Characterization of Diabetic Microaneurysms by Simultaneous Fluorescein Angiography and Spectral-Domain Optical Coherence Tomography

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• PURPOSE: To correlate spectral-domain optical coherence tomography (SD-OCT) findings of perfused diabetic microaneurysms with leakage status on fluorescein angiography (FA) using simultaneous FA and SD-OCT.

• DESIGN: Retrospective, observational case series.

• METHODS: A total of 173 microaneurysms were analyzed in 50 eyes (14 mild nonproliferative diabetic retinopathy [NPDR]; 22 moderate NPDR; 9 severe NPDR; 5 proliferative diabetic retinopathy) of 40 diabetic patients using simultaneous FA and SD-OCT. The characteristics of microaneurysms were evaluated by 2 masked observers using SD-OCT and correlated with leakage status on FA.

• RESULTS: External diameter of microaneurysms averaged 104 μm (range 43-266 μm). Some microaneurysm centers (15/173; 9%) and the outermost extent of microaneurysms (113/173; 68%) were localized to the outer half of the retina. Almost all microaneurysms spanned more than 1 retinal layer (157/173; 91%). Most microaneurysms had an internal lumen with homogeneous reflectivity (109/173; 63%) and moderate reflectivity (87/173; 50%). Retinal thickness through microaneurysms as well as the presence of adjacent hyporeflectivity on SD-OCT correlated with increasing leakage status seen on FA ($P < .001$). Microaneurysm dimensions, percent depth within the retina, retinal layer location, and internal reflectivity by SD-OCT did not correlate significantly with FA leakage status.

• CONCLUSIONS: Simultaneous FA and SD-OCT allows detailed characterization of perfused diabetic microaneurysms. Increased FA leakage of diabetic microaneurysms positively correlated with perianeurysm fluid and retinal thickness. Perfused microaneurysms seen by SD-OCT were localized deeper than the inner nuclear layer. (Am J Ophthalmol 2012;153:861–867. © 2012 by Elsevier Inc. All rights reserved.)

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DIABETIC RETINOPATHY (DR) IS A LEADING CAUSE of visual loss among working-age individuals in developed countries. This vision loss is often the result of macular edema from leaking microaneurysms[1,2] and may be very difficult to treat.

Clinically, diabetic microaneurysms appear as superficial red dots on fundus examination and as hyperfluorescent spots by fluorescein angiography (FA).[3,4] Most knowledge regarding structure and localization of diabetic retinal microaneurysms is derived from histologic and pathologic studies.[5–10] These studies have shown that diabetic microaneurysms are incompetent vascular outpouchings of the macular capillary bed that primarily arise from the deep part of the inner retinal capillary plexus[6,7] and are located in the inner nuclear layer (INL), extending infrequently to the outer plexiform layer (OPL).[7,8] However, most histopathologic studies have been based on trypsin-digested retinal flat mounts with light microscopy or electron microscopy.[7,9,10]

More recently, diabetic microaneurysms were characterized using spectral-domain optical coherence tomography (SD-OCT).[11] However, understanding of structural differences between nonleaking microaneurysms and leaking microaneurysms that may lead to clinically significant macular edema has not been well delineated. Better understanding of structure and location of nonleaking or leaking diabetic microaneurysms may improve current treatment approaches to macular edema.

As a noninvasive and noncontact imaging technique, high-resolution SD-OCT with eye tracking allows us to use simultaneous scanning laser ophthalmoscopy (SLO) to co-localize angiographic findings with SD-OCT images. This makes it possible to correlate angiographic features and SD-OCT morphology in retinal diseases.[12] In this study, we characterize perfused diabetic aneurysms with no, mild, or severe leaking using simultaneous FA and SD-OCT. Our goal is to determine the size, distribution, and reflectivity of these aneurysms and compare angiographic and SD-OCT features.

