Diabetic retinopathy (DR), a diabetic microangiopathy, often leads to severe visual impairment worldwide.1,2 Diabetes disrupts the function and structure of the retinal vasculature, which exacerbates the vascular hyperpermeability and loss of the capillary beds.3–5 These changes contribute clinically to the pathogenesis of diabetic macular edema (DME) and the nonperfused areas (NPAs) and concomitant neovascular complications, which promote neuroglial dysfunction or retinal degeneration in patients with diabetes.6–12

In healthy eyes, the neuroglial cells in the inner retinal layers are perfused by retinal vasculature mainly in the nerve fiber layer (NFL)/ganglion cell layer (GCL) and the inner and outer borders of the inner nuclear layer (INL), whereas the outer retinal layers are nourished by the chorioidal vessels.13,14 Disappearance of the retinal capillaries results in nutrient and oxygen deficiencies or waste accumulation in the inner retinal layers, referred to as retinal ischemia. Clinical studies have identified visual impairment associated with NPAs, which might be supported by histologic studies that documented loss of neuronal cells and gliosis in ischemic retinas with DR.7–12,15,16 However, it is unknown how ischemia exacerbates the in vivo morphologic changes in the neuroglial tissues. Despite its invasiveness, fluorescein angiography (FA) is the gold standard for the clinical evaluation of the morphologic and pathophysiologic changes in the retinal vasculature.17,18 Fluorescein angiography enables recognition of the details of the vascular lesions including microaneuorysms, venous beading, intraretinal microvascular abnormalities, and neovascularization.19 Fluorescein angiography is the only method to adequately delineate NPAs whether the circulatory disruption depends on emboli resulting from blood cells or apoptotic changes in vascular cells induced by the biochemical pathways and cytokines.20–25 However, despite the distinct delineation of vascular lesions, FA cannot directly show the structural changes in the neuroglial tissues of the NPAs.

Several studies have proposed noninvasive methods to assess the NPAs rather than invasive FA. Dark areas on the red-free images were reported to correspond to NPAs on FA images in retinal vascular diseases.26 Phase-variance optical coherence tomography (OCT) delineates the retinal vasculature, which might allow us to evaluate the capillary loss.27 In addition to the vascular lesions, spectral-domain (SD)-OCT showed neuroglial changes (i.e., the decreased thickness of the GCL in eyes with diabetic ischemic maculopathy).28 A few studies have reported changes in the thickness of the inner or outer retinal layers measured by OCT, although the relevance remains controversial.9,11,29,30 In eyes without retinal edema, NPAs are accompanied by thinning of the inner layers and thickening of the outer layers.10 Vascular hyperpermeability resulting from diabetes often exacerbates edematous changes in the retinal parenchyma and modulates the thicknesses of the inner and outer retinal layers. However, qualitative OCT findings that correspond to the pathohistologic changes in neuroglial tissues in NPAs remain largely unknown.10

Purpose. To investigate morphologic changes on spectral-domain optical coherence tomography (SD-OCT) images in nonperfused areas (NPAs) in diabetic retinopathy (DR).

Methods. One hundred eight consecutive eyes of 80 patients with diabetic ischemic maculopathy were retrospectively reviewed. The boundary between the nerve fiber layer (NFL) and the ganglion cell layer (GCL)/inner plexiform layer (IPL) and the status of Henle’s layer were characterized on the vertical sectional images of SD-OCT. These findings were compared with the NPAs on the FA images and the logMAR visual acuity (VA).

Results. The SD-OCT images showed that most areas of capillary nonperfusion had an indistinct boundary between the NFL and GCL/IPL in DR, regardless of high or moderate OCT reflectivity. The total transverse length of the NPAs was correlated positively with that of the areas with no boundary between these layers in all 108 eyes (R = 0.860, P < 0.001). Sixty-four eyes that had center-involved diabetic macular edema (DME) also had a significant association between them (R = 0.764, P < 0.001), and the most significant correlation was seen in eyes without DME (R = 0.955, P < 0.001). The macular transverse length of the areas with no boundary between the NFL and GCL/IPL was associated modestly with the logMAR VA (R = 0.334, P < 0.001). The indistinct Henle’s layer on SD-OCT images often was delineated specifically in the NPAs rather than in the perfused areas.

Conclusions. Nonperfused areas were associated significantly with the absence of a boundary between the NFL and GCL/IPL on SD-OCT images in DR.

