length of these lesions in the NFL along the 10 lines on the B-scan images. Cotton-wool spots are structural changes in focal NPAs. However, we did not evaluate these lesions, which were observed mainly around the vascular arcade but rarely within the central 3 × 3-mm square.

We also individually quantified the transverse length of the areas with cystoid spaces in the INL and HFL.20 Cystoid spaces in the fovea, where inner retinal layers were absent, were allocated to those in the HFL. Most cystoid spaces were delineated in either the INL or HFL, although we rarely observed enlarged cystoid spaces extending from the INL to the HFL.24 The areas with such counts were counted as cystoid spaces in the INL and HFL.

**Statistical Analysis**

The results are expressed as the median (interquartile range, IQR). The agreement of qualitative parameters measured by two retina specialists was confirmed using the intraclass correlation coefficients (ICCs). The data were analyzed using the Mann-Whitney U test or Kruskal-Wallis test with Bonferroni correction to evaluate the differences among groups. The sampling distribution was evaluated using Fisher’s exact test. Spearman’s correlation coefficient was calculated to test the statistical correlation. \( P < 0.05 \) was considered significant.

**RESULTS**

**Three Patterns of Lamellar Capillary Nonperfusion**

After we excluded 45 eyes with poor-quality OCTA images, we retrospectively reviewed 101 eyes of 69 patients with DR, whose characteristics are shown in the Table. The sNPA, dNPA, and bNPA were delineated along 10 horizontal lines (30 mm in total length) within a 3 × 3-mm square centered on the fovea in 37, 56, and 101 eyes, respectively (ICCs, 0.962, 0.975, and 0.986, respectively; Fig. 1). The transverse lengths of the sNPA and dNPA were 0.703 mm (0.244–1.664) and 0.184 mm (0–0.598), respectively (2.34% [0.81–5.55] and 0.61% [0–1.99]) (Fig. 2a). The length of the bNPA was 1.789 mm (1.206–3.264) (5.96% [1.02–10.88]). The transverse length of the sNPA was correlated modestly (\( r = 0.366, P < 0.001 \)) with that of the bNPA (Fig. 2b), whereas the dNPA was not associated with the bNPA or sNPA (Figs. 2c, 2d).

We compared the transverse lengths of the NPAs in eyes with center-involved DME to those in eyes without DME. Thirty eyes with DME had longer transverse length of the dNPA or bNPA (Figs. 2c, 2d). We compared the transverse lengths of the NPAs in eyes with center-involved DME to those in eyes without DME. Thirty eyes with DME had longer transverse length of the dNPA or bNPA (Figs. 2c, 2d). We compared the transverse lengths of the NPAs in eyes with center-involved DME to those in eyes without DME. Thirty eyes with DME had longer transverse length of the dNPA or bNPA (Figs. 2c, 2d). We compared the transverse lengths of the NPAs in eyes with center-involved DME to those in eyes without DME. Thirty eyes with DME had longer transverse length of the dNPA or bNPA (Figs. 2c, 2d).
on the OCT sectional images. The areas with no boundary between the NFL and GCL/IPL, which included the foveal center, were observed and quantified in all 101 eyes (ICC, 0.918; Figs. 3, 4). The proportion of the horizontal length of this OCT finding in the sNPA, dNPA, and bNPA was significantly ($P < 0.001$ for all comparisons) higher than that in the perfused area (PA) (Fig. 5a). The transverse lengths of the sNPA, dNPA, and bNPA also were correlated significantly with the length of no boundary between the NFL and GCL/IPL in the corresponding NPAs ($\rho = 0.836$, $P < 0.001$, $\rho = 0.747$, $P < 0.001$, and $\rho = 0.865$, $P < 0.001$, respectively; Figs. 6a, 6d, 6g).

Inner retinal spots with inverted OCT reflectivity were seen and measured in 48 eyes (ICC, 0.948; Fig. 7). The percentages of the transverse lengths of such spots in the sNPA were significantly ($P < 0.001$ for all comparisons) higher than in the PA, dNPA, and bNPA (Fig. 5b). A significant correlation also was seen between the transverse length of the sNPA and this OCT finding within the sNPA ($\rho = 0.836$, $P < 0.001$; Fig. 6b). Cotton-wool spots correspond to the focal ischemia around the vascular arcades in the FA images, whereas we rarely found them in the central 3 × 3-mm square.