METHODS

IN A RETROSPECTIVE CASE SERIES FROM SEPTEMBER 1, 2008 to October 31, 2010, microaneurysms (N = 173) in

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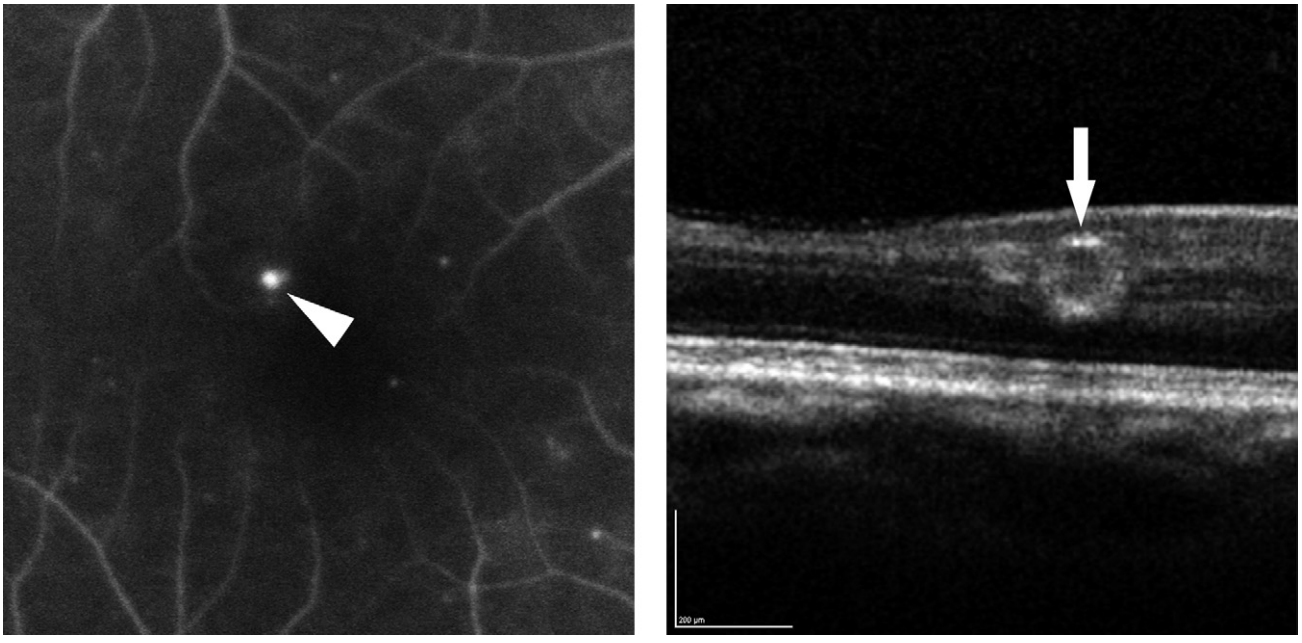

FIGURE 1. Diabetic microaneurysms visualized by simultaneous fluorescein angiography (FA) and spectral-domain optical coherence tomography (SD-OCT). (Left) Mild leakage of a microaneurysm (arrowhead) seen by FA. (Right) A microaneurysm was bisected by vertical scanning plane on SD-OCT (arrow). 1:1 axial: horizontal.

diabetic eyes (N = 50) that underwent simultaneous FA and SD-OCT imaging were evaluated by 2 masked retina specialists. Eyes from diabetic patients with both nonproliferative diabetic retinopathy (NPDR) (N = 45; mild =14, moderate=22, severe = 9) and proliferative diabetic retinopathy (PDR) (N = 5) were evaluated. Patients who received anti–vascular endothelial growth factor (VEGF) treatment in either eye or eyes that received focal or grid laser within 6 months were excluded.

We performed simultaneous FA and SD-OCT (Heidelberg Spectralis, Carlsbad, California, USA), which allows real-time imaging to co-localize angiographically visible microaneurysms. Microaneurysms were detected as hyperfluorescent dots in the early phase of FA imaging and leakage was graded as no, mild, or severe by comparing the FA images of the microaneurysms in the arteriovenous phase with the images in the late phase. OCT protocol used raster sections of the macula. SD-OCT images were selected with either the vertical or horizontal scanning plane bisecting the center of each microaneurysm. All images were evaluated using a 1:1 vertical-to-horizontal aspect ratio. The external and internal diameters of each microaneurysm were measured and the wall thickness was calculated (wall thickness = [external diameter − internal diameter]/2). To analyze depth distribution of microaneurysms, retinal thickness (RT) through the center of each microaneurysm was measured, and the percent depth of each microaneurysm from the retinal surface was calculated. To assess the span of each microaneurysm, the innermost and outermost retinal layers to which each microaneurysm extended were determined.