Keywords: diabetic retinopathy, nonperfused area, spectral-domain optical coherence tomography.
In the current study, we characterized the OCT findings in the NPAs in eyes with DR, focusing on the absence of a boundary between the NFL and GCL/IPL and the indistinct Henle’s layer.

METHODS

Patients

We reviewed retrospectively 108 consecutive eyes of 80 patients (mean age, 60.4 ± 11.8 years; range, 31–85) with diabetic ischemic maculopathy (39 eyes with moderate non-proliferative diabetic retinopathy [NPDR], 30 with severe NPDR, and 39 with proliferative diabetic retinopathy) for which FA and OCT images of sufficient quality were obtained in the Department of Ophthalmology of Kyoto University Hospital from November 2007 to February 2014. The inclusion criteria were ischemic maculopathy of grade 2, 3, or 4 defined by the Early Treatment Diabetic Retinopathy Study (ETDRS) Report Number 11, which was determined based on the agreement of two independent masked graders. The major exclusion criteria were the presence of any other chorioretinal diseases, the presence of a pathology in the optic nerve including glaucoma, severe media opacity including preretinal hemorrhage, a history of treatment of macular pathology, and a history of cataract surgery within 3 months or any major surgery other than cataract extraction within 1 year of entry into the study. All research and measurements adhered to the tenets of the Declaration of Helsinki. The institutional review board/ethics committee of our institution approved the study. Written informed consent was obtained after full explanation of the nature and possible consequences of this study.

Imaging

After comprehensive ophthalmologic examinations including measurement of the best-corrected VA, color fundus photography, and slit-lamp biomicroscopy, FA images of the macula (30° × 30° centered on the fovea) were acquired using a scanning laser ophthalmoscope (Heidelberg Retina Angiograph 2; Heidelberg Engineering, Heidelberg, Germany) as described previously. We measured the transverse length of the NPAs on FA images and compared them to sectional SD-OCT (Spectralis OCT; Heidelberg Engineering) images. Briefly, we determined the 7-mm vertical line on the FA images, which corresponded to the vertical sectional images of the cross-hair mode of OCT images. Two masked graders measured the transverse lengths of the areas with no distinct retinal vessels, which contained both the pathological NPAs and the foveal avascular zone, regardless of whether or not it was enlarged. The mean length measured by two independent graders in a masked fashion (intraclass correlation coefficient [ICC], 0.872) was applied for further analyses.

Figure 1. Optical coherence tomographic findings in NPAs in DR (A) NPAs involving the fovea are delineated mainly in the temporal and inferior subfields on FA image. (B) An SD-OCT image along the arrow in (A). The bidirectional arrow indicates the NPAs. (C–F) Magnified images of (B). (C) An OCT image depicts the distinct boundary between the NFL and GCL/IPL (black arrowheads) and Henle’s layer (white arrowheads) in the perfused areas. (D) No boundary is visible between the NFL and GCL/IPL in the NPAs without retinal edema. (E) The boundary between the NFL and GCL/IPL is indistinct in NPAs accompanied by retinal edema, and enlarged cystoid spaces sometimes disrupt Henle’s layer (area between the arrows). The arrowheads indicate Henle’s layer. (F) The boundary between the NFL and GCL/IPL (black arrowhead) is observed in the NPAs around the arteriole (green bidirectional arrow). The red bidirectional arrow indicates the NPAs.

In the current study, we characterized the OCT findings in the NPAs in eyes with DR, focusing on the absence of a boundary between the NFL and GCL/IPL and the indistinct Henle’s layer.
Retinal sectional images of the macula were obtained using SD-OCT, and the vertical sections in the cross-hair mode (30°) were evaluated further. We first determined the presumed foveal center where the inner retinal layers from the NFL to INL were absent as described previously. It was sometimes difficult to determine the individual retinal layers in eyes with severe ischemic maculopathy, in which the INL or Henle’s layer was unidentifiable. In such cases, we determined that the point on the horizontal line dissecting the center of the optic disc was the foveal center. The sectional images within 3.5 mm of the (presumed) foveal center were evaluated qualitatively and quantitatively. The mean retinal thickness in the central 1-mm subfield of the ETDRS grid was assessed on a two-dimensional OCT map constructed by raster scans, as described previously, followed by diagnosis of center-involved DME according to the criteria in a recent publication.