Cystoid spaces, a major finding in the middle or outer retinal layers, are mainly in the INL and HFL and were seen in the INL in 38 eyes (Fig. 8). Twenty-two eyes (57.9%) with cystoid spaces in the INL had center-involved DME, whereas only 8 (12.7%) of 63 eyes without such cystoid spaces were accompanied by DME ($P < 0.001$). We then quantified the transverse length of cystoid spaces in the INL or HFL (ICC, 0.961 and 0.972, respectively), and compared such lesions to the NPAs. The proportion of horizontal length of cystoid spaces in the INL was significantly higher in the dNPA than in the PA, sNPA, and bNPA ($P < 0.001$, $P = 0.011$, and $P = 0.014$, respectively; Fig. 5c). The length of the dNPA was correlated significantly with that of the cystoid spaces in the INL in the dNPA ($\rho = 0.770$, $P < 0.001$; Fig. 6c). Fifty-eight eyes had cystoid spaces in the HFL, and the proportion of horizontal length of cystoid spaces in the HFL was significantly higher in the dNPA and bNPA than in the PA ($P = 0.004$ and $P < 0.001$, respectively; Fig. 5d). The length of the dNPA was correlated significantly with that of the cystoid spaces in the HFL in the dNPA ($\rho = 0.757$, $P < 0.001$; Fig. 6f), compared to no association in the bNPA ($\rho = 0.256$, $P = 0.053$; Fig. 6i).

**DISCUSSION**

In addition to vascular hyperpermeability, capillary nonperfusion leads to dysfunction of the neuroglial components in DR. The development of OCTA technology has enabled noninvasive appreciation of the three-dimensional structure of the retinal vasculature in DR, whereas FA, the invasive classical modality, provides only two-dimensional fluorescent signals derived mainly from the superficial retinal layers.9,10,17,30 The current study showed the systematic relationship between the lamellar capillary nonperfusion on OCTA images and structural changes in the corresponding neuroglial components in the OCT images. Intriguingly, the sNPA had inner retinal lesions, that is, inner retinal spots with inverted OCT reflectivity and no boundary between the NFL and GCL/IPL.26,27 The dNPA was accompanied frequently not only by neuroglial changes in the
corresponding layer, that is, cystoid spaces, but also by morphologic changes in other layers. These data suggested the clinical feasibility of a three-dimensional evaluation of the disruption in the neurovascular units in DR. Previous publications demonstrated the relationship between retinal dysfunction and circulatory disturbance on FA images, which may be consistent with the structural changes in the lamellar NPAs on OCTA images in this study.31,32 Further investigation

![Diagram](http://arvojournals.org/)

**Figure 3.** No boundary between the nerve fiber layer (NFL) and ganglion cell layer/inner plexiform layer (GCL/IPL) within the nonperfused area in the superficial layer (sNPA) in a 68-year-old patient with proliferative diabetic retinopathy. En face optical coherence tomography angiography images of the superficial layer (a), the deep layer (b), and the merged image (c) (red: superficial, green: deep). (d, e) The sectional images with and without a decorrelation signal along the arrow in (a–c) show the absence of a boundary between the NFL and GCL/IPL within the sNPA (arrowheads). (f) A magnified image of the square in (d). The double-headed arrow indicates the area with no boundary between the NFL and GCL/IPL.

![Diagram](http://arvojournals.org/)

**Figure 4.** No boundary between the nerve fiber layer (NFL) and ganglion cell layer/inner plexiform layer (GCL/IPL) within the nonperfused area in both the superficial and deep layers (bNPA) in a 42-year-old patient with proliferative diabetic retinopathy. En face optical coherence tomography angiography images in the superficial layer (a), the deep layer (b), and the merged image (c) (red: superficial, green: deep). (d, e) The sectional images with and without a decorrelation signal along the arrow in (a–c) show the absence of a boundary between the NFL and GCL/IPL within the bNPA (arrowheads). (f) A magnified image of the square in (d). The double-headed arrows indicate the areas with no boundary between the NFL and GCL/IPL.
We thus speculated that the NPAs in the diabetic retinas, and inconsistencies often are seen between the structure and function of the retinal vessels. For example, the movement of the erythrocytes is unstable in diabetic retinas, and inconsistencies often are seen between the structure and function of the retinal vessels. For example, the decorrelation signals in the microaneurysms can appear or disappear in sequentially acquired images, and the redelineation of the decorrelation signals in the NPA represents the reperfusion. We thus speculated that the NPAs in the OCTA images correspond to either complete loss of the capillary beds or reduced velocity of the erythrocyte movement. In addition, the projection artifacts in hyperreflective lesions or segmentation error and shadow artifacts in retina edema do not enable OCTA to depict retinal vascular structure exactly.