To evaluate the heterogeneity of the contents within microaneurysms, internal reflectivity within each lumen was graded as hyporeflective, moderate, or hyperreflective. Since microaneurysm walls are hyperreflective compared to surrounding retinal tissue, the lumen was considered hyperreflective if reflectivity was similar to the microaneurysm wall. Hyporeflective was defined as a similar reflectivity to cystic intraretinal fluid. Moderate reflectivity was determined for reflectivity intermediate to the two. Internal reflectivity was further characterized as homogeneous or heterogeneous. Additionally, immediately external to each microaneurysm within the surrounding retina, we determined whether surrounding hyporeflectivity was present (Yes or No).

Since correlation between 2 masked observers across all parameters for 30 microaneurysms was highly concordant (93%-97%), statistical analyses (SAS version 9.2; SAS Institute Inc, Cary, North Carolina, USA) were performed using data from either observer. Continuous variables were expressed as mean ± standard deviation (SD). Regression analysis using FA leakage grade as a response was performed. A multinomial cumulative ordinal model was simulated using generalized estimation equations (GEE) to deal with the nature of clustered data including inter- and intra-eye associations. A P value $< .05$ was considered to be statistically significant.

RESULTS

• **FLUORESCEIN ANGIOGRAPHY:** A majority of microaneurysms showed mild angiographic leakage (117/173;