We especially evaluated the boundary between the retinal layers, from the NFL to INL, and the status of Henle’s layer. The GCL often had the same OCT reflectivity as the IPL, which prompted us to compare the reflectivity levels of the NFL to those of the combined layers of the GCL and IPL, referred to as the GCL/IPL. Since the OCT reflectivity of the NFL is much higher than that of the GCL/IPL in healthy eyes, we can determine the boundary between these layers (Supplementary Fig. S1). In contrast, OCT images showed that the boundary was indistinct in the NPAs with and without edematous changes in the retinal parenchyma (Figs. 1–3), which was confirmed using the Plotprofile function of the ImageJ software.

Two masked graders measured the transverse length of the areas with no boundary between the NFL and GCL/IPL, which contained the fovea where the NFL was absent and the boundary between these layers was not seen (Fig. 4). The mean length measured by two masked graders was analyzed further (ICC, 0.980). We also assessed the status of Henle’s layer on the vertical section of the OCT images. OCT showed Henle’s layer as a line of high reflectivity between the INL and outer nuclear layer (ONL), both of which had moderate OCT reflectivity in healthy eyes. This layer sometimes was not identifiable because of increased levels of OCT reflectivity in the INL or enlarged cystoid spaces from the INL to the outer plexiform layer (OPL)/ONL (Figs. 1, 5). Henle’s layer was often indistinct at the presumed foveal center, whether the eyes were healthy or not. We included areas with both of these findings into those without a distinct Henle’s layer; two masked graders measured the transverse length (ICC, 0.992), and the average was analyzed further.

The total transverse length of the areas with no boundary between the NFL and GCL/IPL or those with an indistinct
Henle’s layer on the OCT images, whether continuous or not, was compared with that of the NPAs on the FA images. The length of one continuous area with such OCT findings containing the (presumed) foveal center was measured and defined as the macular transverse length. We further evaluated its association with the logMAR VA.

**Statistical Analysis**

The results were expressed as the mean ± SD. Linear regression analysis was performed to determine a statistical correlation. \( P \) less than 0.05 was considered significant.

**RESULTS**

**Indistinct Boundary Between the Nerve Fiber Layer and Ganglion Cell Layer/Inner Plexiform Layer in Nonperfused Areas**

Individual retinal layers have individual levels of OCT reflectivity in healthy eyes, which enables us to identify the boundaries between the different layers. In contrast, the boundaries between the retinal layers including the NFL, GCL/IPL, INL, and Henle’s layer were often indistinct in the NPAs (Figs. 1, 2). The boundary between the NFL and GCL/IPL especially was

**Morphologic Changes on SD-OCT Images in NPAs in DR**

**Figure 3.** Higher OCT reflectivity in CWS. (A) Nonperfused areas around the arrow on a FA image correspond to the CWS on the color photograph (B). (C) A retinal sectional image shows swelling of the inner retinal parenchyma with no boundary between the NFL and GCL/IPL. (D) The reflectivity levels are higher and homogeneous in the CWS compared with the different levels of OCT reflectivity between the NFL and GCL/IPL in perfused areas (E).

**Figure 4.** Measurements of the transverse length of the areas with no boundary between the NFL and GCL/IPL. (A) An FA image shows ischemic maculopathy accompanied by NPAs in the parafovea. (B) An OCT image along the arrow in (A). There are three areas with no boundary between the NFL and GCL/IPL (bidirectional arrows). The total (both green and red arrows) or macular (red arrows alone) transverse length of the areas with no boundary between these layers was quantified. (C) A magnified image in the parafovea. The boundary between the NFL and GCL/IPL is indistinct in the areas between the two white arrows. The black arrowheads indicate the boundary between the NFL and GCL/IPL. (D) A magnified image around the fovea. There is no boundary between the NFL and GCL/IPL in the areas between the two white arrows. The inner layers (from the GCL to the INL) and Henle’s layer are absent in the areas between the two white arrowheads and the two black arrows, respectively.
indistinct and the INL was delineated in most areas of capillary nonperfusion, whether the areas were accompanied by retinal edema or not (Figs. 1, 2). In addition, cotton-wool spots, seen as NPAs on the FA images, also appeared as highly reflective lesions with an indistinct boundary between the layers (Fig. 3). However, the OCT images often showed the boundary between the NFL and GCL/IPL in the NPAs near the major vessels (Fig. 1).

We then focused on the OCT reflectivity levels of the retinal parenchyma in which the boundary between the NFL and GCL/IPL disappeared. Such parenchymal lesions around the fovea tended to have the same levels of OCT reflectivity as the GCL/IPL in the adjacent perfused areas and a gradual decrease in the NFL thickness (Fig. 4). In contrast, the typical NPAs in the parafovea or perifovea often had higher levels of OCT reflectivity, similar to those in the NFL (Fig. 4).