A recent study reported an OCT finding in the NPA on FA images, that is, no boundary between the NFL and GCL/IPL, which is consistent with such lesions in the bNPA on OCTA images. Intriguingly, we also observed no boundary between these layers within the sNPA. A novel OCT finding, the inner retinal spots with inverted OCT reflectivity, also was seen in the sNPA. Since capillaries in the deep layer reside mainly in the inner and outer borders of the INL, the lamellar ischemia in the middle layers might disrupt the exchanges in oxygen, nutrients, and wastes and concomitant accumulation of intracytoplasmic or extracellular fluids. Vascular hyperpermeability might result in development of cystoid spaces in the INL, which subsequently might compress the surrounding vessels and reduce the velocity or amount of blood flow. In contrast, capillary loss in the deep layer might disrupt the pump function in the Müller cells or drainage of the extravascular fluids from the capillaries there, which might lead to edematous changes in the neuroglial components. We had to consider that the dNPA around the cystoid spaces might be an artifact, a flow void, although we carefully evaluated the decorrelation signals in both the en face and B-scan images. We showed the association between the dNPA and cystoid spaces in the INL. Since capillaries in the deep layer reside mainly in the inner and outer borders of the INL, the lamellar ischemia in the middle layers might disrupt the exchanges in oxygen, nutrients, and wastes and concomitant accumulation of intracytoplasmic or extracellular fluids. Vascular hyperpermeability might result in development of cystoid spaces in the INL, which subsequently might compress the surrounding vessels and reduce the velocity or amount of blood flow. In contrast, capillary loss in the deep layer might disrupt the pump function in the Müller cells or drainage of the extravascular fluids from the capillaries there, which might lead to edematous changes in the neuroglial components. We had to consider that the dNPA around the cystoid spaces might be an artifact, a flow void, although we carefully evaluated the decorrelation signals in both the en face and B-scan images. We showed the association between the dNPA and cystoid spaces in the INL. Since capillaries in the deep layer reside mainly in the inner and outer borders of the INL, the lamellar ischemia in the middle layers might disrupt the exchanges in oxygen, nutrients, and wastes and concomitant accumulation of intracytoplasmic or extracellular fluids. Vascular hyperpermeability might result in development of cystoid spaces in the INL, which subsequently might compress the surrounding vessels and reduce the velocity or amount of blood flow. In contrast, capillary loss in the deep layer might disrupt the pump function in the Müller cells or drainage of the extravascular fluids from the capillaries there, which might lead to edematous changes in the neuroglial components. We showed the association between the dNPA and cystoid spaces in the INL.
in the HFL, as discussed regarding the cystoid spaces in the INL.

In the current study, center-involved DME was associated with both the dNPA and cystoid spaces in the INL. We might hypothesize that the dNPA in the parafovea contributes to the pathogenesis in both such cystoid spaces in the corresponding areas and retinal thickening in the fovea. The imbalance between the diapedesis and absorption of extracellular fluids in the extrafoveal areas might promote foveal SRD. The correlation between the transverse length of the NPA in the deep layer (dNPA) and individual neuroglial findings in the dNPA. The relationship between the transverse length of the NPAs in both layers (bNPA) and individual neuroglial findings in the bNPA.

In conclusion, we showed an association between lamellar capillary nonperfusion and structural changes in the corresponding neuroglial components, which sheds light on the pathogenesis in the fovea. Since we could not determine the boundary between them, we included the FZ in the NPAs around the fovea. Since OCTA cannot assess vascular permeability, the mechanisms regarding edematous changes including the cystoid spaces are not fully understood. We evaluated the structural changes in the neurovascular components along 10 representative lines in Asian patients with sufficient quality OCTA images in a single center. A future study should evaluate the areas with individual lesions on the two-dimensional plane in larger cohorts to guarantee the generalizability in other institutes in other races.

The areas without capillaries were composed of both normal FAZ and pathologically developed NPAs around the fovea. Since we could not determine the boundary between them, we included the FAZ in the NPAs in this study. As a result, the NPAs did not always correspond to the pathologic changes. Since OCTA cannot assess vascular permeability, the mechanisms regarding edematous changes including the cystoid spaces are not fully understood. We evaluated the structural changes in the neurovascular components along 10 representative lines in Asian patients with sufficient quality OCTA images in a single center. A future study should evaluate the areas with individual lesions on the two-dimensional plane in larger cohorts to guarantee the generalizability in other institutes in other races.

![Figure 6](http://arvojournals.org/)

**Figure 6.** Association between the transverse lengths of neuroglial findings and the lamellar nonperfused areas (NPAs) in diabetic retinopathy. (a–c) The association between the transverse lengths of the NPA in the superficial layer (sNPA) and individual neuroglial findings in the sNPA. (d–f) The correlation between the transverse length of the NPA in the deep layer (dNPA) and individual neuroglial findings in the dNPA. (g–i) The relationship between the transverse length of the NPAs in both layers (bNPA) and individual neuroglial findings in the bNPA.
FIGURE 7. Inner retinal spots with inverted reflectivity in the nonperfused area of the superficial layer (sNPA) in a 43-year-old patient with moderate nonproliferative diabetic retinopathy. En face optical coherence tomography (OCT) angiography images in the superficial layer (a) and the deep layer (b). En face OCT images of the nerve fiber layer (c) and the ganglion cell layer (d) show the spots with inverted OCT reflectivity (arrowheads). (e) A merged image of the capillaries in the superficial and deep layers (red: superficial, green: deep). (f) A merged image of (a) (red) and (d) (grayscale). (g, h) A B-scan image with and without a decorrelation signal along the arrow in (a–f) shows the sectional image of spots with inverted reflectivity in the sNPA (arrowheads).
understanding of the layer-by-layer pathogenesis in the neurovascular units in DR.

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References


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