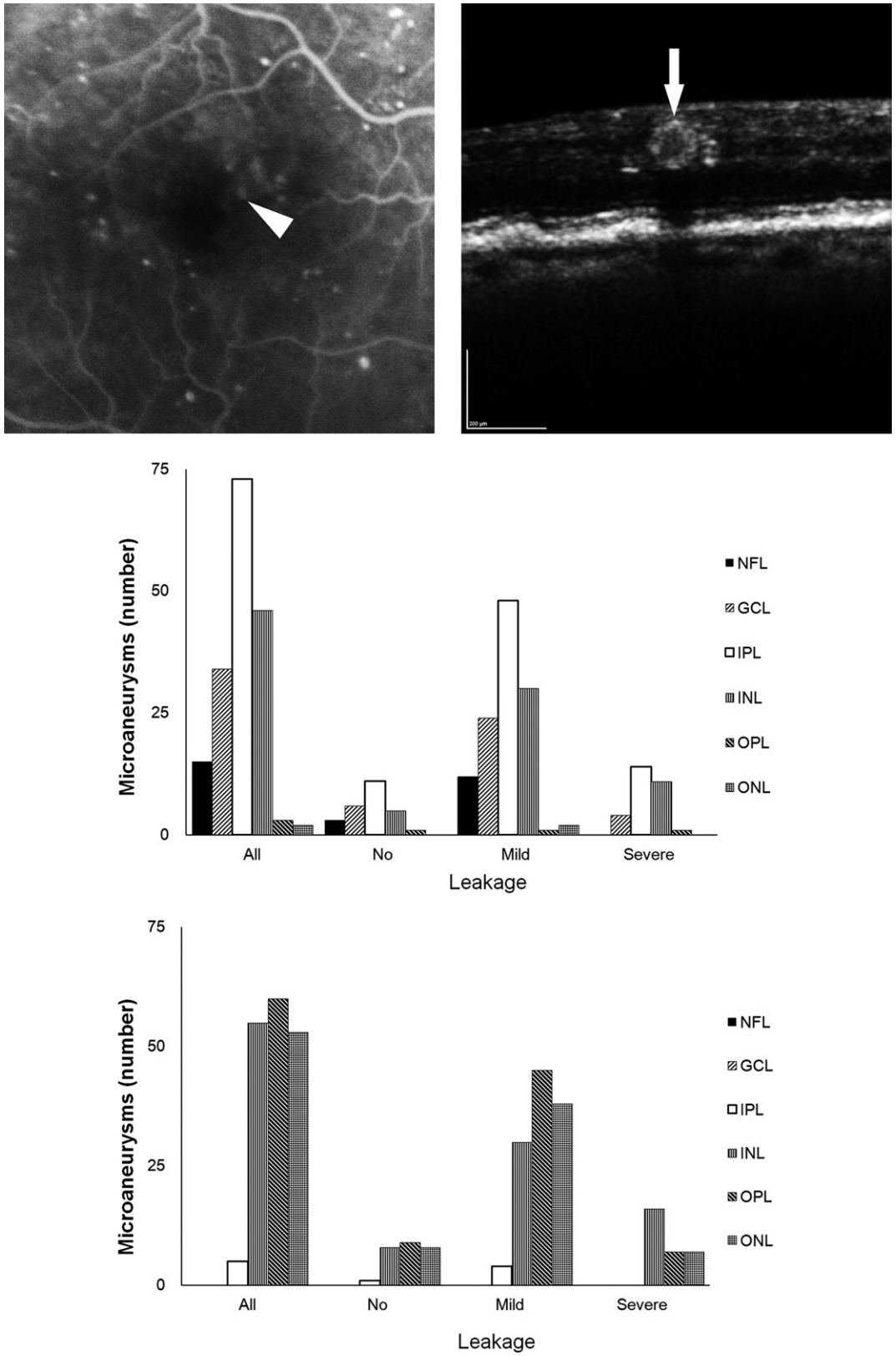

FIGURE 2. Distribution of diabetic microaneurysms (N = 173) by retinal layers. (Top left) Representative mild leakage of a microaneurysm (arrowhead) seen on fluorescein angiography (FA). (Top right) The same microaneurysm (arrow) visualized by spectral-domain optical coherence tomography (SD-OCT) along the vertical scanning plane spanning from inner plexiform layer to outer plexiform layer. (Middle) The innermost distribution of diabetic microaneurysms by retinal layers extended from nerve fiber

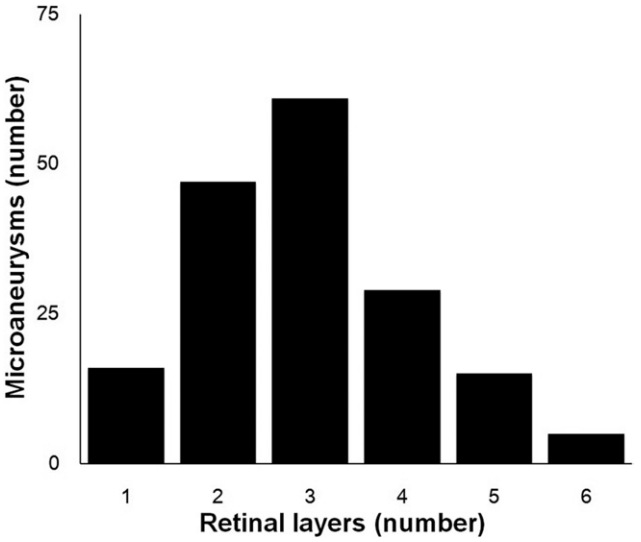

FIGURE 3. Axial extent of retinal layers occupied by diabetic microaneurysms (N = 173). X-axis: number of spanning retinal layers by microaneurysms; y-axis: number of microaneurysms.

68%). The number of microaneurysms with no leakage (26/173; 15%) or severe leakage (30/173; 17%) was similar. Microaneurysms in eyes with NPDR compared to those with PDR revealed no difference in leakage by FA (P = .508).

• **DIMENSIONS OF DIABETIC MICROANEURYSMS:** Overall, diabetic microaneurysms had a well-demarcated round or oval shape visualized by SD-OCT (Figure 1) with an external and internal diameter of 104 ± 35 μm and 55 ± 34 μm, respectively. The calculated mean wall thickness was 23 ± 7 μm. No differences in external (P = .246) and internal diameters P = .349) were observed among aneurysms with no, mild, or severe leakage on FA. The dimension of microaneurysms also did not vary with diabetic retinopathy status (data not shown).

• **DEPTH AND DISTRIBUTION OF DIABETIC MICROANEURYSMS:** To assess the percent depth of microaneurysms within the retina, we evaluated the RT through the center of each microaneurysm. Increasing angiographic leakage was associated with increasing RT around microaneurysms. Microaneurysms with no, mild, and severe leakage seen on FA resulted in RT of 306 ± 45 μm, 363 ± 86 μm, and 439 ± 96 μm, respectively (P < .001, GEE). The majority of diabetic microaneurysms were found at 37% ± 10% depth from the inner retinal surface. No differences in percent depth from the retinal surface were observed among microaneurysms with no, mild, or severe leakage on FA (P = .167). Interestingly, a subset of microaneurysms (15/173; 9%) was localized to the outer half (55% ± 5% depth) from the inner retinal surface.