We measured the total transverse length of the areas with an indistinct boundary between the NFL and GCL/IPL and evaluated the association with the NPAs (Fig. 4). The boundary was indistinct in 88.9 ± 16.7% of the transverse length of the NPAs on FA images, and 88.9 ± 16.2% of the areas with no boundary between these layers corresponded to NPAs on the FA images. This may be compatible to the finding that the total transverse length of the areas with an indistinct boundary between the NFL and GCL/IPL was correlated with that of the NPAs on the FA images in all 108 eyes (R = 0.406, P < 0.001; Fig. 6). The association was more significant in 44 eyes without center-involved DME (R = 0.955, P < 0.001; Fig. 6) rather than those with center-involved DME. We further evaluated the macular transverse length of this finding and found a modest association with logMAR VA in all 108 eyes and 64 eyes combined with center-involved DME (R = 0.354, P < 0.001 and R = 0.406, P = 0.001, respectively; Fig. 7).

**Status of Henle’s Layer in Nonperfusion Areas**

No differences were sometimes seen in the OCT reflectivity between the INL and Henle's layer in the NPAs in diabetic eyes, compared with those in healthy eyes (Figs. 1, 5). The enlarged cystoid spaces extended from the INL to the OPL/ONL in limited areas of the NPAs, indicating discontinuity of Henle's layer (Fig. 5). Most areas (94.5 ± 15.4%) with these findings, referred to as indistinct Henle’s layer, were delineated within the NPAs on the FA images, suggesting higher specificity. However, the total transverse length of the areas without distinct Henle’s layer was not related to that of the NPAs on FA images in all 108 eyes and in 44 eyes without DME (Fig. 8). The macular transverse length of the areas with indistinct Henle’s layer was correlated modestly with the logMAR VA (Fig. 9).

**DISCUSSION**

Diabetes exacerbates the damage in the retinal vasculature, which leads to retinal ischemia and concomitant visual impairment in DR. However, it remains largely unknown how loss of the retinal capillary beds exacerbates the pathogenesis in the neuroglial components and vice versa. The current study found that OCT showed the indistinct boundary between the NFL and GCL/IPL and the absence of Henle’s layer in the NPAs. The absence of a boundary between the NFL and GCL/IPL on OCT images was related significantly to the NPAs on the FA images, suggesting that OCT has potential use for evaluating the NPAs in eyes with DR. In contrast, the indistinct Henle’s layer was located specifically in the NPAs, although no correlation was found between the transverse length of the indistinct Henle’s layer and that of the NPAs.

A few studies have investigated the relationship between the NPAs on FA images and thinning of the inner retinal layers on OCT images in DR. We also observed atrophic changes in the inner retinal layers in eyes without retinal edema, although the retinal thickness can change in response to edema, axial length, age, and so on. Thinning of the inner retinal layers especially might not be useful for evaluating NPAs with retinal edema, which is often exacerbated by...
diabetes-induced vascular permeability. In the current study, OCT delineated the indistinct boundary between the NFL and GCL/IPL in the NPAs with and without retinal edema. In addition, the transverse length of the NPAs was associated significantly with that of the areas without a boundary between these layers on SD-OCT images in 64 eyes with center-involved DME. These data may recommend clinicians to observe the absence of a boundary between the NFL and GCL/IPL for predicting NPAs, whether the retinal parenchyma is edematous or not. In contrast, the association between the VA and this OCT finding was modest, suggesting that other factors also affect visual impairment.

**Figure 6.** A significant association is seen between the total transverse length of the areas with no boundary between the NFL and GCL/IPL and that of the NPAs on FA images in 108 eyes with ischemic maculopathy (A), in 64 eyes with center-involved DME (B), and in 44 eyes without center-involved DME (C).

**Figure 7.** A modest correlation between logMAR VA and the macular transverse length with no boundary between the NFL and GCL/IPL in 108 eyes with ischemic maculopathy (A), in 64 eyes with center-involved DME (B), and in 44 eyes without center-involved DME (C).
We documented the various levels of OCT reflectivity in the retinal parenchyma with no boundary between the NFL and GCL/IPL, which prompted us to hypothesize the several histologic changes in this lesion. The different levels of OCT reflectivity in the NFL and GCL/IPL allowed us to recognize the boundary between these layers in healthy eyes. In contrast, the increased levels of reflectivity in the GCL/IPL or the decreased levels in the NFL may result in the disappearance of the boundary between these layers, which may represent the processes of neuroglial cell death and gliosis as reported in a histologic study. Other possible explanations may be that loss of the ganglion cells and accompanying axons (nerve fibers) corresponds to the OCT findings showing thinning of the inner retinal layers and decreased OCT

**Figure 8.** The relationship between the total transverse length of the areas with no distinct Henle’s layer and that of the NPAs on FA images in 108 eyes with ischemic maculopathy (A), in 64 eyes that also have center-involved DME (B), and in 44 eyes without DME (C).