We further characterized the location and extent of the innermost and outermost retinal layers spanned by each diabetic microaneurysm (Figure 2). The innermost extent ranged from the nerve fiber layer (NFL) to the outer nuclear layer (ONL). The innermost extent of the plurality of microaneurysms (73/173; 42%) was the inner plexiform layer (IPL). The outermost extent of microaneurysms ranged from IPL to ONL, with the majority of microaneurysms (168/173; 97%) having an outermost extent localized between the INL and the ONL. No differences between the outermost (P = .237) and innermost (P = .582) extents of diabetic microaneurysms were seen among microaneurysms with no, mild, or severe leakage.

The majority of microaneurysms spanned more than 1 retinal layer (157/173; 91%) (Figure 3). Thirty-six percent (63/173) spanned either 1 or 2 retinal layers. Similarly, another third spanned 3 retinal layers (61/173; 35%). In addition, nearly one-third of microaneurysms spanned 4 or more retinal layers (49/173; 28%).

• **INTERNAL REFLECTIVITY OF MICROANEURYSMS BY SPECTRAL-DOMAIN OPTICAL COHERENCE TOMOGRAPHY:** To further characterize diabetic microaneurysms by SD-OCT, internal reflectivity and lumen heterogeneity or homogeneity were assessed among microaneurysms with no, mild, or severe leakage by FA (Table 1). Among all diabetic microaneurysms, half (87/173; 50%) had a moderate internal reflectivity, with the remaining microaneurysm lumens well distributed between hyporeflectivity (35/173; 20%) and hyperreflectivity (51/173; 30%). A similar distribution of internal reflectivity by SD-OCT was noted within each category of microaneurysms (no, mild, or severe leakage) (P = .739). The majority of microaneurysm lumens had a homogenous internal reflectivity (109/173; 63%), while over one-third of microaneurysm lumens were determined to have a heterogenous internal reflectivity. Heterogenous or homogeneous internal reflectivity was similar among microaneurysms with no, mild, or severe leakage on FA (P = .186).

• **ADJACENT HYPOREFLECTIVITY OF MICROANEURYSMS BY SPECTRAL-DOMAIN OPTICAL COHERENCE TOMOGRAPHY:** We determined whether there was any adjacent hyporeflectivity surrounding each microaneurysm

layer to outer nuclear layer. The innermost distribution of microaneurysms was not statistically significant among FA leakage status (P = .582). (Bottom) The outermost distribution of diabetic microaneurysms by retinal layers extended from inner plexiform layer to outer nuclear layer. The outermost distribution of microaneurysms was not statistically significant among FA leakage status (P = .237). NFL: nerve fiber layer; GCL: ganglion cells layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer.

TABLE 1. Internal Reflectivity of Diabetic Microaneurysms by Spectral-Domain Optical Coherence Tomography

	All Microaneurysms (N = 173)	No Leakage (N = 26)	Mild Leakage (N = 117)	Severe Leakage (N = 30)
Internal Reflectivity	N (%)	N (%)	N (%)	N (%)
Hyperreflective	51 (30)	7 (27)	34 (29)	10 (33)
Moderate	87 (50)	14 (54)	59 (50)	14 (47)
Hyporeflective	35 (20)	5 (19)	24 (21)	6 (20)
Homogeneous	109 (63)	19 (73)	75 (64)	15 (50)
Heterogeneous	64 (37)	7 (27)	42 (36)	15 (50)

TABLE 2. Adjacent Hyporeflectivity of Diabetic Microaneurysms by Spectral-Domain Optical Coherence Tomography

	All Microaneurysms (N = 173)	No Leakage (N = 26)	Mild Leakage (N = 117)	Severe Leakage (N = 30)
Surrounding Hyporeflectivity	N (%)	N (%)	N (%)	N (%)
Yes	90 (52)	3 (12)	60 (51)	27 (90)
No	83 (48)	23 (88)	57 (49)	3 (10)

using SD-OCT (Table 2, Figure 4). Adjacent hyporeflectivity consistent with perianeurysm edema was observed in half of all microaneurysms (92/117; 52%). Only 3 out of 26 (12%) of non-leaking microaneurysms had adjacent hyporeflectivity, while in the severe leakage group the majority of microaneurysms (27/30; 90%) were surrounded by hyporeflectivity in the retina. Adjacent hyporeflectivity on SD-OCT was associated with increasing FA leakage status ($P < .001$). However, adjacent hyporeflectivity of microaneurysms was not different in relation to their locations in the retina. Out of 158 microaneurysms located in the inner half of the retina, 81 (51%) had adjacent hyporeflectivity on OCT, and 9 out of 15 microaneurysms (60%) located in the outer half of the retina showed adjacent hyporeflectivity ($P = .60$).

DISCUSSION

THIS STUDY OUTLINES PARTICULAR SD-OCT CHARACTERistics of diabetic microaneurysms and correlates these findings with extent of leakage seen by fluorescein angiography. Although previous studies have also evaluated diabetic microaneurysms using SD-OCT, simultaneous FA allows us to exclude involuted or thrombosed microaneurysms as well as lipid or pigmentary changes not likely contributing to visual pathology (macular edema).

Increasing leakage on FA correlated with increased total retinal thickness through the center of each microaneurysm measured using SD-OCT imaging. This affirms that subtle differences in leakage status seen by FA are detectable by differences in total retinal thickness measurements using SD-OCT imaging. There may exist a threshold change in total retinal thickness that may determine progression to clinically significant macular edema and highlight the need for earlier therapeutic intervention.

After normalization of microaneurysm location to total retinal thickness, no differences were seen in percentage depth of microaneurysms between those with no, mild, or severe leakage status by FA. This suggests that the tendency for microaneurysms to leak is not determined by retinal depth/location. In addition, measuring the internal diameter, external diameter, and wall thickness of diabetic microaneurysms revealed no differences among microaneurysms with no, mild, or severe leakage. It is likely that microaneurysm leakage reflects a functional difference in endothelial cell-cell integrity or permeability rather than an anatomic or size variation between microaneurysms.

Interestingly, the presence of adjacent hyporeflectivity around diabetic microaneurysms on SD-OCT correlated with positive leakage status seen on FA (Table 2, $P < .001$). The surrounding hyporeflectivity likely represents an accumulation of perianeurysmal fluid or edema. Thus SD-OCT may be a good alternative to assess "leakage" (perianeurysmal fluid) in patients with a contraindication to fluorescein angiography. Moreover, efficacy of diabetic macular edema treatment may be monitored by changes in surrounding hyporeflectivity of microaneurysms using SD-OCT.

The majority of microaneurysms had an innermost location in the IPL (73/173; 42%) and an outermost location between the INL and ONL (168/173; 97%) (Figure 2). Surprisingly, a group of microaneurysm centers (15/173; 9%) was found in the outer half of the retina. In addition, the outermost extent of the majority of microaneurysms (113/173; 65%) was found in the outer half of