**Figure 9.** A modest correlation between logMAR VA and the macular transverse length of the areas without a distinct Henle’s layer in 108 eyes with ischemic maculopathy (A), in 64 eyes that also have center-involved DME (B), and in 44 eyes without DME (C).
reflectivity around the fovea. This may agree with previous studies that reported apoptotic changes in the ganglion cells in DR or thinning of the GCL in ischemic maculopathy.\textsuperscript{3,4} In contrast, we often observed that the NPAs in the parafovea were accompanied by increased levels of OCT reflectivity in the inner retinal layers. Loss of ganglion cells may be represented by absence of the GCL/IPL, whereas the nerve fibers derived from the distal areas were observed as the inner layers with higher OCT reflectivity.

Diabetes exacerbates the pathogenesis in the neurovascular units (i.e., the degenerative processes in the vascular cells and neuroglial components).\textsuperscript{3,4} It remains ill-defined whether loss of the capillary beds in the NPAs is the primary process or a secondary response after neuroglial atrophy. Since the microcirculation is disturbed in diabetic retinas, vascular damage may precede neuroglial changes.\textsuperscript{20–22} This may be compatible with the OCT findings that the NPAs adjacent to large vessels were accompanied by a boundary between the NFL and GCL/IPL (Fig. 1). In contrast, several clinical and biomedical studies have suggested that the primary processes in the diabetic retinas may be neuroglial dysfunction or degeneration.\textsuperscript{35–37} We thus speculated that neuronal degeneration resulting from diabetes induces a deficiency in vasculogenic factors and concomitant disappearance of vascular cells.\textsuperscript{45} These changes in vasculature and neuronal tissues may promote degenerative processes reciprocally in diabetic retinas.

Although the indistinct Henle’s layer was observed specifically in the NPAs of DR, we did not find an association between the transverse length of the NPAs and that of the areas with the indistinct Henle’s layer, suggesting that this OCT finding is less useful for predicting NPAs. We also considered the possible morphologic changes (i.e., increased OCT reflectivity in the INL, disappearance of the INL, or disrupted Henle’s layer due to enlarged cystoid spaces).\textsuperscript{35} In addition to retinal ischemia, inflammation and extravasated blood components also may promote the chronic neurodegeneration or glialosis in the INL or Henle’s layer, resulting in indistinct Henle’s layer on SD-OCT images.\textsuperscript{5,10} Another mechanism may be that enlarged cystoid spaces extending from the INL to the OPL/ONL disrupt Henle’s layer. The capillary plexus layer in the outer INL may provide structural integrity and separate the cystoid spaces in the INL from those in the OPL/ONL, although loss of capillaries in the NPAs may allow the cystoid spaces to extend between these layers.\textsuperscript{44,45} These possible pathologic mechanisms in the transduction system may contribute to visual impairment, which was supported by the modest correlation between the VA and the indistinct Henle’s layer in the macula (Fig. 9).

Although these OCT findings have potential use for noninvasive evaluation of NPAs and for understanding the pathogenesis, the current study had a few limitations. We assessed the boundary between the NFL and GCL/IPL subjectively, and further objective and automatic methods should be developed. In this study, we measured the transverse length of the areas with an indistinct boundary between the NFL and GCL/IPL on the vertical SD-OCT images dissecting the fovea. We considered that two-dimensional information may provide a better understanding, although the NFL is absent in the temporal raphe, which did not allow us to identify the boundary between the NFL and GCL/IPL.

In summary, the current study documented two OCT findings, that is, the absence of a boundary between the NFL and GCL/IPL and the indistinct Henle’s layer in the NPAs of eyes with DR, suggesting their clinical use in the noninvasive evaluation of NPAs and the pathogenesis in the neurovascular units.

Acknowledgments

Disclosure: Y. Dodo, None; T. Murakami, None; A. Uji, None; S. Yoshitake, None; N. Yoshimura, None

References