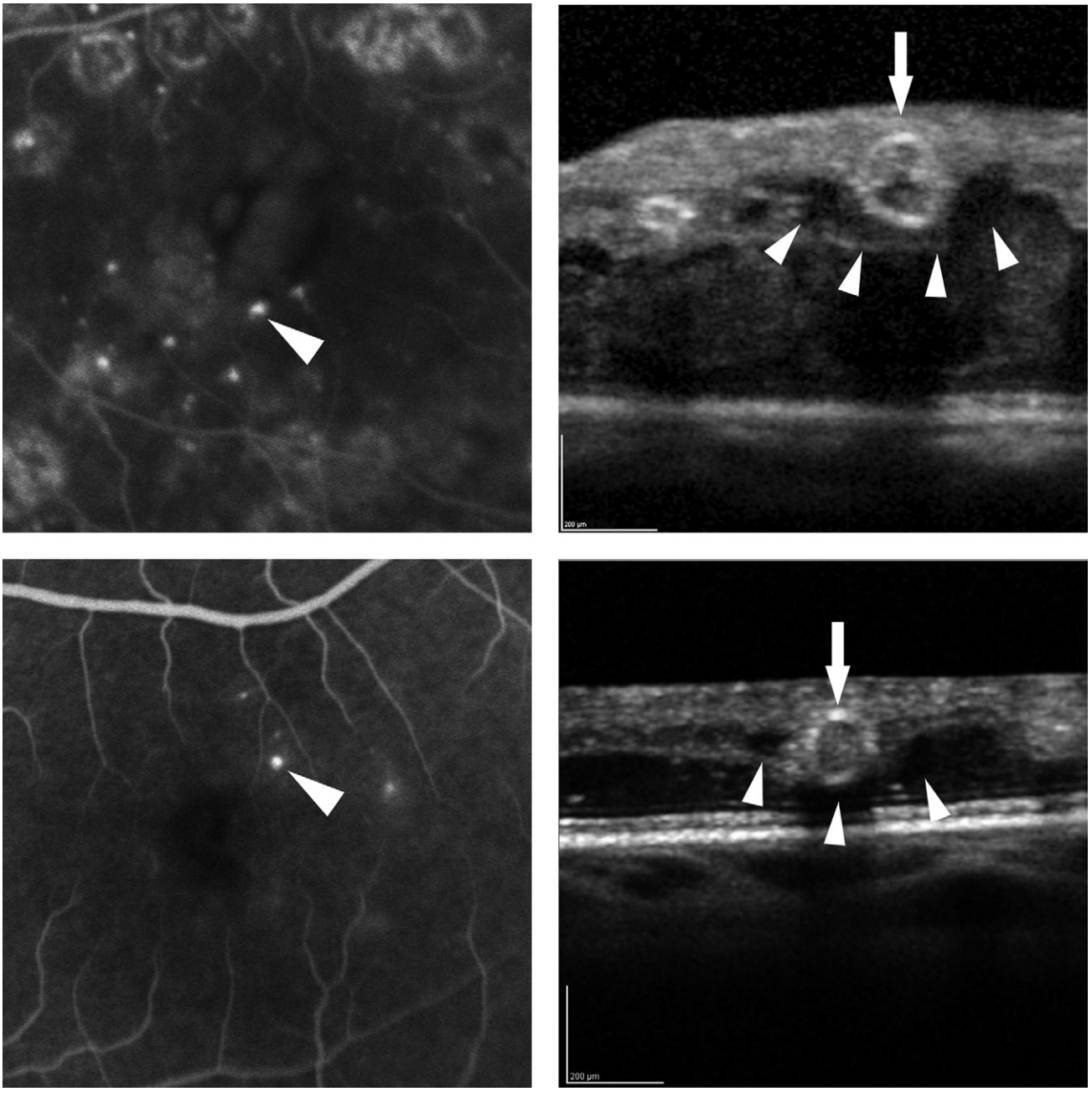

FIGURE 4. The internal and perianeurysm reflectivity of representative diabetic microaneurysms using spectral-domain optical coherence tomography (SD-OCT). (Top left) Severe leakage of a microaneurysm (arrowhead) with macular edema was showed on fluorescein angiography (FA). (Top right) The same microaneurysm (arrow) with perianeurysm hyporeflectivity (arrowhead) and heterogeneous internal reflectivity seen by horizontal scanning on SD-OCT. (Bottom left) Mild leakage of a microaneurysm (arrowhead) seen on FA. (Bottom right) The same microaneurysm (arrow) with internal moderate and homogeneous reflectivity as well as perianeurysm hyporeflectivity (arrowhead) visualized by SD-OCT.

the retina from OPL to ONL. This suggests that patent or perfused (angiographically visible) microaneurysms are actually deeper than previously described.[7,13] In addition, over 90% of diabetic microaneurysms spanned more than 1 retinal layer with only 9% of microaneurysms localized within 1 retinal layer.

Histologic studies have shown that lumen contents of microaneurysms may be composed of polymorphonuclear cells, red blood cells, fibrotic cells, thrombi, or lipid aggregates.[7] Since all microaneurysms analyzed in this study were perfused by fluorescein, it is not surprising that SD-OCT reflectivity and heterogeneity or

homogeneity were similar between microaneurysms with no, mild, or severe leakage seen by FA (Table 1). Currently available software does not permit quantification of internal reflectivity with a numerical scale. Perfused microaneurysms likely contain similar cells with minimal fibrosis or thrombi.

In summary, we have characterized perfused diabetic microaneurysms in detail using simultaneous fluorescein angiography and spectral-domain optical coherence tomography. Subtle differences in retinal thickness and perianeurysmal fluid attributable to individual microaneurysm leakage and edema may be assessed reliably using SD-OCT. We acknowledge the limitations of this retrospective study, which consists of patients with differing disease states and duration of disease. In addition, medical intervention such as laser retinopexy and anti-VEGF treatment may have influenced the leakage of the microaneurysms studied. However, microaneurysms in eyes with either NPDR or PDR revealed no difference in FA leakage ($P = .508$). In addition, our study only included microaneurysms that underwent remote anti-VEGF or laser treatment (more than 6 months); therefore, the impact of medical intervention on characteristic of diabetic microaneurysms may be limited. It may be interesting to evaluate FA leakage with SD-OCT adjacent hyporeflectivity surrounding microaneurysms prospectively following anti-VEGF or laser treatment.

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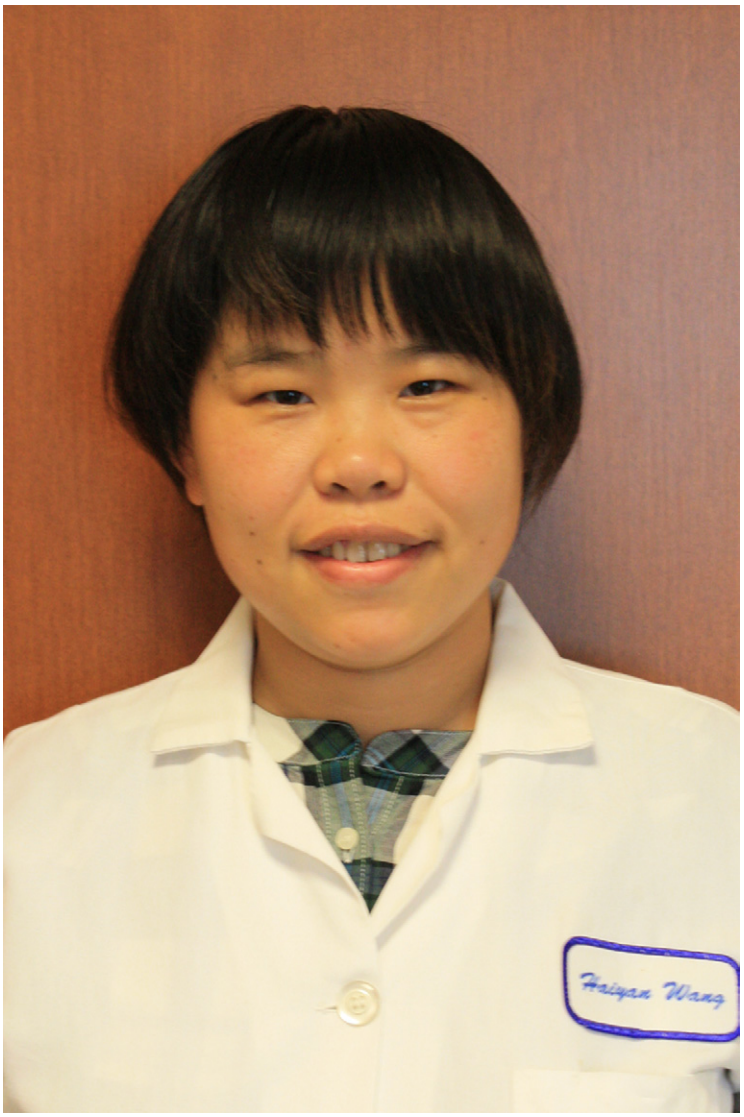

Biosketch

Haiyan Wang, MD, PhD, is an international retina fellow at the Jacobs Retina Center/Shiley Eye Center, University of California, San Diego. She received her MD from Weifang Medical College, China, in 1998. Dr Wang joined the faculty at the Department of Ophthalmology, Xijing hospital, China upon receiving her Master Degree in 2001. She received her PhD from the Fourth Military Medical University of China in 2004. Dr Wang's research interests include medical retina and uveitis.